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Allozyme analysis of genetic diversity, genetic structure and interspecific relationship in genus *Eriobotrya*

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SUMMARY – Levels of allozyme diversity were determined in *Eriobotrya* populations. 24 loci for 12 enzyme systems of 120 materials from 4 species and 1 variant species: *E. japonica* Lindl., *E. prinoides* Rehd. et Wils., *E. serrata* Vidal., *E. dayaoshanensis* Chen and *E. prinoides* var. *daduheensis* H.Z. Zhang, were investigated using isoelectric focus in thin-layer polyacrylamide slab gel. Fifty nine alleles were detected at 24 loci of 12 enzyme systems from 14 populations of *Eriobotrya* with the largest number of five. 15 in 22 loci were significant or extremely significant. Average expected heterozygosity (He) and the portion of polymorphic loci (P) of 10 populations of *E. japonica* were 0.320 and 69.6 respectively. Fixed indexes of the populations imply more heterozygous genotypes than the expected value of Hardy-Weinberg. The highest variation at different loci in 7 populations of *E. japonica* in 19 polymorphic alleles is *Aat-2,* with Fst 0.280. The average of Fst of the 19 loci was 0.085, between long-life woody plants and short-life woody plants. Cluster analysis with BIOSYS-1 population analysis showed that in the dendrograms Japanese population was first clustered to Zhejiang loquat, that all of the cultivars in *E. Japonica* were clustered together and then to *E. prinoides* var. *daduheensis* H.Z. Zhang, that *E. prinoides* Rehd. et Wils. was first clustered to *E. serrata* Vidal. and then to *E. prinoides* var. *daduheensis* H.Z. Zhang, that the cultivars flowering in autumn and winter were clustered together and then to the cultivars in *E. dayaoshanensis* Chen, flowering in spring. Similar results were obtained from NYSYS-pc species analysis.

Key words: *Eriobotrya,* population, isozyme, allozyme, genetic diversity, genetic structure.

RESUME – *"Analyse de la diversité génétique des allozymes, de la structure génétique et de la relation interspécifique chez le genre* Eriobotrya*". Les niveaux de diversité des allozymes ont été déterminés chez les populations de* Eriobotrya*. On a examiné 24 loci pour 12 systèmes enzymatiques de 120 matériels provenant de 4 espèces et 1 espèce variante :* E. japonica *Lindl.,* E. prinoides *Rehd. et Wils.,* E. serrata *Vidal.,* E. dayaoshanensis *Chen et* E. prinoides *var.* daduheensis *H.Z. Zhang, en utilisant une focalisation isoélectrique sur plaque à couche fine de gel de polyacrylamide. Cinquante-neuf allèles ont été détectés sur 24 loci de 12 systèmes enzymatiques pour 14 populations de* Eriobotrya, *le nombre le plus grand étant cinq. 15 des 22 loci étaient significatifs ou extrêmement significatifs. L'hétérozygotie moyenne espérée (He) et la portion des loci polymorphes (P) de 10 populations de* E. japonica *ont été de 0,320 et 69,6 respectivement. Les indices fixes des populations impliquent davantage de génotypes hétérozygotes que la valeur espérée de Hardy-Weinberg. La plus forte variation dans différents loci chez 7 populations de* E. japonica *pour 19 allèles polymorphes est trouvée à* Aat-2, *avec Fst 0,280. La moyenne de Fst pour les 19 loci était de 0,085, entre les ligneuses à longue vie et les ligneuses à courte vie. L'analyse cluster avec BIOSYS-1 pour l'analyse des populations a montré que dans les dendrogrammes la population japonaise était d'abord agglomérée au néflier Zhejiang, que tous les cultivars de* E. Japonica *étaient agglomérés ensemble et ensuite à* E. prinoides *var.* daduheensis *H.Z. Zhang, que* E. prinoides *Rehd. et Wils. était d'abord aggloméré à* E. serrata *Vidal. et ensuite à* E. prinoides *var.* daduheensis *H.Z. Zhang, que les cultivars fleurissant en automne et en hiver étaient agglomérés ensemble et ensuite aux cultivars de* E. dayaoshanensis *Chen, fleurissant au printemps. Des résultats similaires ont été obtenus par analyse des espèces avec NYSYS-pc.*

Mots-clés : Eriobotrya*, population, isozyme, allozyme, diversité génétique, structure génétique.*

Introduction

Eriobotrya japonica Lindl. is an ever-green fruit tree native to south of China. It has long been regarded as delicious and unique fruit for fructifying in spring, ripening in summer, germinating in autumn and flowering in winter (Zhang, 1996). At present, research efforted at analyzing genetic diversity in morphological differences but few in other evidences, even not in population.

Zhang and his colleagues had suspected that *E. prinoides* Rehd. et Wils. evolved to *E. japonica* Lindl., in which *E. prinoides* var. *daduheensis* Zhang as a middle type, and simple classification

system of Chinese *Eryiobotrya* plants based on different time of flowering and fuzz state. However, some researchers indicated their different viewpoints. In the present research, allozyme as molecular markers has been used to study genetic diversity, genetic structure and interspecific relationship in genus *Eriobotrya.* The below provides scientific information for ascertaining the origin of loquat, studying the evolutionary events and planning the conservation strategies.

Materials and methods

Plant materials

120 materials of *Eriobotrya,* representing 4 species and 1 variant species and 10 populations, were obtained from the China Fuzhou National Loquat Germplasm Plantation, Huazhong Agricultural University and Wuhan Institute of Botany of the Chinese Academy of Science (Table 1).

Table 1. Names of accessions

No.	Names	Origin	No.	Names	Origin
001	Qingzhong	Jiangsu	043	Xiangtian	Fujian
002	Baiyu	Jiangsu	044	Leigongchui	Fujian
003	Zhaozhong	Jiangsu	045	Shanpai 3	Fujian
004	Ruantiaobaisha	Zhejiang	046	Xiagao 20	Fujian
005	Bai li	Fujian	047	Jianxinbaisha	Fujian
006	Wugongbai	Fujian	048	Honghouben	Fujian
007	Lizhipipa	Guangxi	049	Dazhong	Fujian
008	Guizhou 1	Guizhou	050	Zaozhong 6	Fujian
009	Chiyepipa	Yunnan	051	Yukawa	JJapan
010	Dayaoshanpipa	Guangxi	052	Xialoubaimi	Fujian
011	Liye pipa	Sichuan	053	Xiangzhong 11	Fujian
012	Daduhe pipa	Sichuan	054	Jianoinbaisha	Fujian
013	Min 3	Fujian	055	Suanpanzhi	Fujian
014	Gold stone	America	056	Yadanben	Fujian
015	Mogi	Japan	057	Meihuaxia	Fujian
016	Luoyangqing	Zhejiang	058	Taiwanmogi	JJapan
017	Jiajiao	Zhejiang	059	Hunanbaisha	HHunan
018	Dahongpao	Zhejiang	060	Jiangxiduhe	Jiangxi
019	Jiefangzhong	Fujian	061	Champagne	America
020	Chang Hong 3	Fujian	062	Zhuluohong sha	Jiangxi
021	Taicheng 4	Fujian	063	Hunantianzhong	Hunan
022	Fuyangzhong	Jiangsu	064	Qinbianzhong	Guangdong
023	Guangrongzhong	Anhui	065	Gangkou 8	Guangdong
024	Anhuidahongpao	Anhui	066	Wuqi	Guangdong
025	Xiangzhong 10	Fujian	067	Dawuqi	Guangdong
026	Jiuyuben	Fujian	068	Duobao 2	Guangdong
027	Zaotao	Fujian	069	Baitangzhong	Guangdong
028	Xialoubaimi	Fujian	070	Xiaowuqi	Guangdong
029	Yadanben	Fujian	071	Gangkou 11	Guangdong
030	Hecheben	Fujian	072	Unbeknown	Jiangxi
031	Hongganben	Fujian	073	Dayeyangdun	Zhejiang
032	Taichengbaimi	Fujian	074	Tangqizhong	Zhejiang
033	Kengben	Fujian	075	Danbianzhong	Zhejiang
034	Longcaibai	Fujian	076	Huangyan 5	Zhejiang
035	Jinlizhi	Fujian	077	Xiyeyangdun	Zhejiang
036	Xianmei	Fujian	078	Baozhu	Zhejiang
037	Xiangzhong 25	Fujian	079	Hongmaowuer	Zhejiang
038	Huangzao	Fujian	080	Tangqizaofeng	Zhejiang
039	Jianzuibai	Fujian	081	1-1-3	Zhejiang
040	Baoyuanbai	Fujian	082	Shaohedahongpao	Zhejiang
041	Shanliben	Fujian	083	Touzao	Zhejiang
042	Zaohuang	Fujian	084	Tangqichihong	Zhejiang

Table 1 (cont.). Names of accessions

No.	Names	Origin	No.	Names	Origin
085	Munuopipa	Guanxi	103	Tanaka	Japan
086	Zhuonan 1	Guanxi	104	Oobusa	Japan
087	Liuzhouguangrongben	Guanxi	105	Tsukumo	Japan
088	Mitangpipa	Guanxi	106	Mizuho	Japan
089	Xiaomaopipa	Guanxi	107	Tohi	Japan
090	Bainangpipa	Guanxi	108	Togoshi	Japan
091	Liuzhoumaomu	Guanxi	109	Morimoto	Japan
092	Yantangpipa	Guanxi	110	Tesoowase	Japan
093	Damaopipa	Guanxi	111	Kusunoki	Japan
094	Huabao 3	Hubei	112	Matui	Anhui
095	Zaobai	Hubei	113	Baihua	Anhui
096	Hubei Liuer	Hubei	114	Duanbingbianhe	Anhui
097	Biqizhong	Jiangsu	115	Changbingbianhewe	Anhui
098	Muyu	Jiangsu	116	ChaoBao	Anhui
099	Huangpi	Jiangsu	117	Huabao 7	Hubei
100	Duanbingzhaozhong	Jiangsu	118	Huabao 2	Hubei
101	Jidanbai	Jiangsu	119	Unbeknown-2	Jiangxi
102	Sensin	Japan	120	Moriowase	Japan

Electrophoresis and isozyme staining

The isozyme assays were conducted on ripe leaf. The isoelectric focusing polyacrylamide slabgel system developed by Huang (2000) was used. The extraction, electrophoresis and isozyme staining procedure, as described by Huang *et al.* (1994). Populations were assayed for the following enzyme system:

Acid phosphatase (ACP; EC.3.1.3.2), Orthophosphoric-monoester phosphohydrolase (I.U.B; E.C.3.1.3.2), Aspartate aminotransferase (AAT; E.C. 2.6.1.1), NAD(P)H-Diaphorase (DIA; E.C.1.6.2.2), Esterase (colorimetric) (EST; E.C.3.1.1), Isocitrate dthydrogenase (IDH; E.C.3.1.1), Malate dehydrogenase (MDH; E.C.1.1.1.37), Malic enzyme (ME; E.C.1.1.1.40), Peroxidase (PER; E.C.1.11.1.7), Phosphoglucoisomerase (PGI; E.C.5.3.1.9), Phosphoglucomutase (PGM; E.C.5.4.2.2), Phosphogluconate dehydrogenase (PGD; E.C.1.1.1.44), Shikimate dehydrogenase (SKD; E.C.1.1.1.25).

Allozyme designation and data analysis

The allozyme analysis on loquat have not been described by other researchers. Therefore, the loci of allozyme system were designated by number of chromosome (all accessions are diploid 2n=34 except Ming-3 is tetraploid), number of subbase at each enzyme systems, and the relationship between allozyme phenotype and genotype. The methods applied in other plants were also referenced. The genetic control of allozymic phenotypes was postulated based on below. The loci for a given multilocus enzyme system were designated sequentially by number starting with 1 for the most anodal locus; at each locus, the alleles were designated sequentially by letter starting with a for the most anodal loci (Wang, 1996). The effective isozyme locus (Fig. 1) and allozymes (Fig. 2) will be transformed into genotype.

Computations were performed using the BIOSYS-1 program, (Swofford *et al.,* 1989) based on genotype per locus of individuals, which calculated the frequency distribution of alleles in different populations, in which accessions were classified into 14 populations from different regions and species.

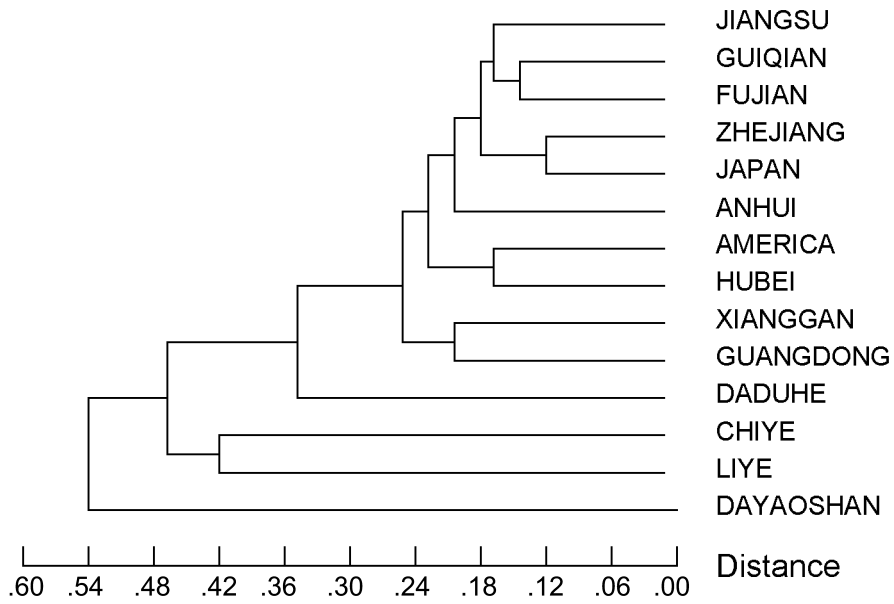

Fig. 1. UPGMA dengrogram of allozyme genetic distance based on modified Roger's genetic distance among 14 populations of Eriobotrya.

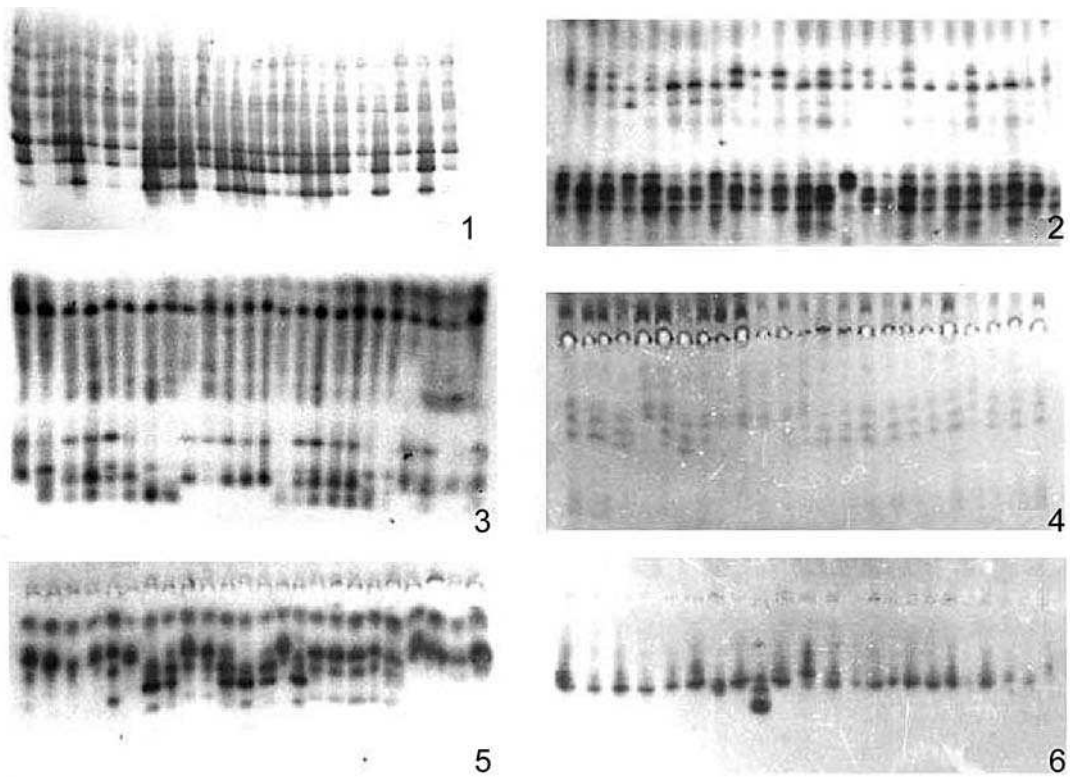

Fig. 2. Zymograms of loquat. 1. Zymogram of DIA respectively (from the left to the right are the accessions from No. 25 to No. 48); 2. Zymogram of ACP (from the left to the right are the accessions from No. 60 to No. 49 and from No. 61 to No. 72); 3. Zymograms of MDH respectively (from the left to the right are the accessions from No. 1 to No. 24); 4. Zymogram of AAT (from the left to the right are the accessions from No. 1 to No. 24); 5. Zymogram of PGI (from the left to the right are the accessions from No. 97 to No. 120); 6. Zymogram of IDH (from the left to the right are the accessions from No. 1 to No. 24).

Result and discussion

Population analysis of genetic diversity

Alleles per locus (A), the polymorphic percentage of loci (P), heterozygosity (H) were key characters for reflecting diversity of population. Table 2 implied that the mean of alleles and the portion of polymorphic loci were the highest in *E. japonica* Lindl. of 5 *Eriobotrya* species (varieties), reflecting high level of poly-morphology in *E. japonica* Lindl. A was 1.8, P was 69.6%, Ho amounted to 0.419. Through comparing each parameter in different regions, we observed that Fujian population's was highest, followed by Guangxi's. The mean number of A, P, Ho and He was 1.9, 77.3, 0.350 and 0.348 respectively. Table 2 also showed high genetic diversity of *E. prinoides* Rehd. et Wils. and *E. prinoides* var. *daduheensis* from active region of variation on species, east and south slopes of Gagong Mountain in west of Sichuan. Especially, expected heterzygosity (He) of *E. prinoides* var. *daduheensis* amounted to highest value of 0.682.

Table 2. Genetic variability at 22 loci in all of the populations (standard errors in parentheses)

	Population	Mean sample size per Locus	A Mean No. of alleles per locus	P Percentage of loci poly-morphic	Mean heterozygosity		Fixation-index
					Ho Direct count	He Hdywbg expected	
1	Gangshu	9.0 (.0)	1.8 (.1)	72.7	.409 (.087)	.313 (.510)	-0.307
2	Zhegiang	16.0 (.0)	1.8 (.1)	68.2	.398 (.086)	.312 (.049)	-0.276
3	Guiqin	11.0 (.0)	1.9 (.1)	77.3	.401 (.080)	.348 (.044)	-0.152
4	Meiguo	2.0 (.0)	1.6 (.1)	59.1	.432 (.095)	.356 (.066)	-0.213
5	Riben	14.0 (.0)	1.8 (.1)	72.7	.403 (.086)	.309 (.050)	-0.304
6	Anhui	7.0 (.0)	1.7 (.1)	68.2	.370 (.092)	.298 (.051)	-0.331
7	Xianggan	4.0 (.0)	1.6 (.1)	59.1	.432 (.095)	.292 (.056)	-0.479
8	Guangdong	8.0 (.0)	1.8 (.1)	72.7	.426 (.083)	.308 (.049)	-0.383
9	Hubei	5.0 (.0)	1.7 (.1)	68.2	.455 (.089)	.317 (.052)	-0.403
10	Fugian	40.0 (.0)	1.9 (.1)	77.3	.465 (.077)	.350 (.046)	-0.329
11	Chiyepipa	1.0 (.0)	1.3 (.1)	27.3	.273 (.097)	.273 (.097)	0.000
12	Dayaosanpipa	1.0 (.0)	1.2 (.1)	22.7	.227 (.091)	.227 (.091)	0.000
13	Liyepipa	1.0 (.0)	1.5 (.1)	45.5	.455 (.109)	.455 (.109)	0.000
14	Daduhepipa	1.0 (.0)	1.7 (.1)	68.2	.682 (.102)	.682 (.102)	0.000
	Mean		1.7	61.4	0.416	0.346	

Inheritance and variation in *E. japonica* Lindl. and *Eriobotrya* populations

F-statistics parameter (Table 3) was used to scale genetic variation at loci. The basic formation of F-statistics recommended by Nei was $1-F_{IT} = (1-F_{IS})(1-F_{ST})$ (Nei, 1978). If $F_{ST} = 0$, there was no variation between sub-populations, or absolute differences if $F_{ST} = 1$. From Table 3, the variation in Ant-2 and Acp-1 were 0.280 and 0.244 respectively, but no variation in Pgm-2, Dia-1, Dia-2 and Est-3 between the sub-population. It meant more than 20% of inheritances and variations were distributed between populations, but less than 80% distributed in populations. And the total average of Fst was 0.085, namely 8.5% of total genetic variability were distributed between populations and 91.5% in

populations. The result was in accordance with Hamick and Godt's results from 655 plants' statistics, in which the genetic variability was 0.766 in long-life woody plant and 0.088 in short-life woody plant (Hamick and Godt, 1989). But from Table 4, given calculating the gene frequency of all accessions, the variation of populations (species) increased to 0.299 of total average of Fst, which obviously accounted for most difference among *E. japonica* Lindl., *E. prinoides* Rehd. et Wils., *E. prinoides* var. *daduheensis* Zhang, *E. serrata* Vidal. and *E. dayaoshanensis* Chen.

Table 3. Summary of F-statistics at 19 loci in 7 populations

Locus	F_{IS}†	F_{IT}††	F_{ST}†††
Pgm-2	-1.000	-1.000	.000
Pgd-1	.606	.656	.128
Acp-1	-.063	.196	.244
Acp-2	-.176	-.044	.112
Acp-3	-.413	-.194	.155
Acp-4	.006	.082	.077
Dia-1	-1.000	-1.000	.000
Dia-2	-1.000	-1.000	.000
Dia-3	-.483	-.432	.035
Idh-1	.669	.714	.134
Mdh-3	-.360	-.283	.056
Me-1	.434	.535	.178
Aat-2	-.013	.271	.280
Aat-3	-.725	-.683	.024
Est-2	-.161	-.046	.098
Est-3	-1.000	-1.000	.000
Prx-3	-.105	-.047	.053
Skd-1	-.160	.289	.153
Skd-2	-.127	-.065	.055
Mean	-.371	-.254	.085

†F_{IS}: fixation index of sub-populaiton.
††F_{IT}: fixation index of total population.
†††F_{ST}: variance of gene frequency in populations.

Table 4. Summary of F-statistics at 19 loci in 14 populations

Locus	F_{IS}†	F_{IT}††	F_{ST}†††
Pgm-2	-1.000	-.579	.210
Pgd-1	.683	.772	.280
Acp-1	-.341	.083	.316
Acp-2	-.362	-.178	.396
Acp-3	-.528	-.079	.294
Acp-4	-.535	-.175	.234
Dia-1	-1.000	-.647	.176
Dia-2	-1.000	-.647	.176
Dia-3	-.613	-.336	.172
Idh-1	-.401	.235	.454
Mdh-3	-.345	-.129	.160
Me-1	-.074	.213	.268
Aat-2	-.391	.434	.593
Aat-3	-.833	-.524	.169
Est-2	-.702	-.441	.672
Est-3	-.991	-.166	.414
Prx-3	-.121	-.123	.217
Skd-1	-.233	.075	.250
Skd-2	-.354	-.058	.219
Mean	-.547	-.084	.299

†F_{IS}: fixation index of sub-population.
††F_{IT}: fixation index of total populaiton.
†††F_{ST}: variance of gene frequency between populaitons.

Genetic similarity and distance within and among populations

In order to compare populations' genetic variety, Nei's method was applied in analyzing the genetic distance and genetic identification showed in Table 5 (Nei, 1978; Swofford and Selander, 1989). From that, genetic distance between loquat populations range from 0.000 to 0.072, in which the distance between Japanese loquat and Zhejiang loquat was 0.000. It proved that Japanese loquat was introduced from Zhejiang province. As for American population with few cultivars, each genetic distance (D) was 0.000 among Zhejiang, Jiangsu, etc., showing it derived from China. For the closer philogenetical relationship, more similar in alleles frequencies are nearer to 1 in genetic similarity (I) and nearer to 0 in D. Otherwise, "Huabao" series as Hubei population were breeded with seeds introduced from Zhejiang and Jiangsu Province, which represent near genetic distance between American and Japanese. Nevertheless, the remotest D value was 0.072 between Hubei and Guangdong suggesting lacking of intercourse.

Table 5. Genetic similarity (I) and genetic distance (D) between loquat populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*****	.985	.998	.666	.639	.633	.830	1.000	.993	.958	.955	.959	.959	.992
2 Zhejiang	.015	*****	.992	.712	.640	.689	.846	1.000	1.000	.994	.969	.959	.986	.980
3 Guiqian	.002	.008	*****	.746	.666	.697	.875	1.000	.995	.964	.988	.987	.973	.995
4 Chiye	.407	.340	.292	*****	.688	.807	.783	.808	.736	.711	.814	.761	.755	.746
5 Dayaoshan	.447.	.446	.406	.373	*****	.728	.684	.714	.662	.654	.710	.591	.634	.674
6 Liye	.458	.373	.360	.215	.318	*****	.796	.693	.681	.734	.734	.668	.648	.706
7 Daduhye	.187	.167	.134	.244	.380	.228	*****	.924	.845	.859	.890	.842	.854	.854
8 America	.000	.000	.000	.214	.336	.367	.079	*****	1.000	.992	1.000	1.000	1.000	1.000
9 Japan	.007	.000	.005	.306	.413	.384	.168	.000	*****	.994	.991	.968	.995	.989
10 Anhui	.043	.006	.037	.341	.424	.309	.152	.008	.006	*****	.985	.947	.957	.955
11 Xianggan	.046	.031	.012	.206	.342	.310	.116	.000	.009	.015	*****	.986	.959	.980
12 Guangdong	.042	.042	.013	.273	.525	.403	.173	.000	.032	.054	.014	*****	.931	.983
13 Hubeil	.042	.014	.014	.027	.281	.434	.158	.000	.005	.044	.042	.072	*****	.948
14 Fujian	.008	.020	.005	.293	.394	.349	.157	.000	.011	.046	.020	.017	.054	*****

As far as species concerned, D value located from 0.116 to 0.525 except for D value less than 0.1 between *E. prinoides* var. *daduheensis* H.Z. Zhang and *E. japonica* Lindl., indicating obvious differences among species.

Cluster analysis on populations

D was calculated by "Modified Roger's distance" prescribed by Wright (1978). Cluster analysis showed that in the dendrograms Japanese loquat was first clustered to Zhejiang loquat, that all of the species in *E. japonica* were clustered together and then clustered to *E. prinoides* var. *daduheensis* H.Z. Zhang, that *E. prinoides* Rehd. et Wils. was first clustered to *E. serrata* Vidal. and then clustered to *E. prinoides* var. *daduheensis* H.Z. Zhang, that the cultivars flowering in winter were clustered together and then clustered to the cultivars in *E. prinoides* var. *daduheensis* H.Z. Zhang flowering in winter. Similar results were obtained from population analysis of BIOSYS-1 and species analysis of NTSYS-pc, indicating that *E. prinoides* var. *daduheensis* H.Z. Zhang was a relatively independent population that was located in between *E. japonica* and *E. prinoides* Rehd. et Wils. and served as the key for binding *E. japonica* and the others in *Eriobotrya,* which supports the simple classification system of Chinese *Eryiobotrya* plants put forward by Zhang *et al.* (1990) and Zhang (1996).

Allozyme analysis and genetic diversity

As far as genetic diversity, it was abundant according to information from National Loquat Germplasm Plantation, "Records of Loquat" on biology, ecology and morphology. However, we have to confront with one question: were all diversities of phenotype caused by inheritance? This study was to answer it, in which fifty-five alleles were detected at 24 loci of 12 enzyme systems in 14 populations of *Eriobotrya* with the largest number of five. Analysis of allele frequency variation of 7 populations

from *E. japonica* showed that 9 in 22 loci were significant and very significant. As genetic diversity of 14 populations, average expected heterzygosity (He) and the portion of polymorphic loci (P) were 0.346 and 61.4 respectively, with mean number of alleles per locus of 1.7. Comparison of genetic variation of the populations in *E. japonica* demonstrated that the populations from Fujian had the highest genetic diversity, with He, P and A being 0.350, 77.3 and 1.9 respectively. Fixed indexes of the populations were negative, implying they had more heterozygous genotypes than expected value of Hardy-Weinberg which may result from non-random outcrossing.

Zhang suggested that Chinese loquat were distributed along Changjiang River. After the theory was brought forward, Huang (private letter) advanced that the original loquat spreaded from primal zone to Zhujiang River, besides to Chang River. The germplasm could communicate with each other after the two ways converge in Fujian, where induced various germplasm resource. His opinion could explain partly why the genetic diversity of Fujian population was highest and why the genetic similarity between Fujian population and Guangxi population were so high. In this study, the allozyme analysis supported Huang's hypothesis.

The protect strategy and measurement for loquat germplasm resource

Understanding species' genetic diversity and genetic structure contributed to make out efficient protection strategies. The genetic variability of *E. japonica* Lindl. was low in populations. Average Fst of 7 main populations' was 0.085, with 8.5% of total genetic variability among populations and 91.5% in populations. This suggested that genetic diversity distributed in populations, and that protect relative fewer in number but higher in genetic diversity to realize the purpose for protecting germplasm. Besides this, reseachers should collect plants as many as possible everywhere in order to cover the gene bank as well from abundant resources of China as well. But for a long time, people did not pay more attention to the protection of resources so that many forests were destroyed. According to our investigation in recent years, the wild resources of *Eriobotrya* Lindl. were less than before greatly. *E. japonica* Lindl. and other species of *Eriobotrya* Lindl., with their unique gene banks were destroyed cruelly in their original zone. Holding a deep research on the actual loquat resources in China and providing scientific evidences for protecting loquat resources were important projects for protecting genetic diversity of loquat against destroy.

The role of *E. prinoides* var. *daduheensis* H.Z. Zhang on classification

E. prinoides Rehd. et Wils. var. *daduheensis* H.Z. Zhang was found in 1980s in the west of Sichuan province of China (Zhang *et al.,* 1990). It drew attention at home and abroad and arose debate concerning its classification. X.L. Li suggested that *E. prinoides* var. *daduheensis* H.Z. Zhang wasn't a sort of population on morphology, and that was classified in one species with *E. japonica* Lindl., more original only (Li *et al.,* 1992). After that, B. Tang analyzed the nuclear type and measure the activity of the isozyme of peroxidase. According to the result, *E. prinoides* var. *daduheensis* H.Z. Zhang was believed as an independent variety and came from hybrid of *E. japonica* Lindl. and *E. prinoides* Rehd. et Wils. with scientific name *E. daduheensis* (H.Z. Zhang) B. Tang (Tang, 1997). Furthermore, the result of our study proved the role of *E. daduheensis* located in between *E. japonica* Lindl. and *E. prinoides* Rehd. et Wils. and as a variety of *E. prinoides* Rehd. et Wils. in taxonomy should be maintained. In our study, BIOSYS-1 and NTSYS-pc to analyze population and individual, UPGMN cluster to relationship among populations were used by investigating allozyme of 11 isozyme. Consequently a similar result was found: all of the species in *E. japonica* were clustered together and then to *E. prinoides* var. *daduheensis* H.Z. Zhang, that *E. prinoides* Rehd. et Wils. was first clustered to *E. serrata* Vidal. and then to *E. prinoides* var. *daduheensis* H.Z. Zhang, that the cultivars flowering in autumn and winter were clustered together and then to the cultivars in *E. dayaoshanensis* Chen flowering in spring.

Two methods on computation produced similar result, which is different in approach but equally. As high genetic diversity of *E. prinoides* Rehd. et Wils. and *E. prinoides* var. *daduheensis* of 14 populations from active region of variability on species, He amounted to highest value 0.682. D between *E. japonica* flowering in autumn and winter and *E. dayaoshanensis* were above 0.45, except for 0.370 between *E. dayaoshanensis* and *E. prinoides* var. *daduheensis* (Cai, 2000), indicating that *E. prinoides* var. *daduheensis* H.Z. Zhang was a relatively independent population that was located in

between *E. japonica* and *E. prinoides* Rehd. et Wils. and served as the key for binding *E. japonica* and the others in *Eriobotrya,* which supports the simple classification system of Chinese *Eryiobotrya* plants put forward by Zhang *et al.* (1990). But there are no satisfying evidences to interpret if *E. prinoides* var. *daduheensis* was the hybrid of *E. japonica* Lindl. and *E. prinoides* Rehd. et Wils. At present, electrophoresis of enzyme system is a effective method to prove hybrid or speculate the parents without interfere of middle type (Wang, 1996). Nevertheless, we can't confirm the location of hybrid in *E. prinoides* var. *daduheensis* in this study based on allozyme analysis of 11 isozyme, for neither Est-2^b, Est-3^b of *E. prinoides* var. *daduheensis* with unique genotype being found in *E. prinoides* Rehd. et Wils. nor in *E. japonica* Lindl.

The relationship between Japanese loquat and Chinese loquat

Loquat named as *Eriobotrya japonica* Lindl. was used with long history. Usually, it was misunderstood as native to Japan for its name implies. Loquat never named as ancient language, not as peach as "momo", pear as "nashi". Tanaka reported that loquat were introduced from China by ancients. Through analyzing the genetic structure in this study, from Table 5 and Fig. 1, we confirmed the nearest relationship between Japanese loquat and Chinese loquat at molecular levels with I = 1.000 and D = 0.000, making Japanese loquat first clustered to Zhejiang loquat in Cluster analysis. In addition, Zhejiang province was important producing area famous for high quality of products, where tributes produced early in Tang Dynasty. Moreover, frequent culture and commercial intercourse between Zhejiang Province and Japan all along may be proved by a truth: *Citrus unshiu* was introduced from China and developed in Japan. For all, we may draw a conclusion: Japanese loquat was introduced from Zhejiang province of China.

Conclusions

(i) Fifty nine alleles were detected at 24 loci of 12 enzyme systems in 14 populations of *Eriobotrya* with the largest number of five.

(ii) The 120 accessions could be distinguished by 11 enzyme systems.

(iii) The average of Fst of the 19 loci was 0.085, in between long-life woody plants and short-life woody plants.

(iv) Fujian population had the highest genetic diversity.

(v) *E. prinoides* var. *daduheensis* H.Z. Zhang was a relatively independent population that was located in between *E. japonica* and *E. prinoides.* Rehd. et Wils. and served as a link for binding *E. japonica* and the others in *Eriobotrya*.

(vi) Japanese loquat was introduced from Zhejiang province of China.

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