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Genetic Diversity and Utilization of Triploid Loquats (*E. japonica* Lindl)

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1. Introduction

Eriobotrya is a genus of at least 22 species and 10 varieties or *forma* of evergreen fruit trees in the family of Rosaceae (Lin, 2007). *Eriobotrya* is native to east and southeast of Asia, of which only loquat (*Eriobotrya japonica* Lindl.) is cultivated for its valuable fruit. The hypothesized center of origin and center of diversity of loquat are located in the medium to lower Daduhe River and the southeast slope of Gongga Mountain in Southwestern China (Qiu and Zhang, 1996). Loquat was domesticated in China at least 2000 years ago and has been widely cultivated for fresh and processed fruit, as well as for its medicinal effect (Qiu and Zhang, 1996). Loquat was introduced into Japan, France, England, United States and various Mediterranean countries between 12th to 19th century. Today loquat is mainly distributed between latitudes 20 and 35° north or south from the equator, but it can be cultivated up to latitude 45° under marine climates (Lin et al., 1999). There are more than 30 loquat producing countries in the world and the production is distributed in Asia, Europe, Africa, Australia, and the America. In addition to the utilization of its fruit, loquat flower is a superior honey source and it mostly blooms in fall and early winter. The white flower is aromatic thus is appreciate as ornamental tree as well.

The global planting area was about 130,000 hectares, with the production over 549,220 tons in 2005. The main producing countries are China, Spain, India, Japan and Pakistan, which account for 97% of the planting area and 94% of the output respectively. In China, the planting area of loquat is more than 120,000 hectares with an output of more than 400,000 tons. The production in China is distributed in 20 provinces and the leading producers include Sichuan, Fujian, Chongqing, Zhejiang, Hunan, Guangdong and Guizhou. Many well-known loquat varieties originated from these provinces and are widely planted in China among which include 'Dawuxing', 'Longquan No.1', 'Zaozhong No.6'(Lin 2007a).

Genetic diversity and the relationships among different varieties of loquat are of great importance for the conservation of genetic resources, breeding, national and international exchange of germplasm (He et al., 2011). Research on genetic diversity of loquat based on pomological traits and molecular markers have been widely carried out (Badenes et al.,

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2000; Cai 2000; Vilanova et al. 2001; Soriano et al. 2005; Dong 2008; Qiao 2008; Watanabe et al. 2008; Gisbert et al. 2009; Yang 2009). These studies significantly enhanced our understanding about the distribution and structure of genetic diversity in loquat germplasm around the world.

2. Germplasm and breeding research of loquat

Loquat is mainly self-compatible, self-incompatibility has only been found in a few varieties (Chen & Chu, 2008). The traditional propagation from seed has provided a range of varieties adaptable to different environments and planting regions. *Ex situ* germplasm collections have been established in China, Japan and Spain (Zheng, 2007; Badenes et al., 2009). Among these collections, the Chinese collections possess highest diversity. There are more than 1000 accessions described in the various Chinese germplasm collections (Zheng, 2007). The largest Chinese collection is located in Fuzhou, with a total of more than 250 accessions. The major European loquat germplasm collection is held at Instituto Valenciano de Investigaciones Agrarias (IVIA) of Spain. The IVIA collection includes more than 74 accessions, of which 49 accessions are Spanish varieties. In addition, Italy and Greece also have small collections of mainly local germplasm (Lin, 2003).

Understanding of genetic diversity in loquat germplasm has been greatly improved through the application of molecular marker technology (He et al., 2011). Using internal transcribed spacer (ITS) region of the 18S–5.8S–26S nuclear ribosomal cistron, Li et al. (2009) analyzed 15 *Eriobotrya* accessions and six close related species. Result of cluster analysis suggested that *E. malipoensis* had closer relationship with *E. japonica* Lindl. This result was further supported by Yang et al. (2009) who analyzed *Eriobotrya* taxa using AFLP markers. However, Zhao et al. (2011) also used ITS region of the 18S–5.8S–26S nuclear ribosomal cistron and assessed the phynogenetic relationship among the *Eriobotrya* species represented by 25 loquat cultivars and seven wild taxa. Their result suggested that the evolution order of the studied taxa was *E. bengalensis* f. *angustifolia*, *E. prinoides var. dadunensis*, *E. prinoides* (*E. bengalensis*), *E. dayaoshanensis* and *E. japonica* Lindl. And *E. bengalensis* f. *angustifolia* was found the likely ancestral taxa of *E. japonica* Lindl.

The intraspecific genetic diversity in loquat has been analyzed by various molecular markers as well. Soriano et al. (2005) assessed genetic relationships among 40 loquat accessions that originated from different countries, including accessions from the European loquat germplasm collection maintained at IVIA in Valencia, Spain. Thirty pairs of microsatellites previously identified in *Malus* × *domestica* (Borkh.) were used. The expected and observed heterozygosities were 0.46 and 0.51 on average, respectively, showing a high level of out crossing behavior in loquat germplasm. However their result also indicates a low degree of genetic diversity in the set of loquat accessions analyzed, in comparison to other members of the Rosaceae family.

He et al. (2011) used apple SSRs that are transferable to loquat and assessed the level of genetic diversity within loquat. They used 39 SSRs transferable to loquat and genotyped 54 loquat accessions from Japan, Spain, and four Chinese provinces, as well as two wild species (*E. prinoides* var. *Daduheensis* and *E. prinoides*). In total they identified 155 different alleles with a mean value of 3.38 per locus, and the mean observed heterozygosity was 0.47. These values indicate a high degree of genetic diversity in the set of Chinese loquat accessions analyzed. Cluster analysis grouped the accessions into cultivated and wild loquats. The

cultivated loquat can be subdivided into three subgroups which generally reflect their geographic origin in China.

The Chinese loquat germplasm is traditionally classified according to the flesh color. Varieties can be classified as either "white flesh" or "orange flesh". The former accounts for about 30 percent of the total Chinese varieties. The texture of the white-fleshed loquat is generally more delicate and tender. Varieties in this group include 'Ruantiaobaisha' and 'Baiyu', both are the leading varieties of Zhejiang and Jiangsu province in China. Several Japanese varieties, such as 'ShiroMogi', canbe placed in the white flesh group. Orange-fleshed varieties, such as 'Mogi', 'Tanaka', and 'Nakasakiwase' account for 95% of the total crop area of Japan. Spanish commercial production depends on four orange flesh varieties, including 'Algerie', 'Magdal', 'Golden Nugget', and 'Tanaka' with 'Algerie' varieties (Badenes et al., 2009).

Crop improvement, both through breeding for new varieties and selection of accessions from existing germplasm, have been carried out in China, Japan and Spain (Zheng, 2007; Shih, 2007; Badenes et al., 2009). However, so far the varieties used in production were mostly farmer selections made by growers in the local areas. Surveys and selection of germplasm accessions have been carried out in China (Zheng, 2007), Mediterranean countries, Turkey and Pakistan (Badenes et al., 2009). Seedless or fewer seeds is one of important objectives in today's fruit breeding programs. Loquat fruit has a relatively smaller fruit than other fruit tree species. The average weight of a loquat fruit is about 30 to 40 grams. Some large-fruited varieties can have a fruit size of 70 grams, and 34 usually with 3 to 4 large seeds. The low edible rate (less than 70%, is an important quality constraint for fresh consumption of loquat (Lin et al, 2007b). Therefore, seedless loquat fruit is highly desired by consumers.

The classical approach to eliminate seeds in many crops is to produce triploids through ploidy manipulation (Ollitrault et al., 2008). And there are three routes to obtain triploidy genotypes, including use of nonreduced megaspore or microspore, crossing induced tetraploids with diploids and in vitro culture of nuclear tissue (Janick, 2011). Tetraploid varities can be spontaneously formed or induced by colchicines (Yahata et al., 2004). In 1978, Chinese researchers of Fruit Research Institute of Fujian Academy of Agricultural Science developed a tetraploid varities 'Min No.3' using colchicine-induced polyploidization. However the tetraploid variety was not well adopted due to the performance in other agronomic traits and quality. Between 1983 and 1985, researchers in the same institute obtained triploid loquat plants through endosperm culture. In early 1990s, the Southern Prefectural Horticulture Institure, Chiba Prefectural Agriculture and Forestry Research Center of Japan hybridized diploid variety "Nagasakiwase" 6 with tetraploidy variety "Tanaka", and developed the first triploid loquat variety "Kibou", as well as a series of triploidy seedlings. From 1997 to present, Liang and his team in the Southwest University of China have made significant progress in identification of natural triploid loquats (Guo et al., 2007).

3. Occurence of natural triploid loquats

Exploitation of triploid plant to induce seedlessness is a promising breeding technique since triploid is a naturally existing ploidy status as found in other Rosaceae species. For example, approximately 10% of apple and pear varieties are triploids although the frequency of natural occurrence is less than one percent. In 1993, Liang et al. discovered that substantial

frequency of natural triploid individuals exists in loquat germplasm. Since then, massive screening have been conducted in Chinese loquat germplasm, which has led to the selection of 352 natural triploid individuals out of 99,542 seeds in 36 varieties, the frequency of occurrence is about 0.35% (Table 1).

Crop	No. 2n × 2n seedlings	Distribution of seedlings, percentage and number			
		Diploid	Triploid	Tetraploid	Pentaploid
Apple	6,825	99.63	0.28 (19)	0.09 (6)	
Loquat	99,542	99.57	0.35 (352)	0.07 (74)	0.01 (10)

Table 1. Comparison between apple and loquat for the frequency of ploidy levels in natural crosses.

4. Morphological characters of triploid loquats

As compared to diploidy loquat, the triploids have stronger growth vigor, characterized by the thick trunk and branches as well as larger leaves. Moreover, phenotypic differences are also found in flowers, triploid plants usually have larger flowers and flower buds than diploid ones. The transverse and length diameters of flowers, flower buds, anthers and ovaries of triploidy loquat were significantly larger than those of diploids. And fruits of triploidy loquat are significantly larger and seedless, with an edible rate of more than 80% (Table2-4, Figure 1). However, the morphological characters of different seedlings have a wide range of variation. For example, the triploid seedlings of Changbai No.1, Q21 has a ovoid-shaped fruits, whereas plant Q27 and Q11 have long ovoid fruits and the fruit weight of Q27 was significantly heavier than that of Q11 and Q21(Table 4).

5. GISH (Genomic *in situ* hybridization) analysis of triploid loquats

GISH is an efficient and accurate technique for the determination of levels and incorporation positions of alien chromatin. This technique has been widely applied to numerous interspecific and intergenomic plant hybrids (Snowdon et al., 1997). The GISH analysis of

Cultivar	Trunk circum. (cm)	No. branches	Annual branch diam (cm)	Leaf		
				Length (cm)	Width (cm)	Leaf index
Dawuxing (2x)	25.5b[z]	7.2a	5.3b	27.5b	7.3b	3.8a
3x seedling	50.0a	3.6b	7.1a	45.8a	15.6a	2.9b
Longquan No.1 (2x)	29.7b	6.0a	6.5b	22.2b	7.0b	3.2b
3x seedling	50.0a	2.4b	8.5a	36.3a	13.4a	2.7a
Jinfeng (2x)	24.9b	7.0a	6.4	25.6b	7.2b	3.5a
3x seedling	45.0a	3.0b	7.5	43.2a	15.6a	2.8b
Zaohong No.3 (2x)	36.0b	7.0a	5.8b	25.7b	7.7b	3.4a
3x seedling	49.0a	4.0b	7.4a	38.7a	14.1a	2.8b

Table 2. Comparison of plant morphology of diploid loquats and their related triploid seedlings. z Mean separation of 2x and related 3x means at 5% level (Liang et al., 2011).

Varieties	Flower width (cm)	Flower length (cm)	No. single flowers	Bud width (mm)	Bud length (mm)	Filament length (mm)
Dawuxing2x	7.34b	4.46b	72.8b	4.50b	4.30b	2.35b
3x seedling	20.20a	14.60a	104.8a	5.85a	6.90a	5.05a
Longquan No.1 (2x)	8.24b	7.18a	68.8b	4.70b	4.90b	2.90b
3x seedling	13.0a	15.0b	82.0a	6.15a	6.70a	3.85a
Jinfeng (2x)	10.00b	9.60b	51.40b	4.77b	4.77b	2.92b
3x seedling	21.80a	18.8a	98.40a	6.85a	7.00a	3.93a
Zaohong No.3 (2x)	11.60b	8.62b	57.6b	4.60b	5.40b	3.00b
3x seedling	20.20a	12.00a	116.2a	7.60a	7.05a	3.75a
	Anther length (mm)	Anther width (mm)	Style length (mm)	Ovary width (mm)	Ovary length (mm)	
Dawuxing2x	1.65b	1.20b	2.50b	1.84b	1.29b	
3x seedling	2.25a	1.55a	4.05a	3.51a	2.91a	
Longquan No.1 (2x)	1.85b	1.30b	3.05b	1.97b	1.35b	
3x seedling	2.60a	1.95a	3.95a	3.56a	2.95a	
Jinfeng (2x)	1.91b	1.93b	2.54b	1.95b	1.35b	
3x seedling	2.57a	2.56a	4.11a	3.62a	2.94a	
Zaohong No.3 (2x)	1.85b	1.70b	2.73b	2.30b	1.44b	
3x seedling	2.56a	2.00a	4.04a	3.60a	2.91a	

Table 3. The comparison of flowers of natural triploid and diploid loquats. The lower cases in the table means of the significant level (P<5%) of multiple range test.

Varieties	Shape[1]	Flesh color[2]	No. seeds	Weight (g)	Length (mm)	Width (mm)	Shape index	Edible portion (%)
Raotiaobaisha2x	R	W	4-6	25.2 (34)[3]	37.8	38.4	0.97	62.0
3x seedling(H324)	LO	OY	0	50.3 (68)	56.2	40.1	1.40	86.0
Jinfeng2x	O	OY	4-6	61.1 (133)	58.2	46.9	1.24	65.0
3x seedling(D425)	LO	OY	0	79.3 (103)	78.5	52.7	1.49	84.9
3x seedling(D327)	LO	OY	0	78.1 (85)	73.8	41.3	1.78	85.6
Dawuxing2x	O	OY	4-6	58.7 (96)	62.5	45.1	0.97	65.0
3x seedling(A322)	LO	OY	0	65.8 (85)	73.0	50.0	1.56	83.5
3x seedling(A313)	LO	OY	0	62.2 (83)	70.0	49.0	1.49	82.5
3x seedling(A35)	LO	OY	0	63.1 (85)	73.0	49.0	1.78	85.2
Changbai No.1 2x	R	W	4-6	36.2(39)	37.0	42.0	0.88	59.6
3x seedling(Q21)	O	W	0	40.0(50.2)	45.0	41.0	1.10	84.9
3x seedling(Q27)	LO	W	0	65.0(78.3)	61.0	46.0	1.33	85.2
3x seedling(Q11)	LO	W	0	41.6(62.5)	61.0	38.5	1.58	84.5

Table 4. Characteristics of fruit in diploid loquats and their related triploids.
[1]LO= long ovoid, O= ovoid, R=roundish; [2]OY= orange yellow, W=white;
[3]Maximum fruit weight (Liang et al., 2011).

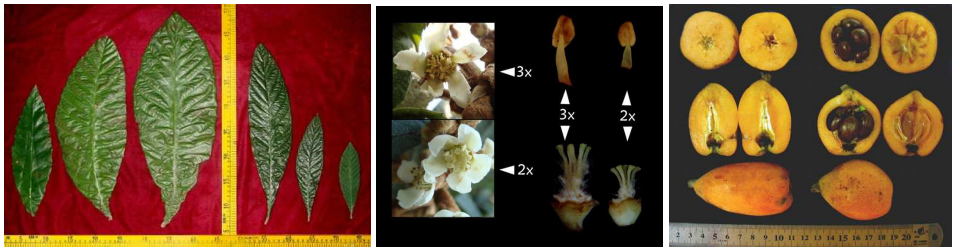

Fig. 1. Compariation of morphological characteristics in leaf, flower and fruit between triploid seedling and diploid (left: 3x, right: 2x)

the natural triploid loquat revealed three types of hybrid (Table 5). In the first type, hybrid signals were detected throughout all 51 chromosomes. In the second type, only 34 chromosomes were detected with hybrid signals. In the third one, only areas around the centromere of all the 51 chromosomes showed hybrid signals. These results showed that these triploids were either homogenous or heterogenous triploids. The different types of hybrid signals also indicated the genetic diversity of natural triploid loquats. Wang (2008) used GISH analysis on artificial triploid loquats, the results showed that the source of hybrid somatic chromosomes can be accurately distinguished using genome DNA of one parent as probe, revealing 17 chromosomes from male parent, and 34 from maternal parent. No significant variation in chromosome structure, such as interchange and inversion was found.

Type of GISH	Individuals	Genome composing
Ⅰ	Dawuxing (A322, A376), Longquan No.1 (B4-331, B316, B349, B352, B378), Jinfeng (D425), Zaohong No.3 (E39), Ganlu No.1 (I315), Huangrou (G320), Luzhou No.6 (C321)	＋＋＋
Ⅱ	Dawuxing (A368, A379), Longquan No.1 (B347, B372, B374, B441)	＋＋－
Ⅲ	Dawuxing (A332), Longquan No.1 (B38, B329, B333, B338, B339, B345, B350, B351, B356, B375), Zaohong No.3 (E310), Ruantiaobaisha (H324), Longquan No.5 (K381, K459), Longquan No.6 (J367), Donghuzao, Jianyangtezao, Xiangzhong No.11, Zaozhong No.6	＋＋＋

Table 5. The results of GISH and composing of genomes of different varieties of natural triploid loquats. "+, -" means of different genomes. (Liang, 2006)

6. Molecular marker analysis of natural triploid loquats

Molecular marker is an effective technique to assess genetic diversity both dominant and co-dominant molecular markers such as ISSR (inter-simple sequence repeat), AFLP (amplified fragment length polymorphism), MSAP (methylation sensitive amplified polymorphism) and SSR (simple sequence repeat) have been used in genetic diversity analysis of loquat germplasm, including natural triploid loquats.

6.1 ISSR (Inter-simple sequence repeat) analysis of natural triploid loquats

Liang (2006) used ISSR markers to analyze genetic diversity of loquat. Result based on twelve ISSR primers showed that similarity of 'Dawuxing', 'Longquan No.1', 'Longquan No.5', 'Longquan No.6' ranged from 0.65 to 0.98, 0.64 to 0.95, 0.76 to 0.96 and 0.83 to 0.93 respectively. As showed by the amplification pattern of primer 835, unique bands were detected in some seedlings (Figure 2, Table 6), indicating significant genetic differentiation among the triploidy accessions.

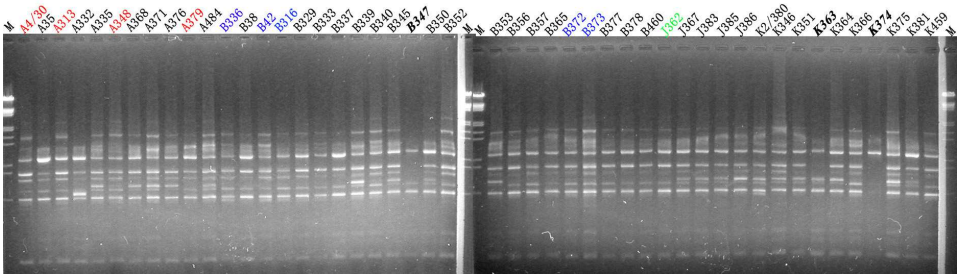

Fig. 2. The amplification pattern of ISSR primer 835. (M: λDNA/*Hind* III + *EcoR* I marker)

Band size	Exist	Missing
1500bp	A4/30(2x), A313, A335, A348, A368, A371, A376,A484	A35, A332, A379
770bp	K346, K366, K375, K381, K459; J367, J386	K2/380, K351, K363, K364, K374; J362(2x), J383, J385
700bp	A335, A368, A371, A376, A484; B339, B345, B350, B353; K2/380, K346, K351, K363, K364, K375, K381; J367, J383, J385, J386	A4/30(2x), A35, A313, A332, A348, A379; B336(2x), B38, B329, B333, B337, B340, B347, B352, B356, B357, B365, B377, B378, B460; K366, K374, K459; J362(2x)
660bp	A4/30(2x), A35, A313, A335, A348, A368, A371, A376, A379, A484	A332
550bp	B336(2x); K2/380	B38, B329, B333, B337, B339, B340, B345, B347, B350, B352, B353, B356, B357, B365, B377, B378, B460; K346, K351, K363, K364, K366, K374, K375, K381, K459

Table 6. The characteristic bands of primer 835.

6.2 AFLP (amplified fragment length polymorphism) analysis of natural triploid loquats

Wang (2008) assessed the effectiveness of AFLP markers in loquat using 6 natural triploid loquats and their maternal parents. With results indicated that, 12 pairs of AFLP primers amplified 2454 bands, as contrasted with maternal parent, there were 112 added bands and 96 lost bands of triploids, the number of amplified bands also differed among the clones of triploid loquats (Table 7). A369 has the greatest difference from its parents (25 additional bands and 19 missing sites), followed by A348 (20 new bands and 22 missing sites), A35 (22 new bands and 14 missing sites), A368 and A322 have the same number of different sites

(A368 has more missing bands and A322 has more new bands), and A313 has 12 new bands and 10 missing bands (Wang et al., 2011).

Types	Numbers of polymorphic bands						Numbers of polymorphic loci and their ratios[a]		Total bands
	A348	A368	A313	A35	A322	A369			
Added bands	20	14	12	22	19	25	112	53.9%	2454
Missing bands	22	18	10	14	13	19	96	46.1%	
Total	42	32	22	36	32	44	208	8.5%[b]	

Table 7. Statistics of polymorphic bands of natural triploids by AFLP analysis. [a] the ratios of one type of polymorphic bands and total polymorphic bands; [b] the ratios of polymorphic bands and total bands.

6.3 MSAP (methylation sensitive amplification polymorphism) analysis of natural triploid loquats

A total of 3879 bands were amplified with 12 pairs of primers within the group of six natural triploid loquat clones and their maternal plant, in which, 363 bands were fullly methylated and 241 bands were hemimethylated. The methylation ratios of six triploid lines were between 12.9% and 18.3%, 15.8% on average, full methylation ratios were between 7.6% and 11.7%, 9.7% on average, hemimethylation ratios were between 4.7% and 6.1%, 5.6% on average. All these belong to four patterns of methylation, monomorphism, demethylation, hyper-methylation and hypo-methylation, the number of sites and frequency were 251 and 29.2%, 171 and 19.9%, 334 and 38.9%, 103 and 12.0% respectively, and all of them exisited in all triploid lines (Table 8). All these indicted that, during the process of genome recombination and triploidization, a great number of hyper-methylation, demethylation, hypo-methylation and maintained methylation were proceeded (Wang, 2008).

	2x	A348	A368	A313	A35	A322	A369
Total methylated bands[a]	573	551	567	583	539	531	535
Full methylated bands[b]	44	69	43	59	63	51	53
Hemimethylated bands[c]	37	32	30	34	33	25	31
Total methylated bands	81	101	73	93	96	76	84
Full methylation ratios(%)	7.7%	12.5%	7.6%	10.1%	11.7%	9.6%	9.9%
Hemimethylation ratios(%)	6.5%	5.8%	5.3%	5.8%	6.1%	4.7%	5.8%
Total methylation ratios(%)	14.1%	18.3%	12.9%	16.0%	17.8%	14.3%	15.7%

Table 8. Genomic DNA methylation of natural triploid loquats and their female parent. [a] including full methylated and hemimethylated sites; [b] Full methylation denotes 5′-C^{m}CGG-3′in double strands; [c] Hemimethylation denotes 5′-^{m}C CGG-3′in single strand.

6.4 SSR (simple sequence repeat) analysis of natural triploid loquats

Fifty five pairs of polymorphism primers were screened, and a total of 135 alleles were detected with ten clones of 'Dawuxing'. The allele with 222 base pairs of CH01h02 was only found in the triploids. And there's three alleles of 238 bp, 236 bp and 230 bp with primer Hi15h12 of A322 (Figure 3). Similar results were obtained by Watanabe et al. (2008). New alleles emerged as compared diploid and each one of triploid individuals, indicating foreign genes maybe introgressed along with the formation progress of triploid individuals. All ten clones were completely distinguished from each other, the highest SM similarity coefficient was between A2x and A313, with 0.926, and the contrast one was between A313 and A332, with the similarity coefficient of 0.496. Principal component analysis divided 10 strains into three groups (Figure 4), Group I, including the A484, A376, A379 and A368 of four lines, A35, A322 and A332 are three lines constitute the group II, group III consists of A2x, A313 and A484 (He, 2010).

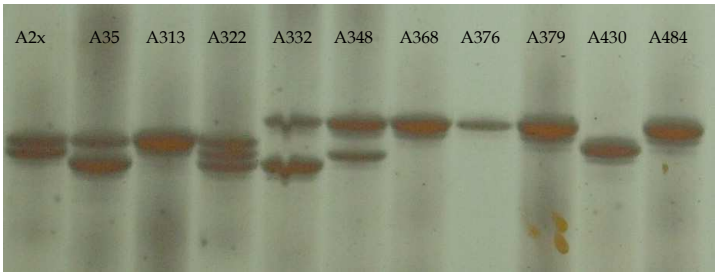

Fig. 3. The amplification pattern of SSR primer Hi15h12.

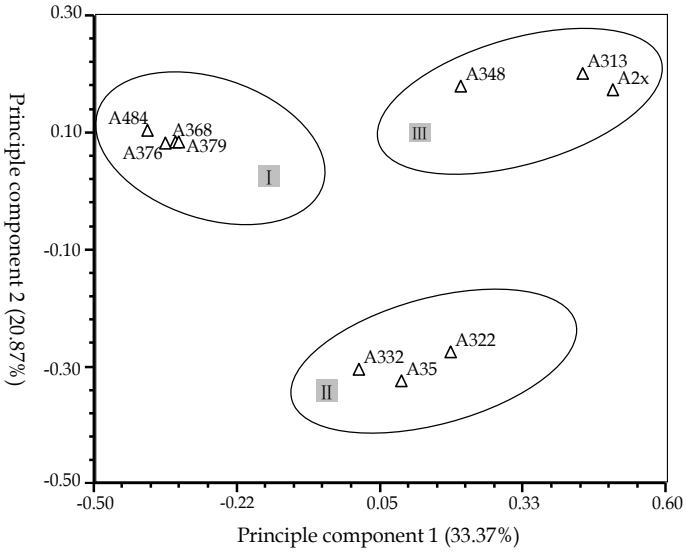

Fig. 4. Two dimensional plot of the principal components analyzed of 10 loquat individuals with 55 primer pairs, using the similarity matrix obtained with SM coefficient.

7. Molecular characterization of artificial triploid loquats

Seven artificial triploid loquats were obtained by sexual hybridization between tetraploid "Jiefangzhong" and diploid "Hunanzaoshu". Eleven ISSR primers amplified 1989 bands, to contrast with parents, the added, lost, agnate and maternal bands were 1, 4, 9 and 19 respectively, indicating that the genomes of hybrid progenies are more similar to maternal parent which provided more chromosomes, the added and lost bands different from both parents , suggesting substantial genome variations among the artificial triploid loquats.

Similar result was observed using AFLP analysis. A total of 3122 bands were amplified with 12 pairs of AFLP primers. The numbers of added, lost, agnate and maternal bands were 82, 58, 49 and 105 respectively. All these indicated that substantial degree of genome variation occurred druing the process of triploid formation (Table 9).

DNA methylation analysis of artificial triploid loquat individuals and their parents showed that, a total of 5302 bands were amplified with 12 pairs of primers, of which 605 bands were full methylated and 233 bands were hemimethylated. Total methylation ratios of triploidy F1 hybrids were between 13.2% and 17.8%, 15.4% on average, full methylation ratios were between 10.5% and 12.2%, 11.7% on average, hemimethylation ratios were between 2.2% and 5.5%, 3.9% on average. Relative to parental plants, transmutation tendency of toal methylation and full methylation ratios of the artificial triploids was not significant. However, the hemimethylation ratios in the artificial triploids all decreased. The frequencies of the five types of methylation, demethylation, hyper-methylation, hypo-methylation and intermediate pattern were 24.2%, 28.8%, 38.5%, 6.6% and 1.9% respectively (Table 10). Hyper-methylation occurred mainly during the development of artificial triploids, then demethylation, accompanied hypo-methylation, and methylation maintain and intermediate pattern that methylation state maintained between parent plants.

Types	Patterns of band			Numbers of polymorphic bands							Numbers of polymorphic loci and their ratios[a]			Total bands
	4x	2x	3x	H1	H2	H3	H4	H6	H8	H19				
Added bands	-	-	+	14	5	10	14	11	18	10	82		27.9%	
Lost bands	+	+	-	7	7	6	9	9	12	8	58		19.7%	
Agnate bands	-	+	+	1	5	5	4	6	6	5	32	49	16.7%	3122
	+	-	-	1	7	2	3	2	2	0	17			
Maternal bands	+	-	+	12	4	11	10	11	11	13	72	105	35.7%	
	-	+	-	8	4	4	5	3	3	4	33			
Total				43	32	38	45	42	52	40	294		9.4%[b]	

Table 9. Statistics of polymorphic bands of artificial triploids by AFLP technique. [a] the ratios of one type of polymorphic bands and total polymorphic bands; [b] the ratios of polymorphic bands and total bands.

	Parents		Artificial triploid loquats						
	4x	2x	H1	H2	H3	H4	H6	H8	H19
Total methylated bands[a]	567	596	599	547	592	614	609	599	579
Full methylated bands[b]	65	66	70	63	74	67	64	68	71
Hemimethylated bands[c]	35	35	15	29	30	14	27	13	32
Total methylated bands	100	101	85	92	104	81	91	81	103
Full methylation ratios(%)	11.5%	11.1%	11.7%	11.5%	13.5%	10.9%	10.5%	11.4%	12.3%
Hemimethylation ratios(%)	6.2%	5.9%	2.5%	5.3%	5.1%	2.3%	4.4%	2.2%	5.5%
Total methylation ratios(%)	17.6%	16.9%	14.2%	16.8%	17.6%	13.2%	14.9%	13.5%	17.8%

Table 10. Genomic DNA methylation of artificial triploid loquats and their parents. [a] including full methylated and hemimethylated sites; [b] Full methylation denotes 5′-C^{m}CGG-3′in double strands; [c] Hemimethylation denotes 5′-^{m}C CGG-3′in single strand.

8. Future research prospect

Loquat originated from China, and there's diverse loquat germplasm, including related species need to be investigated. Most of the selected natural or artificial triploidy seedlings were leading varieties, which only covers a very small fraction of the loquat diversity. Additional populations or germplasm groups should be used for breeding triploid loquat, and the hybridization between polyploidy and diploidy accessions should be explored. Furthermore, detailed characterization of all triploid seedlings should be carried out using new generation of molecular tools. As the genome sequencing of loquat will be finished, techniques based on transcriptomics and comparative genomics will enrich the genetic research of triploid loquats.

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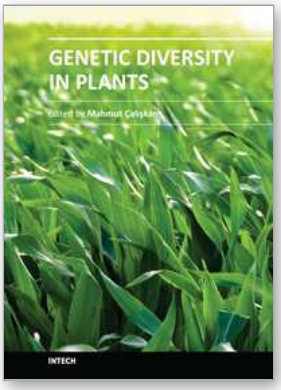

Genetic Diversity in Plants

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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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