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藕莲种质资源形态与农艺性状的遗传变异分析

郭宏波[1*], 柯卫东[2], 李双梅[2]
(1. 西北农林科技大学生命科学学院, 陕西杨凌 712100; 2. 武汉市蔬菜科学研究所, 武汉 430065)

摘 要: 藕莲是莲(*Nelumbo nucifera Gaertn.* ssp. *nucifera*)的3种类型之一,在我国作为水生蔬菜栽培的历史愈两千年。对国家种质资源圃中来自17个省和直辖市的68份藕莲资源的19个形态和农艺性状进行了观察,并对其遗传变异进行了分析。结果发现整藕重与主藕重、主藕长和第三节粗成极显著正相关。通径分析结果表明主藕重对整藕重的正向效应最高(0.982)。主成分分析结果显示前4个成分占总变异的77.33%。结果还显示对整藕重负效应最高的是主藕重/整藕重(−0.296)。聚类分析发现所有藕莲资源可分为5组。

关键词: 农艺性状; 聚类分析; 相关性分析; 形态性状; 莲; 通径分析; 藕莲
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Genetic Variability and Interrelationships among Morphological and Agronomical Characteristics in Rhizome Lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) Germplasms

GUO Hong-Bo[1*], KE Wei-Dong[2], LI Shuang-Mei[2]
(1. *College of Life Science*, *Northwest A & F University*, Yangling, Shaanxi 712100, China;
2. *National Garden of Aquatic Vegetable*, *Aquatic Vegetable Division of National Vegetable Research Center*, *Wuhan Institute of Vegetable Science*, Wuhan 430065, China)

Abstract: Rhizome lotus (*Nelumbo nucifera Gaertn*. ssp. *nucifera*) has been cultivated for more than two thousand years as a food in China. The genetic variability in sixty-eight rhizome lotus germplasms from different regions of China, were evaluated using nineteen morphological characteristics. The associations of whole rhizome weight (TW) with main stem weight (MW), main stem length (ML), and width of the main stem section between the third and fourth knot (RW) were positive and highly significant. The path analysis revealed that MW had the highest positive direct effect on TW (0.982). Four principal components analyses (PCA) accounted for 77.33% of the total variability. Characteristics that showed high negative direct effect on TW were MW/TW (−0.296). A cluster analysis based on the morphological characteristics revealed five continuous groups according to evolutionary direction.

Key words: Agronomical characteristics; Cluster analysis; Correlation coefficient; Morphological characteristics; *Nelumbo nucifera* Gaertn. ssp. *nucifera*; Path analysis; Rhizome lotus

The lotus plant (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) is a valuable aquatic plant used widely as food, ornamentals, medicine, and packing material in China. It is classified into three types according to its different purposes and morphological distinctions[1,2]. One type, rhizome lotus, is primarily used as a food based on its nutritive and medicinal properties[2].

Rhizome lotus is widely distributed in China and has adapted to many edaphic-climatic con-

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Biography: Hongbo Guo, born in 1977, male, PhD, assosciate professor, mainly engaging in conservation of genetic resources and their utilization (E-mail: zouguoge@yahoo.com.cn).
* Author for correspondence.

ditions, from southern Hainan to northern Heilongjiang Province, and from eastern Taiwan to western Tian Mountain, Xinjiang Province[2,3]. Like many other plant species, this broad range of adaptation is due to the existence of a high number of local genotypes rather than a single ubiquitous genotype[1,4-6]. The evolutionary processes leading to the development of rhizome lotus landraces depends on various factors, such as natural and artificial selection, domestication history, and thousands of years of adaptation to cultivation environments. The apparent loss of genetic diversity in many crop plants has triggered widespread interest in niche environments where novel genes are often preserved in landraces. The collection, conservation, and use of landraces have, therefore, often been linked to breeding programs[7].

The main aim of rhizome lotus breeders is the development of cultivars with competitive rhizome yield, quality components, and adaptation to diverse agro-climatic regions. Breeding a crop for new and targeted environments requires the use of a range of landraces /genotypes as it allows intraspecific variability quantification for different characteristics and their interactions. In spite of the immense nutritive and medicinal importance of rhizome lotus, reports on variability and association among different morphological characteristics are lacking. Although its genetic diversity has been investigated based on fewer rhizome lotus accessions or cultivars by using allozyme, ISSR, nuclear ribosomal ITS sequence, and RAPD markers[5,6,8,9], many landraces from China's provinces recorded in the National Garden of Aquatic Vegetables have not been evaluated for morphological characteristics.

The purpose of this investigation was to evaluate the genetic variability and interrelationship among rhizome lotus germplasms using nineteen morphological characteristics. The present study was conducted with the following objectives: (1) To evaluate the morphological characteristics of rhizome lotus germplasms and to determine the selection criteria for increasing rhizome yield; (2) To elucidate interrelationships among yield and yield components using correlation analysis and supplementing correlation results using path coefficient analysis.

1 Materials and methods

1.1 Plant materials and experimental design

A total of 68 rhizome lotus landraces, including two cultivars from the Wuhan Institute of Vegetable Science in China, were included in the study (Table 1). The landraces were collected from fifteen provinces and one city, ranging from the tropical Hainan Province to the frigid-temperate Heilongjiang Province, and from western Sichuan to eastern Jiangsu Province.

The experiment was conducted during the 2002–2003 growing season at the experimental field of the National Garden of Aquatic Vegetable, Wuhan Institute of Vegetable Science, Hubei Province. The experimental site was situated 10 m above sea level at 30.52°N latitude and 114.31°E longitude. Sixty-eight landraces were sown in late March, 2002, and harvested in early March, 2003. The experimental field was disc ploughed and harrowed before sowing. The experimental design was a randomized block with three replications. The plot size for each replication was 10 m ×6.7 m. Each plot had three rows spaced 1.6 m apart and each row had four plants separated 2 m from each other. Each landrace was sown in a separate concrete plot and irrigation was applied as needed. No fungicide or insecticide was used during the experiment. Five plants from each landrace in each replication were randomly tagged and data were recorded on these plants for the 19 morphological traits (Table 2 and Figure 1).

1.2 Statistical analysis on morphological characteristics

The raw data was compiled by taking the

Table 1 List of 68 rhizome lotus germplasms (*Nelumbo nucifera* Gaertn. ssp. *nucifera*)

S. No.	Germplasm code	Germplasm line	Origin*	S. No.	Germplasm code	Germplasm line	Origin*
1	I-0147	Hongjianlian	Fujian province	35	I-0251	Huxianou	Huxian, Shaanxi province
2	I-0433	Wuzhierhao	Wuhan, Hubei province	36	I-0235	Xixiangtangou	Guangxi
3	I-0055	Baheou	Xishui, Hubei	37	I-0252	Weiyangou	Xi'an, Shaanxi
4	I-0407	Zhaoyangbailian	Zhaoyang, Hubei	38	I-0441	Hongshanhonglian	Wuhan, Hubei
5	I-0215	Jianghanou	Qianjiang, Hubei	39	I-0447	Ziguitianou	Zigui, Hubei
6	I-0003	Mata	Shashi, Hubei	40	I-0149	Wuyi	Jianli, Hubei
7	I-0148	Baipaozi	Xiaogan, Hubei	41	I-0434	Xuzhoubailian	Xuzhou, Jiangsu
8	I-0019	Wuliansihao	WIVS	42	I-0456	Yulinnanjiang-2	Yulin, Guangxi
9	I-0463	Zoumayang	Chongqing	43	I-0451	Haikou-1	Haikou, Hainan province
10	I-0058	Makoubailian	Hanchuan, Hubei	44	I-0446	Gufubailian	Yichang, Hubei
11	I-0040	Damaojie	Wuhan	45	I-0406	Wuhuyeshengou	Wuhu, Anhui
12	I-0018	Elian-1	WIVS	46	I-0450	Linwangou	Hainan
13	I-0044	Chongqingou	Chongqing	47	I-0455	Bobailianou	Bobai, Guangxi
14	I-0211	Honghulian	Honghu, Hubei	48	I-0002	Shuangjiajiang	Shashi, Hubei
15	I-0207	Dazihong	Baoying, Jiangsu province	49	I-0110	Yingchengbailian	Yingcheng, Hubei
16	I-0202	Gongantianou	Gongan, Hubei	50	I-0001	Micheng	Dangyang, Hubei
17	I-0395	Yangguzi	Shashi, Hubei	51	I-0109	Hongpaozi	Xiaogan, Hubei
18	I-0390	Jianshuihehua	Jianshui, Yunnan province	52	I-0237	Wuzhoutianou	Wuzhou, Guangxi
19	I-0453	Nanjingbaihe	Nanjing, Jiangsu	53	I-0236	Zaoshi	Yingcheng, Hubei
20	I-0190	Huanganbailian	Hongan, Hubei	54	I-0392	Huaiyanglian	Huaiyang, Henan
21	I-0232	Xingguotianou	Zhaoqing, Guangdong province	55	I-0388	Wuyangbailian	Wuyang, Henan
22	I-0228	Xiantaozaoou	Xiantao, Hubei	56	I-0469	Shibeihuaou	Chenggong, Yunan
23	I-0192	Xuqiaoyeou	Wuchang, Hubei	57	I-0472	Chengjianglian	Chengjiang, Yunan
24	I-0408	Hefeizhougang	Hefei, Anhui province	58	I-0470	Lijiagaoshan	Yuxi, Yunan
25	I-0240	Xichuancunou	Nanning, Guangxi province	59	I-0476	Shangzhihonglian	Tongjiang, Heilongjiang province
26	I-0219	Lichuanou	Lichuan, Hubei	60	I-0383	Yuxi-1	Yuxi, Yunan
27	I-0401	Nanpingmalian	Changde, Hunan province	61	I-0402	Baoanlianziou	Baoan, Hunan
28	I-0244	Xianningbailian	Xianning, Hubei	62	I-0238	Guilinjiashanou	Guilin, Guangxi
29	I-0193	Guandiou	Wuchang, Hubei	63	I-0462	Zhejiang-5	Hangzhou, Zhejiang province
30	I-0435	Yunmengbailian	Yunmeng, Hubei	64	I-0394	Sifangdengzi	Shashi, Hubei
31	I-0458	Guiganghonglian	Guigang, Guangxi	65	I-0454	Dawolong	Jinan, Shandong province
32	I-0389	Nanyanglian	Nanyang, Henan province	66	I-0464	Yudongfanbeizhou	Baxian, Sichuan province
33	I-0248	Jinziba	Enshi, Hubei	67	I-0119	Hongxiayeou	Wuhan, Hubei
34	I-0249	Huangniba	Enshi, Hubei	68	I-0126	Diaochahu-2	Hanchuan, Hubei

* WIVS, abbreviation of Wuhan Institute of Vegetable Science.

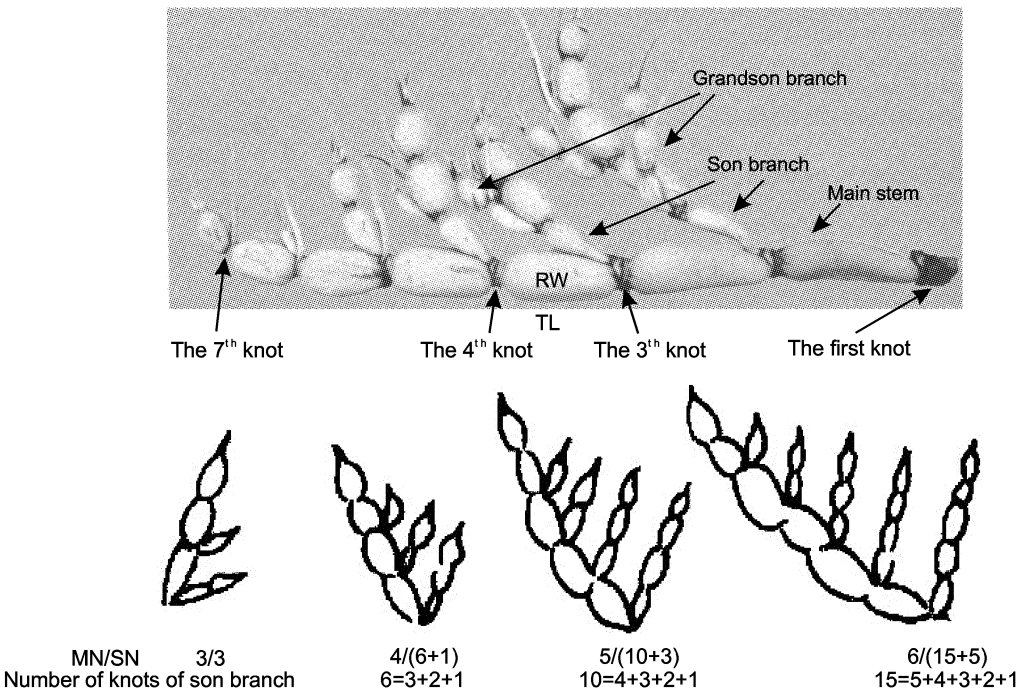
Table 2 List of descriptors used for the characterization of rhizome lotus (*N. nucifera* Gaertn. ssp. *nucifera*) germplasms

Characteristic*	Abbr.	Unit of measure or weight**
Plant height	PH	cm
Leaf length	LL	cm
Leaf width	LW	cm
Flower type	FT	0 – no flower; 1 – Da; 3 – Qb
Flower color	FC	0 – absent; 1 – white; 2 – white with red top; 3 – pink; 4 – red; 5 – rose red
Number of flowers	FA	0 – absent; 3 – few; 5 – intermediate; 7 – many
Length of main stem of rhizome	ML	cm
Knot number of main stem of rhizome	MN	number
Length between the third and fourth knot of main stem	TL	cm

Table 2 Continuted

Characteristic*	Abbr.	Unit of measure or weight**
Width of the section between the third and fourth knot of main stem	RW	cm
TL / RW ratio	DW	number
Weight of main stem of rhizome	MW	kg
Weight of whole rhizome	TW	kg
Percentage of MW/TW	MT	%
Skin color of rhizome	SC	1 – white; 3 – faint yellow; 5 – straw yellow; 7 – yellow
Stoma character on skin of rhizome	SA	3 – few; 5 – intermediate; 7 – many
Top color of rhizome	TB	1 – straw yellow; 3 – yellow; 5 – orange yellow; 7 – aubergine
Depth underground of ripe rhizome	DG	cm
Mature character of rhizome	MC	1 – precocious; 2 – mid-precocious; 3 – intermediate; 4 – mid-serotinous; 5 – serotinous

* See detailed explanation of these characteristics in Guo (2009) and Fig. 1.
** Da, The number of petals less than and equal to 20; Qb, more than one thousand petals in total during all the period of flowering (stamen and pistil are completely transformed into petals and receptacle disappeared).



MN: Number of knots of main stem; SN: Number of knots of son and grandson branch
$Y = X(X-1)/2 + 2X - 7 = (X^2 + 3X - 14)/2$ (when $X \geqslant 4$); $Y = X(X-1)/2$ (when X = 2 and 3); where Y = SN, X = MN

Fig. 1 The rhizome of lotus (*N. nucifera* ssp. *nucifera*) with seven knots in main stem was employed to describe the related morphological characteristics
(the ratio of numbers of knots on main stem / numbers of knots on son and grandson branch)

means of all the plants collected from each treatment and replication for different traits in the experiment. The mean, standard deviation, and minimum and maximum values were calculated for each morphological character in each landrace. A fixed-effects model ANOVA was performed to determine morphological variations between and within landraces using SPSS11.5. For each character, if the null hypothesis was rejected, multiple comparisons using the Least Significant Difference (LSD) test were carried out to determine differences between landraces. All LSD tests were carried out at $\alpha = 0.05$ significance level. Correlation analysis was performed to determine the relationships between all traits using the Correlate program in SPSS11.5. Path coefficients were then computed to separate the direct and indirect effects of correlation coefficient, which were initially proposed by Wright (1921, 1923)[10,11] and later described by Dewey & Lu (1959)[12] and by Li (1975)[13].

The data of morphological characteristics were first standardized by the Stand program within the General options of NTSYS-pc 2.1 software[14], and were then used to construct a data matrix of pair-wise dissimilarities between landraces by calculating the Euclidian distance coefficient. Principal components analysis (PCA) was used to depict non-hierarchical relationships among the studied landraces using a correlation matrix as input (calculated using standardized morphological data) and the first and second principal component scores were plotted to generate the two-dimensional model using the NTSYS-pc 2.1 software[14].

2 Results

2.1 Variability studies

The mean, standard deviation, and minimum and maximum values for different morphological characteristics are presented in Table 3. Whole rhizome weight (TW) ranged from 0.1 to 4.5 kg per rhizome with *N. nucifera* ssp. *nucifera* Shibeihuaou showing the lowest yield. Highest TW was shown by both of *N. nucifera* ssp. *nucifera* (Wuliansihao and Makoubailian) (4.5 kg/rhizome), followed by *N. nucifera* ssp. *nucifera* Elian-1 (4.2 kg/rhizome), and *N. nucifera* ssp. *nucifera* Baheou (4.0 kg/rhizome). Main stem weight of rhizome (MW) ranged from 0.1 to 2.75 kg per rhizome, with both *N. nucifera* ssp. *nucifera* (Shibeihuaou and Hongxiayeou) showing the lowest value (0.1 kg/rhizome). Highest MW was exhibited by *N. nucifera* ssp. *nucifera* Makoubailian (2.75 kg/rhizome), followed by both *N. nucifera* ssp. *nucifera* (Xuzhoubailian and Wuliansihao) (2.50 kg/rhizome). The percentage of MW/TW (MT) ranged from 49.16 to 92.59%, with *N. nucifera* ssp. *nucifera* Elian-1 displaying the lowest. Highest percentage was shown by *N. nucifera* ssp. *nucifera* Xuzhoubailian (92.59%), followed by *N. nucifera* ssp. *nucifera* Zaoyangbeilian (90%), and *N. nucifera* ssp. *nucifera* Guilinjiashanou (88.61%).

Table 3 Mean and standard deviations for each characteristic of the 68 rhizome lotus (*N. nucifera* ssp. *nucifera*) germplasms in China

Characteristic*	Mean	Standard deviation	Minimum	Maximum
DG (cm)	35.68	7.24	22	55
DW	2.90	1.00	1.75	6.17
FA	3.32	2.16	0	7
FC	1.09	1.51	0	5
FT	0.91	0.59	0	3
LL (cm)	60.16	18.45	29.00	86.80
LW (cm)	51.46	16.41	23.00	78.00
MC	3.21	1.37	0	5
ML (cm)	83.35	20.88	38.00	135
MN	4.42	0.77	3.00	7.00
MT (%)	73.79	8.42	49.16	92.59
MW (kg)	1.31	0.71	0.10	2.75
PH (cm)	137.82	32.71	67	205
RW (cm)	6.10	1.61	2.10	8.20
SA	4.35	1.74	3	7
SC	2.82	1.79	1	7
TB	2.29	1.46	1	7
TL (cm)	16.61	3.96	7	25.40
TW (kg)	1.83	1.07	0.10	4.5

* Characteristic key see Table 2.

2.2 Correlation studies

The correlation coefficients between various characteristics are presented in Table 4. All morphological characteristics except underground depth of ripe rhizome (DG), flower color (FC), mature character of rhizome (MC), skin color (SC), and top color of rhizome (TB) exhibited significant association with TW. Among these significant characteristics, all except DW (length between the third and fourth knot of main stem/width of the section between the third and fourth knot of main stem, TL/RW), flower type (FT), MT, and skin color of rhizome (SC) showed positive association with TW. Characteristics like leaf length (LL), leaf width (LW), plant height (PH), main stem length of rhizome (ML), MW, and TW showed highly significant positive association amongst themselves. The association of SC with all characteristics, except DG, DW, number of flowers (FA), FC, and FT, was negative. MT was negatively correlated with all characteristics except for DG, DW, MC, SA, and TL.

2.3 Path analysis

In the present study, path coefficient analysis was conducted by taking TW as the dependent variable. The direct and indirect effects of various characteristics on TW are provided in Table 5. The path analysis revealed that MW had the highest positive direct relationship with TW (0.982),

Table 4 Simple correlation coefficient of 19 morphological characteristics in rhizome lotus germplasms (*N. nucifera* ssp. *nucifera*)

Characters	DG	DW	FA	FC	FT	LL	LW	MC	ML	MN	MT	MW	PH	RW	SA	SC	TB	TL	TW
DG	–																		
DW	−0.23	–																	
FA	−0.19	0.39**	–																
FC	0.13	0.46**	0.41**	–															
FT	−0.19	0.39**	0.72**	0.41**	–														
LL	−0.14	−0.18	−0.27*	−0.06	−0.31*	–													
LW	−0.12	−0.18	−0.26*	−0.01	−0.31**	0.98**	–												
MC	0.02	−0.02	−0.15	−0.07	−0.29*	0.21	0.24*	–											
ML	−0.11	−0.29*	−0.24*	−0.09	−0.20	0.48**	0.49**	0.28*	–										
MN	−0.07	−0.42	−0.11	−0.21	−0.05	0.03	0.04	0.07	0.28*	–									
MT	0.09	0.04	−0.16	−0.12	−0.06	−0.26*	−0.25*	0.20	−0.30*	−0.15	–								
MW	−0.02	−0.54**	−0.38**	−0.26*	−0.30*	0.42**	0.44**	0.26*	0.88**	0.29*	−0.13	–							
PH	−0.11	−0.26*	−0.31**	−0.02	−0.23	0.57**	0.60**	0.21	0.58**	0.10	−0.09	0.64**	–						
RW	0.21	−0.68**	−0.48**	−0.29*	−0.42**	0.46**	0.46**	0.22	0.71**	0.19	−0.08	0.88**	0.63**	–					
SA	0.20	−0.03	−0.13	−0.11	−0.17	0.12	0.14	0.11	0.13	−0.16	0.18	0.12	0.23	0.17	–				
SC	0.16	0.16	0.39**	0.14	0.32**	−0.44**	−0.46**	−0.39**	−0.29*	−0.07	−0.04	−0.38**	−0.57**	−0.40**	−0.17	–			
TB	0.32	−0.07	−0.14	0.22	−0.25*	0.43**	0.41**	0.07	0.07	−0.11	−0.12	0.05	0.08	0.11	0.03	−0.05	–		
TL	−0.03	0.31**	−0.19	0.14	−0.16	0.35**	0.36**	0.32**	0.54**	−0.33**	0.003	0.42**	0.47**	0.435**	0.31*	−0.31*	0.07	–	
TW	−0.06	−0.53**	−0.32**	−0.23	−0.27*	0.45**	0.45**	0.20	0.89**	0.33**	−0.35**	0.96**	0.60**	0.82**	0.05	−0.32**	0.10	0.36**	–

Table 5 Path coefficient analysis showing direct (in bold) and indirect effects of 18 morphological characteristics over rhizome lotus yield (TW) of *N. nucifera* ssp. *nucifera*

Characters	DG	DW	FA	FC	FT	LL	LW	MC	ML	MN	MT	MW	PH	RW	SA	SC	TB	TL	Pearson correlation
DG	**−0.009**	0.014	−0.011	0.007	0.024	−0.023	0.024	0.001	0.001	−0.003	−0.027	−0.021	−0.002	−0.025	0.000	0.009	−0.015	0.000	−0.056
DW	0.002	**−0.059**	0.025	0.018	−0.073	−0.044	0.062	−0.008	0.003	0.004	0.207	−0.687	−0.005	0.099	0.000	0.019	0.013	0.002	−0.678**
FA	0.002	−0.025	**0.058**	0.022	−0.115	−0.038	0.048	−0.011	0.001	−0.004	0.060	−0.228	0.000	0.053	0.000	0.014	0.011	−0.004	−0.156
FC	−0.001	−0.021	0.026	**0.051**	−0.074	0.000	−0.003	−0.005	0.000	0.003	0.007	−0.185	0.000	0.030	0.000	0.015	−0.010	0.000	−0.167
FT	0.002	−0.033	0.051	0.029	**−0.131**	−0.053	0.069	−0.013	0.002	−0.004	0.029	−0.412	−0.003	0.075	0.000	0.017	0.014	−0.007	−0.370*
LL	0.001	0.016	−0.013	0.000	0.042	**0.166**	−0.20	0.008	−0.002	0.014	0.067	0.389	0.012	−0.044	0.000	−0.033	−0.024	0.004	0.402*
LW	0.001	0.018	−0.014	0.000	0.044	0.162	**−0.205**	0.010	−0.003	0.015	0.058	0.450	0.013	−0.049	0.000	−0.032	−0.021	0.005	0.453**
MC	0.000	0.020	−0.027	−0.012	0.073	0.057	−0.085	**0.024**	−0.002	0.010	−0.056	0.455	0.008	−0.052	0.000	−0.032	0.000	0.007	0.391*
ML	0.001	0.028	−0.007	−0.000	0.042	0.062	0.088	0.009	**−0.006**	0.009	0.056	0.887	0.013	−0.086	0.000	−0.026	−0.013	0.008	0.904**
MN	0.001	0.022	−0.006	0.003	0.013	0.052	−0.071	0.005	−0.003	**0.044**	0.059	0.521	0.008	−0.035	0.000	−0.020	−0.010	−0.004	0.581**
MT	−0.001	−0.001	−0.012	−0.001	0.013	−0.038	0.040	0.005	0.001	−0.009	**−0.296**	−0.075	−0.005	0.005	0.000	0.007	0.014	0.003	−0.354*
MW	0.000	0.041	−0.013	−0.010	0.055	0.066	−0.094	0.011	−0.005	0.023	0.022	**0.982**	0.013	−0.102	0.000	−0.031	−0.011	0.006	0.954**
PH	0.001	0.014	−0.000	−0.001	−0.008	0.095	−0.128	0.010	−0.004	0.018	0.070	0.598	**0.021**	−0.051	0.000	−0.041	−0.009	0.005	0.621**
RW	−0.002	0.049	−0.026	−0.013	0.032	0.060	−0.084	0.010	−0.004	0.013	0.012	0.838	0.009	**−0.120**	0.000	−0.026	−0.016	0.007	0.790**
SA	−0.002	0.000	−0.007	−0.008	0.010	0.015	−0.027	0.008	−0.001	−0.013	−0.032	0.103	0.005	−0.058	**0.000**	−0.012	−0.008	0.006	0.039
SC	−0.001	−0.019	0.014	0.013	−0.039	−0.092	0.112	−0.012	0.003	−0.015	−0.034	−0.520	−0.015	0.054	0.000	**0.059**	0.005	−0.004	−0.493*
TB	−0.003	0.017	−0.014	0.011	0.041	0.088	−0.094	0.000	−0.002	0.009	0.093	0.229	0.004	−0.040	0.000	−0.006	**−0.046**	0.002	0.290
TL	0.000	−0.005	−0.013	0.001	0.020	0.040	−0.057	0.010	−0.003	−0.010	−0.048	0.349	0.006	−0.052	0.000	−0.015	−0.007	**0.017**	0.265

followed by LL (0.166), and SC (0.059). Characteristics that showed high negative direct effect were MT (−0.296), LW (−0.205), and FT (−0.131). MW showed a positive indirect effect on TW via all characteristics except DG, DW, FA, FC, FT, MT, and SC. However, RW exerted a negative indirect influence on TW via all the morphological characteristics except DW, FA, FC, FT, MT, and SC.

2.4 Cluster analysis on morphological characteristics

The phenotypic distance index based on morphological characteristics ranged from 0.047 (between *N. nucifera* ssp. *nucifera* Xiantaozaoou and Xingguotianou) to 0.429 (between *N. nucifera* ssp. *nucifera* Zhaoyangbailian and Hongjianlian). Cluster analysis grouped the original 68 rhizome lotus landraces into well-defined phenotypes. Starting at the trunk of the tree, which was the entire domain of variation studied, five successional groups were distinguished (Figure 2). The means and standard deviations of the morphological characteristics in each group were calculated (Table 6). Group II exhibited the highest values among characteristics like TW, MW, ML, and RW, followed by Group III. In terms of these economical characteristics, group IV was lowest, followed by group V.

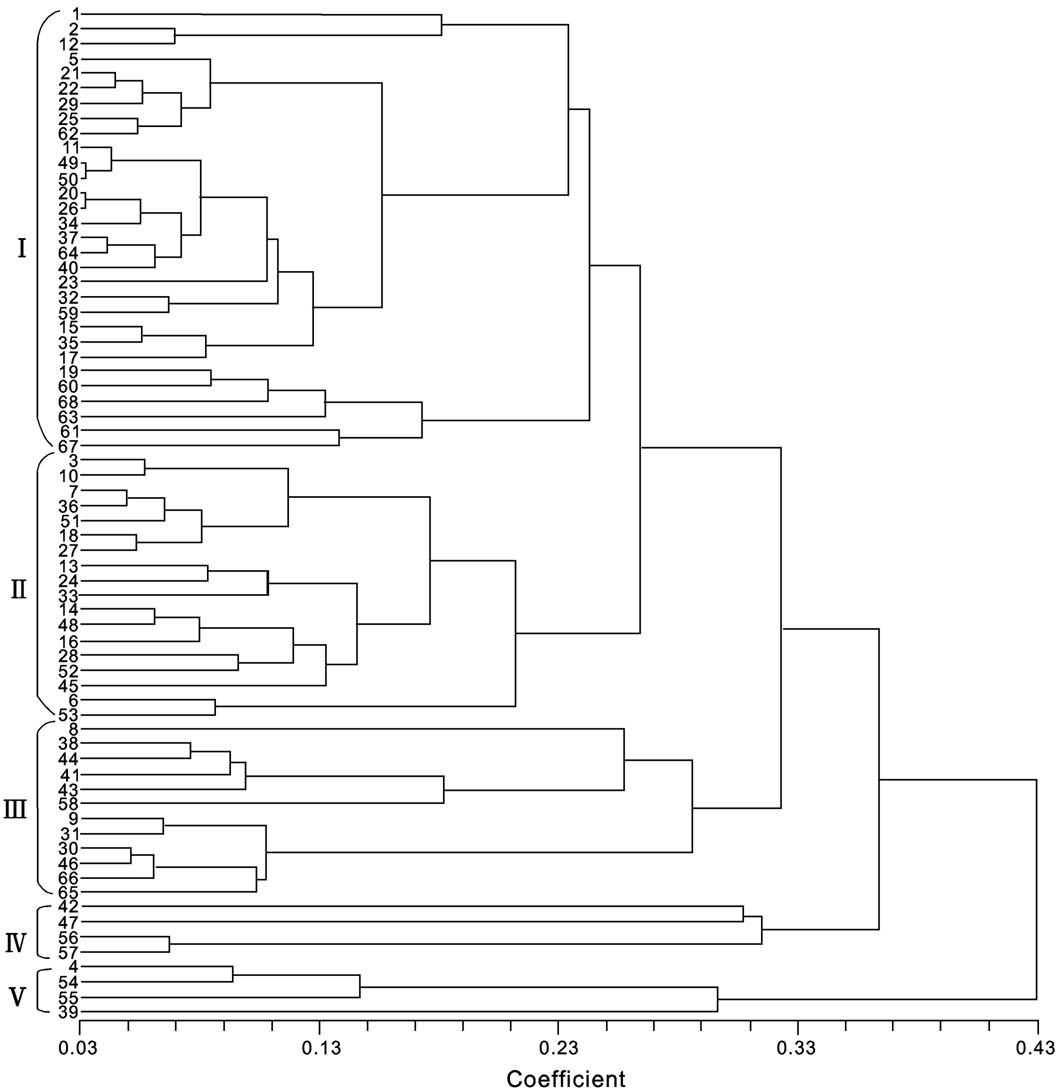


Fig. 2 Dendrogram showing the results of a cluster analysis based on 19 morphological characteristics of 68 rhizome lotus germplasms (*N. nucifera* ssp. *nucifera*) from China

Table 6　Means and standard deviations of morphological characteristics in each cluster obtained from UPGMA dendrogram (Fig. 2) with 68 rhizome lotus (*N. nucifera* ssp. *nucifera*) germplasms

Characteristics	Clusters				
	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ
DW	3.04 ±1.16	2.60 ±0.51	2.97 ±1.09	3.53 ±1.33	2.41 ±0.53
LL	66.39 ±14.26^{b}	74.73 ±8.49^{a}	37.73 ±3.79^{ab}	42.48 ±9.19^{ab}	32.73 ±1.74^{ab}
LW	56.75 ±13.48^{b}	64.32 ±7.61^{b}	31.89 ±3.43^{ab}	34.00 ±6.63^{ab}	26.75 ±3.30^{ab}
ML	80.33 ±17.65^{ac}	97.54 ±15.35^{ab}	88.17 ±18.70^{abc}	54.75 ±12.61^{d}	56.20 ±21.94^{d}
MN	4.33 ±0.74	4.56 ±0.57	4.50 ±1.00	4.13 ±0.63	4.63 ±1.25
MT	71.95 ±7.76^{a}	72.06 ±7.05^{a}	75.51 ±10.31^{a}	75.84 ±3.59^{a}	86.97 ±3.39^{b}
MW	1.10 ±0.62^{a}	1.83 ±0.46^{b}	1.56 ±0.62^{b}	0.46 ±0.43^{c}	0.67 ±0.96^{ac}
PH	137.94 ±30.59^{a}	150.78 ±25.70^{a}	150.98 ±24.96^{a}	97.25 ±16.78^{b}	79.65 ±11.41^{b}
RW	5.91 ±1.58^{a}	7.04 ±0.62^{ac}	6.51 ±1.31^{a}	3.73 ±1.86^{b}	4.43 ±2.06^{b}
TL	16.69 ±3.74^{a}	18.11 ±2.68^{a}	17.98 ±3.49^{a}	11.38 ±1.25^{b}	10.40 ±4.32^{b}
TW	1.57 ±0.97^{a}	2.58 ±0.76^{b}	2.11 ±1.01^{ab}	0.60 ±0.56^{ac}	0.76 ±1.09^{ac}

Note: a, b, c and d represent significant difference (p <0.05).

2.5 PCA analysis

The first four principal components in the PCA of the 19 morphological characteristics accounted for 37.27, 13.60, 10.01, and 8.78% of the total variation, respectively, and together explained 77.33% of the total variation among the 68 rhizome lotus landraces (Table 7). MW, TW, RW and ML were the most important characteristics contributing to the first principal component, while FA, FT, MT, and MN contributed significantly to the second principal component. For the third principal component TL, DW, SA, LL, and LW were the most important characteristics, while TB and DG contributed to the fourth principal component.

Table 7　Eigenvalue, Eigenvector and scores of the four factors retained from the principal component analysis (PCA) of 19 characteristics performed on 68 rhizome lotus (*N. nucifera* ssp. *nucifera*) germplasms from China

Characteristic	PCA1	PCA2	PCA3	PCA4
DG	1.9E-02	-0.34	-0.16	0.61
DW	-0.70	0.20	0.55	-0.13
FA	-0.46	0.69	6.3E-02	-0.18
FC	-0.26	0.53	0.21	0.34
FT	-0.64	0.66	5.3E-02	-0.14
LL	0.66	0.28	0.41	0.29
LW	0.71	0.26	0.41	0.23
MC	0.62	-0.34	0.25	-0.24
ML	0.82	0.24	-9.2E-02	-0.20
MN	0.50	0.43	-0.40	-9.5E-03
MT	-0.17	-0.53	0.15	-0.26
MW	0.89	0.10	-0.23	-0.20
PH	0.71	0.37	0.30	-0.18
RW	0.85	-0.20	-0.25	4.1E-02
SA	0.21	-0.34	0.53	3.8E-02
SC	-0.68	-0.11	-0.25	0.25
TB	0.42	0.12	9.6E-02	0.80
TL	0.43	-0.24	0.55	-0.15
TW	0.87	0.25	-0.30	-0.13
Eigenvalue	7.08	2.58	1.90	1.67
Total variance (%)	37.27	13.60	10.01	8.78
Cumulative variance (%)	50.87	60.88	69.66	77.33

3 Discussion

Gaining information on correlation, path coefficients, principal component analysis, and heritability estimates of yield and yield contributing characteristics in rhizome lotus germplasms is required to define selection criteria for breeding programs and new cultivar development. While a large variation exists among the germplasms of rhizome lotus in the rhizome yield, it has remained unexplored due to lack of information on the relationships between component characteristics and their contributions towards yield. Most former studies concentrated on a small number of characteristics[15,16], but in this study, 19 morphological and agronomical characteristics have been investigated simultaneously.

Highly significant positive correlation coefficient with rhizome yield was found for leaf length, leaf width, ML, MN, MW, PH, RW, and TL (Table 4). In former studies of rhizome lotus, TL, RW, TW, and MW exhibited strong positive correlations with rhizome yield[16], consistent with our results.

The DW ratio (TL/RW) was negatively correlated with TW (Table 4), although both TL and RW were significantly positive with rhizome yield. This ratio is an important descriptor of rhizome yield. Generally, if the ratio value is more than three, rhizome yield and nutritional content is low and is coupled with poor taste. High DW ratio almost always occurs in wild lotus, flower lotus, and seed lotus (see details in reference [2]) as well as in a minor part of rhizome landraces. Fortunately, this ratio can be reduced through artificial selection and breeding from a desirable gene pool. Group II in Figure 1 consists of two cultivars and those improved landraces with highest yield and lowest DW ratio, while group IV includes traditional landraces which exhibit lowest yield and highest ratio value (Table 6). Both characteristics in the other three groups are intermediate.

The percentage of MT (MW/TW) was also negatively correlated with TW despite the fact that both MW and TW can significantly increase rhizome yield. This is consistent with the findings of Ke *et al*. (2000)[16]. When the MW and TW simultaneously increased, the increase speed of the latter was more than the former (Figure 2). For example, when the MN increased from 3 to 4, the number of knots on the son and grandson branch (SN) increased from 3 to 7. Therefore, the ratio of MN/SN decreased from 3/3 to 4/7. Within the market, only the main stem of rhizome can be sold, while the son and grandson branches are often used as "seed", thus the higher percentage of MT is expected by farmers. If one cultivar has a higher yield, more son and grandson branches would be produced and, except for part used as "seed", abandoned.

Rhizome skin color (SC) was found to be negatively associated with TW. Generally, the SC of wild lotus, flower lotus, seed lotus, and many rhizome lotus landraces are yellow or orange-yellow. This color, however, is considered undesirable by food consumers, with white being the preferred choice. From our results, an increase of yield improved this characteristic. In addition, both flower type (FT) and number of flowers (FA) were also negatively correlated with TW, consistent with our field observations. The cultivar or landraces with high yield often have no or fewer flowers, which are usually flower type Da (number of petals less than and equal to 20) with white color (see explanation in Table 2). This may be attributed to the reduction of reproduction growth, and thus facilitation of vegetation growth of the rhizome. Conversely, wild lotus, flower lotus, or seed lotus more often have FA with red colors and different types of flowers[2,4].

Path coefficient analysis divided the correlation coefficient into a series of direct and indirect effects of morphological characteristics on the TW of rhizome lotus (Table 5). Path analysis showed that only MW and LL had strong direct effects on the enhancement of the TW of rhizome lotus, in that order, while it was strongly negative for MT, LW, FT, and DW. The indirect effects via MW, ML, RW, PH, MN, MC, LL, and TL substantially increased the total correlations between the characteristics and TW. However, those characteristics via MW, DW, SC, FT, FA, and FC reduced the positive correlations with TW considerably.

Rhizome maturity (MC) is an important income factor for farmers, who often use pre-mature cultivars to sell its rhizomes as early as possible for a higher price. During our field survey, however, if a cultivar was pre-mature, its rhizome yield was obviously lower than a serotinous one. From the results of this study, MC was positively correlated with MW, TL, and TW, but not significant with TW (Table 4). Likewise, path analysis showed positive but very weak direct effect on the increase of yield (Table 5). Therefore, MC is not an absolute characteristic to describe which cultivar is pre-mature or serotinous, or to increase the rhizome yield by using serotinous cultivar. These results are consistent with reports by Ke *et al*. (2003)[3]. Indeed, the MC characteristic was closely correlated with underground depth of ripe rhizome (DG). Generally, the rhizome planted at an underground depth ranging from 20 to 30 cm was less mature than that of DG with 50 – 60 cm, and the former's rhizome yield was lower than the latter. Similarly, if one specific cultivar was planted in different water depth aboveground, the rhizome yield also changed. According to our field survey, for example, if the cultivar "Elian 5" (see details in reference [2]) was cultivated in a rice field with a water depth of 10 – 20 cm, the characteristics of the harvest rhizome showed 5 – 6 knots on the main stem (MN), a width of the section between the third and the fourth knot (RW) of 7.0 –7.5 cm, and a mean weight of section between two knots (WT) of 0.4 –0.5 kg. If the same cultivar was planted in a pond with a water depth of more than 1 m, however, the characteristics of its rhizome displayed a MN of 3 – 4, RW of 7.5 –8.5 cm, and WT of 0.75 –1.0 kg. All these field surveys verify that the DG characteristic had no or very little effect on rhizome yield (Table 4 and Table 5). The reason why the cultivar planted in shallow water depth and/or underground depth is less mature may be attributed to the rapid increase of soil temperature when the air temperature is increasing in spring. This increase in soil temperature facilitates the germination of "seed" rhizome and takes advantage of this during the entire growth season till harvest.

A dendrogram based on the 19 morphological characteristics clustered all 68 rhizome lotus germplasms into five groups (Figure 2). Among all samples, only eight clustered in group IV and group V, and they exhibited more genetic distance. This result is in accordance with the reality that these landraces were collected from different regions showing genetic distinctness, with some cultivated in their own specific and remote environments for more than 100 years, such as Shibeihuaou and Chengjianglian from Chenggong and Chengjiang, Yunnan provinces[17]. The morphological characteristics of these five groups were calculated to help determine the evolutionary processes. Based on TW character, the rhizome yield was in this order, group IV < group V < group I < group III < group II (Table 6). All characteristics, except for DW and MT, exhibited the same trend, while these two characteristics displayed the converse order. The evolutionary direction of rhizome lotus may be, therefore, from small to large among all morphological characteristics, except DW and MT. This analysis is in accordance with the evolutionary direction of TW, MN, and MW reported by Ke et al. (2000)[15]. On the other hand, however, both DW and MT characteristics are reduced when the rhizome yield increases (as mentioned above).

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References:

[1] Guo H B, Ke W D, Li, S M, Peng J. Cluster analysis of *Nelumbo* accessions based on RAPD markers [J]. *J Plant Genet Resour*, 2004, 5(4): 328 –332. (in Chinese)

[2] Guo H B. Cultivation of lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) and its utilization in China [J]. *Genet Resour Crop Evol*, 2009, 56: 323 – 330.

[3] Ke W D, Li F, Liu Y P, Sun B. Advance on lotus germplasm resources and current breeding [J]. *J Changjiang Vegetables*, 2003, 4: 5 – 9. (in Chinese)

[4] Guo H B, Li S M, Peng J, Ke W D. Genetic diversity of *Nelumbo* accessions revealed by RAPD [J].

Genet Resour Crop Evol, 2007,54: 741 -748.

[5] Han Y C,Teng C Z,Chang,F H,Robert G W,Zhou M Q,Hu Z L,Song Y C. Analyses of genetic relationships in *Nelumbo nucifera* using nuclear ribosomal ITS sequence data,ISSR and RAPD markers [J]. *Aquat Bot*,2007,87: 141 -146.

[6] Han Y C,Teng C Z,Zhong S,Zhou M Q,Hu Z L,Song Y C. Genetic variation and clonal diversity in populations of *Nelumbo nucifera* (Nelumbonaceae) in central China detected by ISSR markers [J]. *Aquat Bot*,2007,86: 69 -75.

[7] Brown A H D,Frankel O H,Marshall D R,Williams J T. The use of plant genetic resources[M]. Cambridge:Cambridge University Press,1989.

[8] Tian H L,Xue J H,Wen J,Mitchell G,Zhou S L. Genetic diversity and relationships of lotus (*Nelumbo*) cultivars based on allozyme and ISSR markers[J]. *Sci Hortic*,2008,116: 421 -429.

[9] An N,Guo H B,Ke W D. Genetic variation in rhizome lotus (*Nelumbo nucifera* Gaertn. ssp. *nucifera*) germplasms from China assessed by RAPD markers[J]. *Agricult Sci China*,2009,8(1): 31 -39.

[10] Wright S. Correlation and causation[J]. *J Agricul Res*,1921,20:557 -585.

[11] Wright S. The theory of path coefficients:a reply to Niles's criticism[J]. *Genetics*,1923,8,239 -255.

[12] Dewey D R,Lu K H. A correlation and path-coefficient analysis of components of crested wheatgrass seed production [J]. *Agron J*, 1959, 51: 515 -518.

[13] Li C C. Path Analysis:a Primer[M]. Pacific Grove, California:Boxwood Press,1975: 347.

[14] Rohlf F J. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System[M]. Version 2.1. New York,USA:Exceter Software,2000.

[15] Ke W D,Fu X F,Huang X F,Peng J,Zhou G L,Ye Y Y . Systematic cluster analysis of some *Nelumbo nucifera* Gaertn. germplasm resources and its application on breeding[J]. *Acta Horticul Sin*, 2000, 27: 374 -376. (in Chinese)

[16] Ke W D,Huang X F,Fu X F,Zhou G L,Peng J. Genetic analysis of some quality and agronomic traits in *Nelumbo nucifera* Gaertn[J]. *J Wuhan Bot Res*, 2000,18: 519 -522. (in Chinese)

[17] Ke W D,Huang X F,Liu Y P,Fu X F. Some species of aquatic vegetables found in Yunnan province [J]. *China Vegetables*,2005,2: 31 -33. (in Chinese)

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