

Research Article

Genetic Diversity of Flower Lotus (*Nelumbo Adans.*) Cultivars Assessed by SSR Markers

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Citation: Du F, Li N, Chang Y, Li P, Yao D, et al. (2017) Genetic diversity of flower lotus (*Nelumbo Adans.*) cultivars assessed by SSR markers. Asian J Life Sci 2017: AJLS-102.

Received Date: 29 March, 2017; **Accepted Date:** 11 April, 2017; **Published Date:** 17 April, 2017

Abstract

Lotus (*Nelumbo Adans.*) is an important aquatic crop in Asia, Oceania and North America. Flower lotus is widely used in aquatic gardens for its high ornamental value. To determine its genetic diversity and population structure, 36 flower lotus accessions were genotyped with 17 pair SSR primers. A total of 76 alleles were generated and 87.5% were polymorphic. The Polymorphism Information Content (PIC) value ranged from 0.20 to 0.81. Analysis of Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) clustering and Principal Coordinate Analysis (PCoA) distinctly grouped flower lotus accessions from Asian lotus, American lotus and Sino-American hybrids into different clusters. However, the hybrids dispersed among clusters. Population structure analysis further confirmed the clustering of lotus accessions, and it also indicated that there were several subgroups within Asian lotus collection. The genetic diversity and population structure of flower lotus accessions herein are beneficial for germplasm utilization and genetic improvements of flower lotus in the future.

Key words: *Nelumbo*, Flower Lotus; Molecular Marker; Genetic Relationship; Population Structure

Introduction

Lotus (*Nelumbo Adans.*), an aquatic perennial, is a popular ornamental plant in Asia, Oceania and North America. In addition, lotus is widely used for vegetable and medicine in China, Japan, and some Southeast Asian countries [1,2]. In several cultures and religions of the world, lotus is famous for cultural significance and symbolism. Lotus has two species: *Nelumbo nucifera* Gaertn and *Nelumbo lutea* (Willd.) Pers [3]. *N. nucifera* is mainly distributed in Asia and north Oceania, and it appears naturally with the color of red, pink and white [4,5]. While *N. lutea* is found in North America and northern South America, and it is characterized by yellow floral color [6,7]. In China, lotus has a long cultivation history of more than 3,000 years [5,8]. Based on its application, lotus is classified into three types: flower lotus, rhizome lotus and seed lotus. Flower lotus is widely used in aquatic gardens, and its at-

tractive characteristics, such as flower color, flower shape make it a predominant type in flower industry.

Now days, cross breeding is still the most efficient breeding method in flower lotus. It is of great importance to get a better understanding of flower lotus germplasms for suitable breeding programs. Since morphological traits are inevitably affected by external environments, it is a hard work in assessing flower lotuses with complex genetic backgrounds. Currently, the applications of molecular markers have been preferred due to good

Stability and high efficiency [9-11]. Using Inter-Simple Sequence Repeats (ISSR), genetic diversity of wild populations of *N. nucifera* were investigated [12,13]. Random Amplification of Polymorphic DNA (RAPD) and ISSR markers were applied to evaluate the genetic relationships among lotus germplasms from different origins, including China, Thailand and America [14]. Genetic variation among *Nelumbo nucifera* Gaertn accessions were evaluated by Amplified Fragment Length Polymorphisms (AFLP) markers [15]. Comparing with other molecular markers, Simple

Sequence Repeat (SSR) markers have many advantages, such as codominance, high reproducibility, relatively high levels of polymorphism, and abundant distribution in genomes [16]. With the genome sequencing of the China Antique lotus, a great number of SSR motifs were identified and a new set of SSR markers were tested [17,19]. These discoveries advanced our knowledge of the genetic diversity of lotuses to some extent. However, few studies focus on flower lotus cultivars, which have huge potentials in plant breeding and landscape application.

In this study, 36 flower lotus cultivars were genotyped with SSR markers. Genetic diversity and population structure of these cultivars were evaluated using UPGMA, PCoA and STRUCTURE program. Findings of this study will provide important clues for germplasm utilization, and contribute to genetic improvements of flower lotus cultivars as well.

Materials and methods

Plant materials

36 cultivars of flower lotuses were used as experimental materials. The samples comprised 22 cultivars of *Nelumbo nucifera*, 1 cultivars of *Nelumbo lutea*, 8 hybrids and 5 new cultivars. These accessions varied from plant size, petal form and flower color. All samples were collected from institute of botany, Jiangsu province and Chinese academy of sciences, Jiangsu province, China. The morphological characteristics of these lotus cultivars were listed in (Table 1).

Code	Name	Type or Origin information	Plant size	Petal form	Flower color
1	Pi Zhenhong	As	Ms	D	R
2	Xing Huafen	As	Ms	D	R
3	Zhongguohong-Shanghai	As	Ms	F	R
4	Zhongguohong-Xibeipo	As	L	F	R
5	Zhongguohong-Beijing	As	L	F	R
6	Zhongguohong-Jiaxing	As	L	F	R
7	Zhongguohong-Jinggangshan	As	L	D	R
8	Zhongguohong-Shaoshan	As	L	D	R
9	Zhongguohong-Ruijin	As	L	D	R

10	Zhongguohong-Zunyi	As	L	sD	R
11	Xin Jinling Huodu	As	L	sD	R
12	Hong Tailian	As	L	Du	R
13	Yiliang Qianban	As	L	T	R
14	Hong Xianzhi	As	L	F	R
15	Da Sajin	As	L	D	V
16	Yi Xianlian	As	L	F	V
17	Fen Qinglian	As	Ms	F	V
18	Cai Die	As	Ms	F	V
19	Feiyan Chunmian	As	Ms	F	P
20	Fei Tian	As	Ms	F	P
21	Ju Wuba	As	L	D	P
22	Bi Yun	As	L	D	W
23	Cui Yun	H	Ms	D	W
24	Boli Furen	H	Ms	D	V
25	Yu Huaqing	H	Ms	D	V
26	Jing Cai	H	Ms	D	V
27	Youyi Mudan Lian	H	L	D	Y
28	Wu Jian	H	L	D	Y
29	Liuchao Yuye	H	L	F	Y
30	Liuchao Yimeng	H	L	F	Y
31	American Lotus	Am	L	F	Y
32	Jin Taiyang	Un	Ms	D	Y
33	Moling Qiuse	Un	Ms	D	Y
34	Jin Pingguo	Un	L	D	Y
35	Liuchao Jinfen	Un	L	F	Y
36	Zhufeng Cuiying	Un	L	Du	W

Table 1: Morphological characteristics of 36 flower lotus cultivars.

As = Asian lotus species, Am = American lotus species, H = Sino-American hybrids species and Un = unknown; L = large, Ms = medium-small; F = few petals (less than 20 petals per flower), sD = semidouble petals (21-50 petals per flower), D = double petals, in which most of the stamens in the flower are transformed into petals, Du = duplicate petals, in which the stamens and pistils are transformed into petals, and T = thousands of petals, in which the stamens, pistils, and receptacle are all transformed into petals; R = red petals, Y = yellow petals, W = white petals, and V = versicolor petals.

DNA extraction

For each sample, total genomic DNA samples were extracted from fresh leaves of lotus using the Cetyl Trimethyl Ammonium Bromide (CTAB) method [20]. The quality of extracted DNA was evaluated by 0.8% agarose gel electrophoresis, and the concentration was quantified by a One Drop spectrophotometer. After that,

the DNA was diluted to a concentration of 50 ng/μL, and stored at -20°C for further analysis.

SSR analysis

A set of SSR primers developed by Ming et al. [17] were used as candidates, and finally 17 pair primers were selected for further analysis. The fluorescent primers were synthesized by Beijing Dingguo Changsheng Biotechnology Company (Beijing, China). For PCR amplification, each 25-μL reaction mixture contained 50 ng DNA, 2.5 μL 10×PCR buffer, 0.2 mM dNTPs, 4 μM of each primer, and 1U of Taq DNA polymerase (Takara). The PCR reaction was carried out for 5 min at 94°C, followed by 35 cycles of 30s at 94°C, 30s at the appropriate annealing temperature (ranges from 55°C to 60°C, Table 2), 3s at 72°C, and then followed by a supplemental incubation for 10 min at 72°C. The fluorescent PCR products were separated by capillary electrophoresis, and finally detected with ABI3730 sequencer (ABI, California, USA).

Data analysis

To evaluate the polymorphic levels, genetic diversity parameters including the observed Number of Alleles (Na), the effective Number of Alleles (Ne), Shannon's information index (I), the ex-

pected Heterozygosity (He) and the observed Heterozygosity (Ho) were calculated with POPGENE version 1.31. The Polymorphism Information Content (PIC) of each primer was calculated with Microsatellite Tool kit of Microsoft Excel. A dendrogram based on the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) was performed, and the boots trap analysis was constructed using Power Marker version 3.25. Nei's genetic distance matrixes were further subjected to Principal Coordinated Analysis (PCoA) using XLSTAT 2014. Additionally, an admixture model-based population structure was investigated using the software STRUCTURE version 2.2 [21].

Results

Levels of polymorphism

As shown in (Table 2) a total of 76 alleles were scored from PCR amplification of genomic DNA from 36 cultivars, and 87.5% were polymorphic. When scored for all the cultivars, each maker was associated with 2 to 8 polymorphic alleles. The effective number of alleles (Ne) varied from 1.28 (SSR061) to 5.81 (SSR072) with a mean of 3.01. Shannon's Information index ranged from 0.38 (SSR061) to 1.86 (SSR072) with an average of 1.16. The expected and observed heterozygosity (He and Ho) varied from 0.22 (SSR061) to 0.84 (SSR072) with a mean of 0.63, and from 0.14

Primers	Sequence(5'-3')	Tm(°C)	Na	Ne	I	Ho	He	PIC
SSR028	F:CATTTGAAGTTGGGGACAAAAG	57	8	4.36	1.71	0.94	0.78	0.74
	R:GACTAGGCCCAAAGTAGAGGGT							
SSR041	F:GGCAAGGAAGATCTAGCGGTAAGT	58	4	3.30	1.27	0.75	0.71	0.64
	R:CATGCATGAATCTTGAAGTCTTG							
SSR061	F:AAAGACCAATGAGAAGTGGGATGA	58	2	1.28	0.38	0.14	0.22	0.20
	R:TCTCGATGTAGCATTAGAACACCG							
SSR072	F:ATTTTCGAATCTCCCTCCATACT	58	8	5.81	1.86	0.89	0.84	0.81
	R:GAACAACAAAAAGAATGAGGATCTATTG							
SSR078	F:GACTAATGAAACAGGGTTAGGGC	60	6	3.96	1.50	0.81	0.76	0.71
	R:TCTCCAATCTCCAACGACTCTTA							
SSR079	F:TGAATGTGAAAAGAAAAAGTCGCA	55	5	2.65	1.17	0.75	0.63	0.56
	R:TGTACTACTTTCCTCGAAGACGGC							
SSR098	F:TAGACCAGGCTAGGATGGGGTAG	60	5	4.16	1.47	0.58	0.77	0.72
	R:AGTTAATGACATCTGCAGGGTGGT							
SSR110	F:GCCAGCTACACCTTTTGATCTCAG	60	4	3.32	1.27	0.75	0.71	0.64
	R:TTCCCAATTCTCAGTCCAGGTATGT							
SSR155	F:GGGAGACTGTTTTAAAGCCCTA	57	3	1.48	0.59	0.36	0.33	0.29
	R:AAATTGATGGCCATTCTGACTTA							
SSR236	F:CGATTTAACAAAATGGAAAACCGA	55	5	2.42	1.04	0.44	0.60	0.50
	R:TAGTGGAGTAGAGAAGTAGCGGCG							

SSR257	F:AGATGGTCCCTACCATTAACCGAT	60	3	2.87	1.08	0.78	0.66	0.58
	R:GAGTTTTGCTTGGAGGGATAACCT							
SSR301	F:TAGAAAGAGGAAAGAGGGGAGGTG	58	4	2.60	1.09	0.56	0.62	0.55
	R:GAAAAGAGGGATTTAATGGGGATG							
SSR346	F:GGCGAGAATTGAAGAAGAAATCTG	58	4	2.33	0.96	0.36	0.58	0.48
	R:AGAGCAGCCCAAGAATGTTTGAT							
SSR355	F:TTGGATTGAGTAAATGAGGGGAAA	57	4	3.05	1.22	0.97	0.68	0.61
	R:AGAAACTTCTCAGTTTCAAGCA							
SSR362	F:TAGTTATGGCCTTATGGGGAACCT	58	4	2.96	1.16	0.64	0.67	0.60
	R:AAGGTTTCTGTTTGGGGACCTTTA							
SSR460	F:CAAGTGAAGACACCGAAGAGGATT	57	3	1.98	0.85	0.56	0.50	0.44
	R:TATGGAAGCCATAAGAAAATCCGA							
SSR489	F:CCACTCGATTTGTGTTTGTGTTGT	58	4	2.57	1.05	0.75	0.62	0.54
	R:AAGGTTGAGAGGAAGGAAAATTG							
Total		-	76	-	-	-	-	-
Mean		57.88	4.47	3.01	1.16	0.65	0.63	0.57

Table 2: Polymorphism information for SSR makers.

Na, Observed number of alleles; Ne, Effective number of alleles; I, Shannon's Information index; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphism information content.

SSR cluster analysis

Using UPGMA method, the dendro gram for lotus collections was constructed. The 36 cultivars were divided into two clusters (Group I and Group II). Group I was comprised of 33 accessions, including both Asian lotus and Sino-American hybrids. In subgroup Ia, most of the accessions followed the characteristics of Asian lotuses. While the accessions of Ib came in with different colors and sizes, indicating complicated genetic backgrounds of those cultivars. Group II has three accessions, including a typical America originated lotus. And notably the three members were all characterized by yellow floral color. As for the new cultivars, 'Jin Pingguo' and 'Zhufeng Cuiying' were grouped into subgroup Ia, indicating a close relationship with Asian lotus species, 'Moling Qiuse', 'Liuchao Jinfen' and 'Jin Taiyang' showed close relationship with Sino-American hybrids in subcluster Ib2, suggesting a heterozygous genetic background (Figure 1).

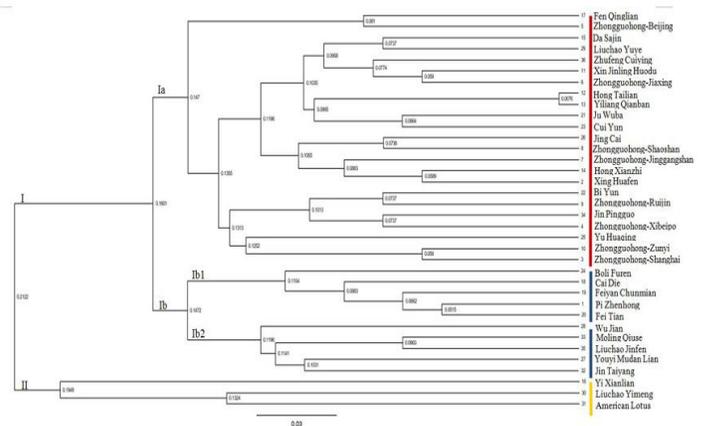


Figure 1: Dendrogram of 36 accessions of lotus, derived from the UPGMA cluster analysis. The clusters are shown with different colors. The accession codes refer to Table 1.

To get a better understanding of lotus clusters, the dendrogram was further confirmed by PCoA. The first two principle components accounted for 24.78% of the total variation. Accessions from Asian lotus, American lotus and Sino-American hybrids were located in three distinct clusters. Meanwhile, the hybrids were further separated into two sub clusters by coordinate F2 (Figure 2). Overall, the results obtained by PCoA were consistent well with the UPGMA cluster analysis.

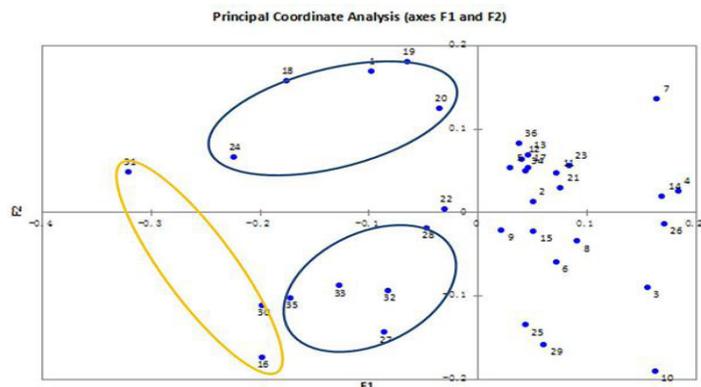


Figure 2: Matrix plot of the first two coordinates of principal coordinate analysis (PCoA). The clusters are shown with different colors. The accession codes refer to Table 1.

Population structure analysis

The population structure of lotus accessions herein was investigated by STRUCTURE program. The optimum K value for the lotus accessions was observed to be four by the Log Likelihood Of The Data [LnP(D)] in the STRUCTURE output (Figure S1). The threshold of membership probability of lotus accessions was defined as 0.70. As a result, 12 accessions were assigned into subgroup C1, 5 accessions to C2, 4 accessions to C3, and 5 accessions to C4 (Figure 3). Ten accessions whose membership probabilities <0.70 were retained in a mixed subgroup.

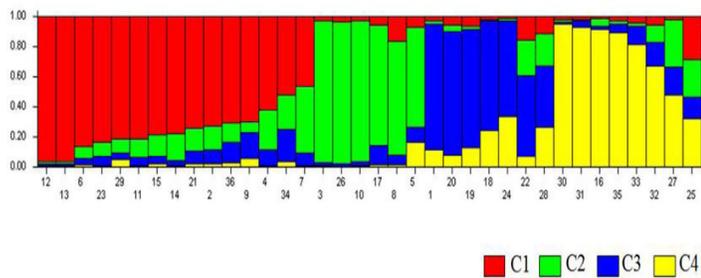


Figure 3: Population structure model of 36 investigated lotus cultivars. The K = 4 ‘subgroups’ (C1, C2, C3 and C4) are shown with different colors. Numbers on the x-axis correspond to the individual plants (1 to 36) listed in Table 1. The y-axis represents the Q value (Percentage of genetic composition).

Discussion

According to the traditional classification system, flower lotus are classified on the basis of plant size, petal form and flower color etc., and these morphological characteristics help to distinguish *N. nucifera* from *N. lutea* [5]. Here, using SSR strategy, Asian lotus and American lotus were clearly separated into distinct clusters. However, the eight Sino-American hybrids were divided into different clusters (Figure 1). The differences of hybrids’ grouping may be caused by different genetic compositions. Due to the preference of breeders for cultivars with yellow flower, the majority of hybrids currently were obtained by continuous inter-specific hybridization. And their genetic composition depends on the ratio of genetic materials from Asian lotus and American lotus.

New cultivars have always been goals for breeders. In China, plenty new cultivars of flower lotus have been created through natural crossing in recent years, and the five new cultivars used in this study are representatives. The clustering and population structure analysis herein contributed to inferring the genetic backgrounds of these new cultivars. ‘Moling Qiuse’ and ‘Liuchao Jinfen’, which were selected from seedlings of the same female parent, were grouped into subgroup Ib2 (Figure 1), indicating heterozygous genetic backgrounds. The cultivar ‘Jin Taiyang’ shows close relationship with its female parent ‘Youyi Mudanlian’. However, the offspring of ‘Jin Taiyang’, also known as ‘Jin Pingguo’, gathered with Asian lotus species (Figure 1 and Figure 3), suggesting generous achievement of genetic information from *N. nucifera* as pollen parent.

A better understanding of population structure is essential for germplasm utilization. So far, population structures have been demonstrated in many plants [9,10,22,23], including wild lotus populations [24,25]. In this study, we employed a Bayesian-based method in the software STRUCTURE to explore population structure for flower lotus cultivars. The method has two parameters, log likelihood L(K) and an ad hoc measure ΔK, but usually the parameter L(K) did not give the real value for population structure [26]. We calculated ΔK and observed the maximum ΔK value for K=4 herein (Figure S1). As a result, the lotus accessions were assigned into four groups, exhibiting a high correspondence with the results of UPGMA and PCoA. However, the accessions of Asian lotus dispersed among groups C1 C2 and C3 (Figure 3). So it is reasonably suggested that there might be several subgroups within Asian lotus as a result of geographical segmentation or historical evolution. Even though, structure analysis retained 10 cultivars into the mixed subgroup, suggesting more complicated genetic compositions of these commercialized lotus cultivars (Figure 3). The possible explanation was that hybridization and selection breeding of flower lotus had been more intensive during the last decades, and introgression took place during artificial breeding programs.

Acknowledgements

This work was supported by Natural Science Foundation of Jiangsu Province (Grant No. BK20151370), National Natural Science Foundation of China (Grant No. 31400604), Jiangsu Agriculture Science and Technology Innovation Fund (JASTIF), CX (16) 1024 and Jiangsu Key Laboratory for the Research and Utilization of Plant Resources (SQ201403).

References

1. Guo HB (2009) Cultivation of lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) and its utilization in China. *Genet Resour Crop Evol* 56: 323-330.
2. Hu M and Skibsted LH (2002) Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nucifera*). *Food Chemistry* 76: 327-333.
3. Savolainen V and Chas MW (2003) A decade of progress in plant molecular phylogenetics. *Trends Genet* 19: 717-724.
4. Wang QC and Zhang XY (1999) *Chinese Lotus Flower Cultivars-continued*. Beijing, China Architecture & Building Press.
5. Zhang XY, Chen LQ and Wang QC (2011) *New Lotus Flower Cultivars in China*. Beijing, China Forestry Publishing House.
6. Huang XQ, Chen JY, Huang GZ (1992) Preliminary studies on biosystematical relationship between the two *Nelumbo* species. *Acta Horticulturae Sinica* 19: 164-170.
7. Yang M, Han YN, Xu LM, Zhao JR, Liu YL (2012) Comparative analysis of genetic diversity of lotus (*Nelumbo*) using SSR and SRAP markers. *Scientia Horticulturae* 142: 185-195.
8. Wang QC and Zhang XY (2004) *Lotus Flower Cultivars in China*. Beijing, China Forestry Publishing House.
9. Zhang F, Ge YY, Wang WY, Shen XL, Liu XJ, et al. (2012) Genetic diversity and population structure of cultivated bromeliad accessions assessed by SRAP markers. *Scientia Horticulturae* 141: 1-6.
10. Zhang J, Chen T, Wang J, Chen Q, Luo Y, et al. (2016) Genetic diversity and population structure in cherry (*Cerasus pseudocerasus* (Lindl.) G. Don) along Longmenshan Fault Zones in China with newly developed SSR markers. *Scientia Horticulturae* 212: 11-19.
11. Mao JX, Luo D, Wang GW, Zhang J, Yang YM, et al. (2016) Genetic diversity of orchardgrass (*Dactylis glomerata* L.) cultivars revealed by simple sequence repeats (SSR) markers. *Biochemical Systematics and Ecology* 66: 337-343.
12. Han YC, Teng CZ, Chang FH, Robert GW, Zhou MQ, et al. (2007) Analyses of genetic relationships in *Nelumbo nucifera* using nuclear ribosomal ITS sequence data, ISSR and RAPD markers. *Aquatic Botany* 87: 141-146.
13. Han YC, Teng CZ, Robert GW, Zhou MQ, Hu ZL, et al. (2009) Mating system and genetic diversity in natural populations of *Nelumbo nucifera* (Nelumbonaceae) detected by ISSR markers. *Plant Systematics and Evolution* 277: 13-20.
14. Li Z, Liu XQ, Gituru RW, Juntawong N, Zhou MQ, et al. (2010) Genetic diversity and classification of *Nelumbo* germplasm of different origins by RAPD and ISSR analysis. *Scientia Horticulturae* 125: 724-732.
15. Hu JH, Pan L, Liu HG, Wang SZ, Wu ZH, et al. (2012) Comparative analysis of genetic diversity in sacred lotus (*Nelumbo nucifera* Gaertn.) using AFLP and SSR markers. *Molecular Biology Reports* 39: 3637-3647.
16. Park YJ, Lee JK, Kim NS (2009) Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. *Molecules* 14: 4546-4569.
17. Ming R, VanBuren R, Liu YL, Yang M, Han YP, et al. (2013) Genome of the long-living sacred lotus (*Nelumbo nucifera* Gaertn.). *Genome Biology* 14: 41.
18. Yang M, Han YN, Van Buren R, Ming R, Xu LM, et al. (2012) Genetic linkage maps for Asian and American lotus constructed using novel SSR markers derived from the genome of sequenced cultivar. *BMC Genomics* 13: 653.
19. Liu ZW, Zhu HL, Liu YP, Kuang J, Zhou K, et al. (2016) Construction of a high-density, high-quality genetic map of cultivated lotus (*Nelumbo nucifera*) using next-generation sequencing. *BMC Genomics* 17: 466.
20. Doyle JL and Doyle JJ (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
21. Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
22. Gawenda I, Schroder-Lorenz A, Debener T (2012) Marker for ornamental traits in *Phalaenopsis orchids*: population structure, linkage disequilibrium and association mapping. *Molecular Breeding* 30: 305-316.
23. Song BH, Windsor AJ, Schmid KJ, Ramos-Onsins S, Schranz ME, et al. (2009) Multilocus patterns of nucleotide diversity, population structure and linkage disequilibrium in *Boechea stricta*, a wild relative of *Arabidopsis*. *Genetics* 181: 1021-1033.
24. Yang M, Liu F, Han YN, Xu LM, Juntawong N, et al. (2013) Genetic diversity and structure in populations of *Nelumbo* from American, Thailand and China: Implications for conservation and breeding. *Aquatic Botany* 107: 1-7.
25. Li C, Mo HB, Tian DK, Xu YX, Meng J, et al. (2015) Genetic diversity and structure of American lotus (*Nelumbo lutea* Willd.) in North America revealed from microsatellite markers. *Scientia Horticulturae* 189: 17-21.
26. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology* 14: 2611-2620.