Analysis of genetic relationships and diversity among mango cultivars and the relative species based on ISSR markers of nuclear DNA and chloroplast DNA

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1 MATERIALS AND METHODS

Genomic DNA and chloroplast DNA were extracted from leaves and chloroplast of 35 mango cultivars and relative species (Table 1) using CTAB method with little modification, respectively. 42 ISSRs primers were screened and UPGMA cluster analysis was performed by NTSYSpc version 2.1e software .

Table 1. Mango cultivars and relative species used in the study

Cultivar	Abbreviation of Cultivar name	Cultivar	Abbreviation of Cultivar name
Red Ivory	GxRI	Zill	UZL
Tianyangxiang Mang	GxTY	Long Mang	YLM
Alphonso	IAL	*M. siamensis* Warbg ex Craib	II 4
Jin Mango	YJM	Long Jin Mango	GdLJ
Gaozhou Carabao	PhGZ	Jinqian Carabao	GdJQ
Burma Mango	BBM	Thailand Mango (unknown cultivar)	ThTM
Guandao Mang	GxGD	Burma Ball Mango No. 3	BBB 3
Indian Mango No.15	IIM 15	Datou Mang (Yunnan)	YDT
Macheso	BMC	Bangalora	IBG
M. himalis J. Y. Liang	GxMH	Xianluo Mango	ThXL
Okrong	ThOR	Sri Lanka 811	SSL
Pakistan Mango	PPM	Jinhuang Mang	TJH
White Flower Mango	ThWF	Xiamao Mang	GdXM
Renmian Mang	GdRM	Neelum	INL
Liuzhou Lusong	PhLZ	Dwarf Mango	ThDM
Zhanjiang Carabao	PhZJ	Yellow Aroemwnis	ThYA
Aroemwnis	ThAS	*M. persiciformis* Wu & Ming	GxMW
Red Aroemwnis	ThRA		

2.RESULTS AND ANALYSES

2.1 Selection of primers and diversity analysis

Of the 42 ISSR primers screened, 9 primers for nuclear DNA and 8 primers for chloroplast DNA (Table 2) were selected in the present analysis for their reproducible and polymorphic DNA amplification patterns (Fig.1). Among the bands amplified with 7 common primers , their polymorphic loci of nuclear DNA ISSR and cpISSR were 53 bands (accounting for 70.7 % of the total bands) and 54 bands (accounting for 79.4 % of the total bands), respectively. The results showed

that nuclear DNA ISSR yielded more bands and less polymorphic loci than cpISSR. In the other words, the genetic diversity of mango by cpISSR was more ample than that by nuclear DNA ISSR.

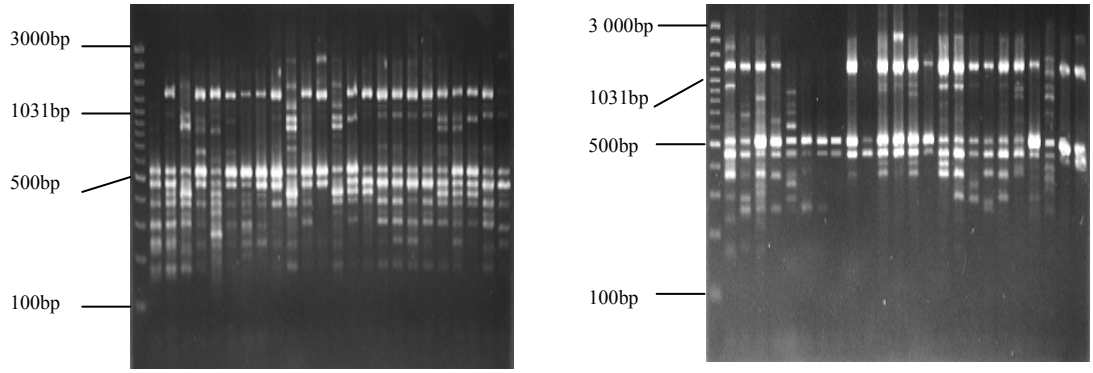

Fig. 1 PCR amplified patterns by UBC-857 primer with nuclear DNA (Left) and chloroplast DNA (Right) of mango

Note: *Lane M: GeneRuler^{TM} 100bp DNA ladder plus, and Lane 1 to 24 represented for cultivars Tianyangxiang Mang, Renmian Mang, Jin Mang, Macheso, Burma Mango, Dwarf Mango, Thailand Mango, Sri Lanka 811, Liuzhou Lusong, Long Mang, Burma Ball Mango No 3, Indian Mango No.15, White Flower Mango, Zhanjiang Carabao, Jinhuang Mango, Guandao mang, Neelum, Xiamao Mango, Okrong, Aroemwnis, Jinqian Carabao, Longjin Mang and *M. himalis* J. Y. Liang, respectively.

Table2 Diversity analysis of ISSR-PCR with nuclear DNA and chloroplast DNA

Primer*	Sequence (5'-3')	Nuclear DNA			Chloroplast DNA		
		Total bands	polymorphic bands	Percentage of polymorphism	Total bands	polymorphic bands	Percentage of polymorphism
UBC-811	$(GA)_8C$	7	5	71.4	7	5	71.4
UBC-835	$(AG)_8YC$	13	10	76.9	10	8	80
UBC-840	$(GA)_8YT$	9	5	55.5	8	5	62.5
UBC-841	$(GA)_8$ YC	12	8	66.7	11	9	81.8
UBC-851	$(GT)_8$ YG	15	10	66.7	11	8	72.7
UBC-857	$(AC)_8YG$	11	9	81.8	10	9	90
GXU-1	$(ACACACAT)_2$	8	6	75	11	10	90.9
UBC-826	$(AC)_8C$	□	□	□	11	7	63.6
UBC-842	$(GA)_8YG$	10	7	70	□	□	□
UBC-876	$(GATA)_2(GACA)_2$	10	8	80	□	□	□
	total	95	68		79	61	

Note: * "UBC" stood for primers designed by Biotechnology Laboratory, University of British Columbia, Canada; "GXU-1" represented primer designed by ourselves; □ represented for poor primer for nuclear DNA or chloroplast DNA.

2. 2 Cluster analysis

Based on the Jaccard coefficient of similarity obtained from the nuclear DNA ISSR band sizes and cpISSR band sizes by 7 common primers, two dendrograms were constructed by the UPGMA method (Fig.2). If the coefficient 0.73 was regarded as a divided line of genetic relationship, the 35 samples with nuclear DNA ISSR could be divided into two groups (Fig.2 left) , in which: group 1 consisted of *M. persiciformis* Wu & Ming only and group 2 included the other 34 cultivars and the relative species, while the 35 samples with cpISSR could be clustered into five groups (Fig.2 right):

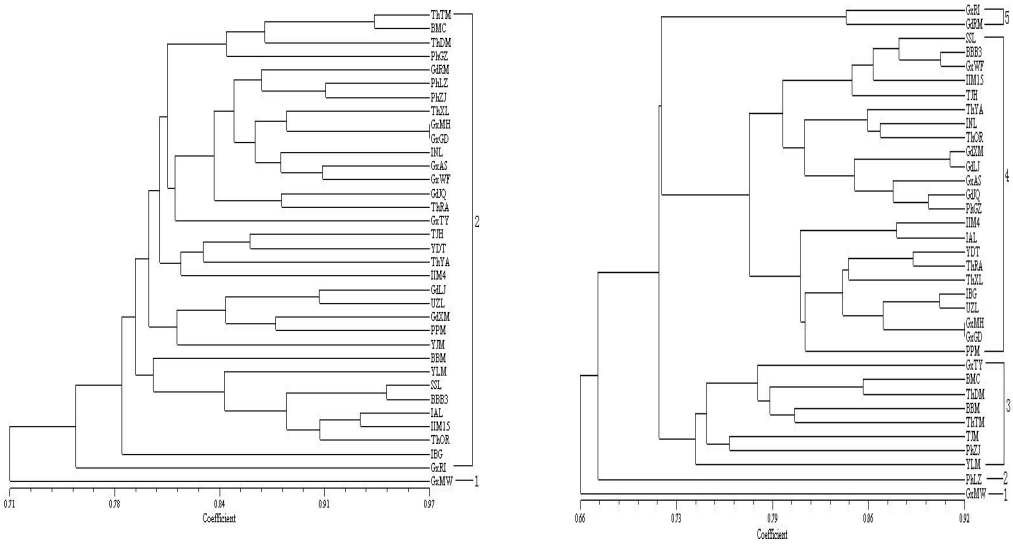

Fig 2 UPGMA dendrograms of relationships among 35 mango cultivars and relative species based on the Jaccard cofficient of similarity obtained from nuclear DNA ISSR and cp ISSR data (left for nuclear DNA ISSR; right for cp ISSR, Abbr. of cultivars seen Table 1)

The results showed that both nuclear DNA ISSR and cpISSR of mango cultivars and relative species were highly informative and there existed huge diversity among them, indicating ISSRs with nuclear DNA or chloroplast DNA were quite valuable and effective for identification of mango cultivars or relative species and evaluation of their genetic diversity. Although the cpISSR had less total bands, the percentage of polymorphism was superior to the nuclear DNA ISSR (79.4％ vs 70.7％), on some extent, the cpISSR showed advantages over the nuclear DNA ISSR makers.

Compared with the nuclear DNA markers, the chloroplast DNA markers are more reliable for mango taxonomic studies because of the conservation and uni-parental inheritance of the chloroplast genome in higher plants. To date, cpISSR have rarely been cross-species amplified in woody plants. The present study demonstrated that cpISSR markers provide a new and efficient tool for mango cytoplasm analysis and has proven to be an important complementary method in studying the genetic relationship of mango and relative species.

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