Full Length Research Paper

A novel strategy for the identification of 73 *Prunus domestica* cultivars using random amplified polymorphic DNA (RAPD) markers

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Despite the usefulness of DNA marker techniques, various DNA markers have not been widely applied for practical cultivar identification. We developed a novel strategy based on DNA molecular fingerprints from the genotyped plant individuals, following which a cultivar identification diagram (CID) was manually generated and used as referable information for quick plant and/or seed sample identification. Based on this, we used random amplified polymorphic DNA (RAPD) markers to identify a total of 73 plum cultivars of different origins. The cultivars could be clearly separated by the fingerprints of 9 RAPD primers. Experimental verification also indicated that the CID generated is referable and workable in the identification of any two or more plum cultivars studied, which remains the main advantage of this CID constructed manually over the phylogenetic trees from cluster analysis used in most reports on plant identification using DNA markers. Furthermore, fewer primers can be used to distinguish all cultivars using this approach. This new strategy developed and employed in plum cultivar identification may be applied in the plum industry to identify and separate plant and seed samples using DNA makers.

Key words: Plum, Cultivar identification diagram, random amplified polymorphic DNA (RAPD) markers.

INTRODUCTION

Plum (*Prunus* sp.) genus is taxonomically diverse and adapted to a broad range of climatic and edaphic conditions (Ramming and Cociu, 1991; Salesses et al., 1993), and contains more than 30 species (Weinberger, 1975) that are diploid ($2n = 2x = 16$) to hexaploid ($2n = 6x = 48$) (Rehder, 1954) in nature. A very large number of plum cultivars are known worldwide (Blazek, 2007). Accurate and rapid identification of plum varieties is therefore necessary for both breeders and commercial companies. When compared to some other fruit crops, plum has not received much attention from geneticists, cytogeneticists and molecular biologists. Reports on plum cultivar identification using molecular markers are limited (Heinkel et al., 1998; Rohrer et al., 2004; Shimada et al.,

2006), most of those reports employ cluster analysis of the banding patterns. Although, these phylogenetic tree based dendrograms could give the genetic diversity levels and separate the plant individuals, they are not able to make easy and referable identification of plum cultivars. Therefore, developing a strategy that can make reliable, easy, and referable identification of plum cultivars is vital for the nursery industry and growers to protect plant patents and provide genetically uniform plants.

Molecular markers are advantageous in that they are not affected by the environment and can provide a powerful tool for proper characterization of cultivars. Recently, various DNA-based markers have been developed and used in studies on genetic diversity, fingerprinting and origins of cultivars in different fruits (Cheng and Huang, 2009; D'Onofrio et al., 2009; Elidemir and Uzun, 2009; Fang et al., 2005; Melgarejo et al., 2009;

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Papp et al., 2010). Among several markers available, the Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990) marker is useful in analysis of cultivars due to simplicity, efficiency, easy operation, and non-requirement of any previous sequence information. If optimization of the RAPD technique by choosing 11 nt primers and strict screening of PCR annealing temperature is done before the technique is employed in fingerprinting of plants, RAPD can be the technique of choice. So far, RAPD markers have been used in the cultivar identification and genetic relationship analysis of a number of fruit species, such as apricot-*Prunus armeniaca* (Ercisli et al., 2009), cherry- *Prunus cerasus* (Demirsoy et al., 2008), pistachio- *Pistacia vera* (Javanshah et al., 2007), pomegranate- *Punica granatum* L. (Hasnaoui et al., 2010), strawberry- *Fragaria ananassa* Duch (Wang et al., 2007). However, in practice fruit plant identification using available powerful DNA markers has not been done yet in efficient, recordable, and easy way due to limitations in analysis strategies of those DNA fingerprints called cluster analysis. The cluster analyses have not made the cultivar or species separation efficient in practice and thus utilization of DNA markers in plant and crop seed identification remains a non-popular practice (Hasnaoui et al., 2010).

The objective of this study was to develop a new method that can make the identification of plum cultivars as a practicable, efficient, recordable, and referable approach as possible using a cultivar identification diagram (CID) generated for the 73 selected plum cultivars. The CID may be employed like that of the periodic table of elements with advantages of highly referable and of use, workability, and flexibility by newer addition of cultivars upon availability of data. In addition, CID will provide valuable information and theoretical basis for identification of cultivars, genetic diversity analysis and genetic improvement of crops at molecular level besides essential requirement in granting of protection to new varieties through DUS (Distinctness, Uniformity and Stability) testing (Lu et al., 2009).

MATERIALS AND METHODS

Plant materials and genomic DNA extraction

A total of 73 plum genotypes (Table 1) were used in this study. Total genomic DNA of each genotype was extracted from young leaves using the modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980; Bousquet et al., 1990). The extracted DNA was diluted to a final concentration of 30 ng μL^{-1} with 1×TE buffer and stored at -20°C pending use.

Amplification of RAPD markers

The reaction mixture (final volume 15 µl) contained 1.5µl 10 buffer, 1.2 µl MgCl₂(25 mM), 1.8 µl dNTP (2.5 mM), 1.2 µl primer (1.0 µM), 0.08µl rTaq Polymerase Dynazyme (5 U/µl) and 30 ng of genomic DNA. Amplification reactions were performed based on the

standard protocol of Williams et al. (1990) with minor modification. The PCR was carried out in a Autorisierter Thermocycler (Eppendorf, Hamburg, Germany), programmed as follows: Pre-denaturation for 5 min at 94°C; then 42 cycles each consisting of a denaturation step for 30 s; an annealing step for 1 min at annealing temperature (Table 2); an extension step for 2 min at 72°C. Amplification was terminated by a final extension of 10 min at 72°C.

RAPD analysis

Out of 54 11 nt RAPD primers, reproducible polymorphic bands were developed by 9 primers (Table 2). The 54 primers had been earlier designed and used for assorted experiments. The PCR products were detected on 1.3% (w/v) agarose gels in 1×TAE (0.04 M *Tris*-acetate, 0.001 M EDTA pH 8.0) buffer at 100 V. The gels were stained with 0.5 µg/ml of ethidium bromide and photographed under ultraviolet light. Polymorphic bands among the cultivars were observed from the photographs. In order to have reproducible, accurate and clear banding patterns, every amplification was repeated at least thrice separately.

Data analysis

Only the clear and unambiguous bands in the photographic prints of gels were manually chosen and scored for each cultivar by each primer. When some cultivars had a specific band in the fingerprint generated from one primer, they could be separated singly, and those cultivars sharing the same banding pattern were separated into the same sub-group, while the others were separated into another sub-group. On this basis, all the plum cultivars were step by step completely separated from each other with more primers being employed.

Test of use and workability of the cultivar identification diagram (CID)

Several plum cultivars, which were randomly chosen from the inter- and intra-groups, were used to verify the utilization and workability of CID showing the separation of 73 cultivars. The corresponding primers to be used for the separation of each group were easily picked out from the diagram.

RESULTS

Cultivar identification

To determine the suitability of the RAPD technique in identifying the plum cultivars, fifty four 11 nt primers were employed and the annealing temperatures for each primer were screened based on the quality and reproducibility of banding patterns 11 nt. Finally, all the 73 plum cultivars were successfully identified by the joint use of several 11 nt primers (Table 2). A notable example of the RAPD pattern, obtained with primer Y12, is shown in Figure 1A. The RAPD primer Y12 was the first to be used to amplify the 73 plum cultivars. The electrophoresis results showed that forty-nine plum cultivars (The lane numbers correspond to the codes in Figure 2, refer Table 2 for cultivar names) generated uniform, clear and reproducible bands, which were absent in the other

Table 1. 73 plum cultivars used in this study.

| No. | Cultivar | Origin | | No. | Cultivar | Origin | |
		Province	Country			Province	Country
1	Youyi	Liaoning	China	38	Beijingwanhong	Beijing	China
2	Bulin	unknown	America	39	Xiguali	Hebei	China
3	Haolaiwu	unknown	America	40	Qiuxiaojie	unknown	China
4	Qiyuehong	Hebei	China	41	Kelsey	unknown	America
5	Faguohong	Liaoning	China	42	Guangfunai	unknown	unknown
6	Chuandaojiuhong	unknown	Japan	43	Moerteli	unknown	America
7	Dashizaosheng	unknown	Japan	44	Taiyangli	unknown	Japan
8	95-6	Liaoning	China	45	Kaiseman	unknown	America
9	Aoli	unknown	America	46	Misili	unknown	America
10	Xiangjiaoli	Liaoning	China	47	American-hongxinli	unknown	America
11	Gaixiandali	unknown	America	48	Conghua-sanhuali	Guangdong	China
12	Changli-15	Jilin	China	49	Aodaliya-14	unknown	America
13	Hongbaoshi	unknown	America	50	Hongmenli	unknown	America
14	Heibaoshi	unknown	America	51	Liwang	unknown	Japan
15	Muhuangli	Heilongjiang	China	52	Xianfeng	unknown	America
16	Zaoyan	unknown	Japan	53	Heihupo	unknown	America
17	Angenuo	unknown	America	54	Taoli	unknown	China
18	Qiulizi	Liaoning	China	55	Suoruisi	unknown	Italy
19	Dalimei	Heilongjiang	China	56	Laluoda	unknown	America
20	Dongbeili	Dongbei	China	57	Meiguili	Yunnan	China
21	Taoyeli	unknown	China	58	Dazili	Hebei	China
22	Xiaosuli	Liaoning	China	59	Liyanghuangli	Jiangsu	China
23	Xiaoheli	Beijing	China	60	Yueguangli	unknown	Japan
24	Suilinghong	Heilongjiang	China	61	Hongliangjin	unknown	Japan
25	Fali	Yunnan	China	62	Kaersai	unknown	America
26	Fenghuali	Zhejiang	China	63	Hongmeili	unknown	America
27	Guiyang	unknown	China	64	Dahongli	Guangdong	China
28	Xiaoganyuhuangli	Shandong	China	65	Weikexun	unknown	America
29	Liheli	unknown	China	66	Zuili	Zhejiang	China
30	Meiguodali	Beijing	China	67	Hubeili	Hubei	China
31	Owent	unknown	America	68	Aozhakeshouxiang	unknown	America
32	Wuxiangli	Huabei	China	69	Shengmeigui	unknown	unknown
33	Meiguihuanghou	unknown	America	70	Jiaqingzi	Jiangsu	China
34	Taihouli	unknown	unknown	71	Shandong-yuhuangli	Shandong	China
35	Hongbulin	unknown	America	72	Aodeluoda(Green)	unknown	America
36	Aodeluoda(Red)	unknown	America	73	Haoyun	unknown	America
37	Furongli	Fujian	China				

cultivars. The fragment size of the specific band was about 650 bp (Figure 1A). When this special band was selected for cultivar identification, the cultivars could be separated into two groups. Another primer Y22 was chosen to differentiate the two groups of plum cultivars respectively, but there were no polymorphic bands, meaning that the 73 cultivars in the two groups could not be differentiated using the primer Y22 and hence, the primer Y4 (Figure not shown) was chosen to further distinguish cultivars within the two groups. The first and second group could be successfully separated into several secondary groups by the use of primer Y4 (Figure 2). Primer Y40 was used to amplify all of the secondary groups where 11 plum cultivars were directly identified from each other and the other smaller groups could also be further separated into many more sub-groups (Figure 1B). A further example of such separation is the RAPD pattern obtained with primer Y41 which had several polymorphic bands, meaning that the cultivars in this group could be successfully differentiated from each other (Figure 1C). After following this trend and eventually utilizing all the 9 primers, the original group of

Table 1. 11 nt primers chosen for further fingerprinting of 73 plum genotypes.

| Primer	Nucleotide sequence (5'–3')	Annealing temperature (°C)
Y-4	GTTTCGCTCCT	44.8
Y-12	CTGCTGGGACC	40.4
Y-24	GGACCCAACCC	44.4
Y-30	GTGTGCCCCAC	44.4
Y-34	AAGCCTCGTCT	44.4
Y-39	AGCGTCCTCCA	44.4
Y-40	AGCGTCCTCCT	42.8
Y-41	AGCGTCCTCCG	44.4
Y-60	ACCCCCGACTC	42.8
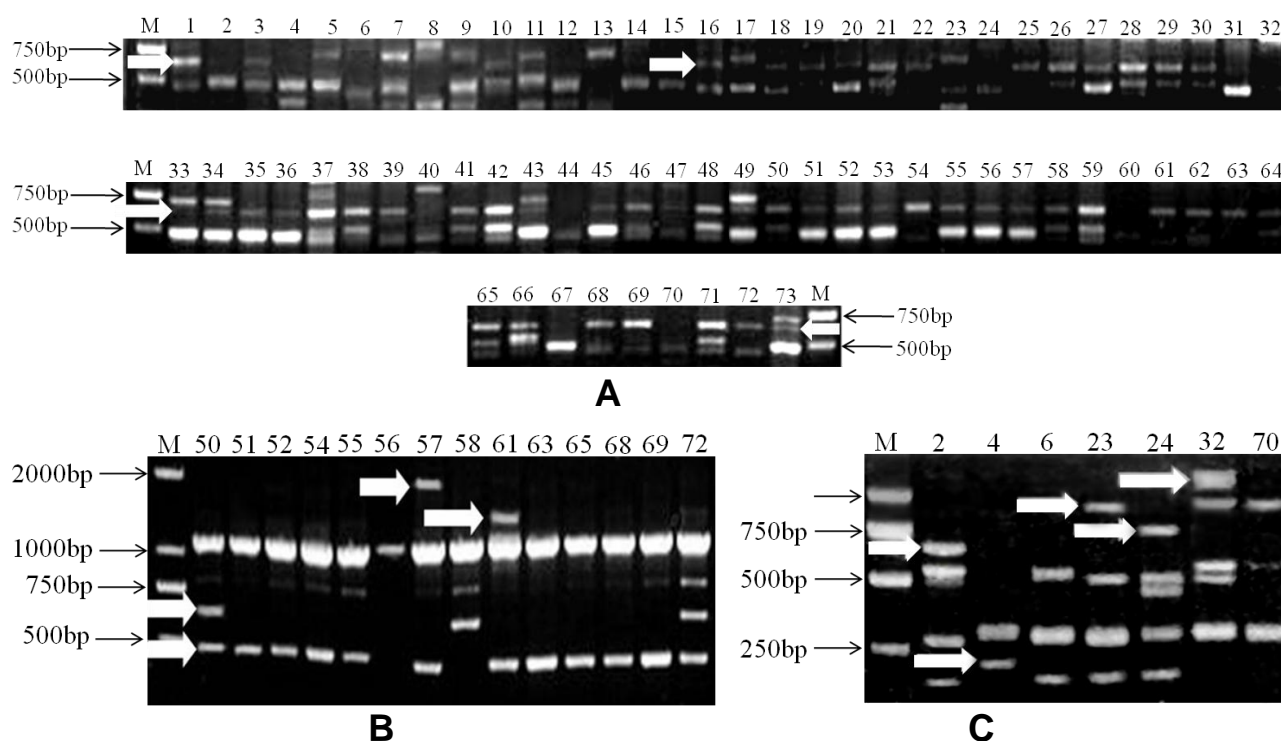
Figure 1. A: RAPD patterns of 73 plum genotypes obtained with primer Y12. Horizontal arrows indicate the specific bands. The lane numbers correspond to the codes in Table 1. M: DNA size marker. B and C: RAPD profiles obtained with RAPD primers. Horizontal arrows indicate the specific bands. The lane numbers correspond to the code in Table 1. M: DNA size marker. B obtained with the prime Y40, C obtained with the primeY41.

73 plum cultivars could be completely differentiated from each other (Figure 2).

Test of the utilization and workability of the diagram in cultivar identification

An important aim of this study was to learn how to use the RAPD marker to distinguish the 73 plum cultivars. In addition, we endeavored to generate a referable plum cultivar identification diagram (CID) for identification of cultivars in future nursery industry practice and cultivar-

right-protection. Four plums 'Conghua-sanhuali' and 'Kaersai' from the first and 'Owent', and 'Hubeili' from second group were chosen and used to verify the scientific aspects of this method using primers Y12 and Y4 (Figure 2). The PCR results clearly showed that the four plum cultivars could be identified by three specific bands as anticipated in Figure 2. Firstly, primer Y12 was used to amplify genomic sequence of the four plums cultivars (Figure 3A), where a specific band (~ 650 bp) was used for cultivar identification. The cultivars 'Conghua-sanhuali' and 'Kaersai' were separated into the same group due to a specific band that was absent in
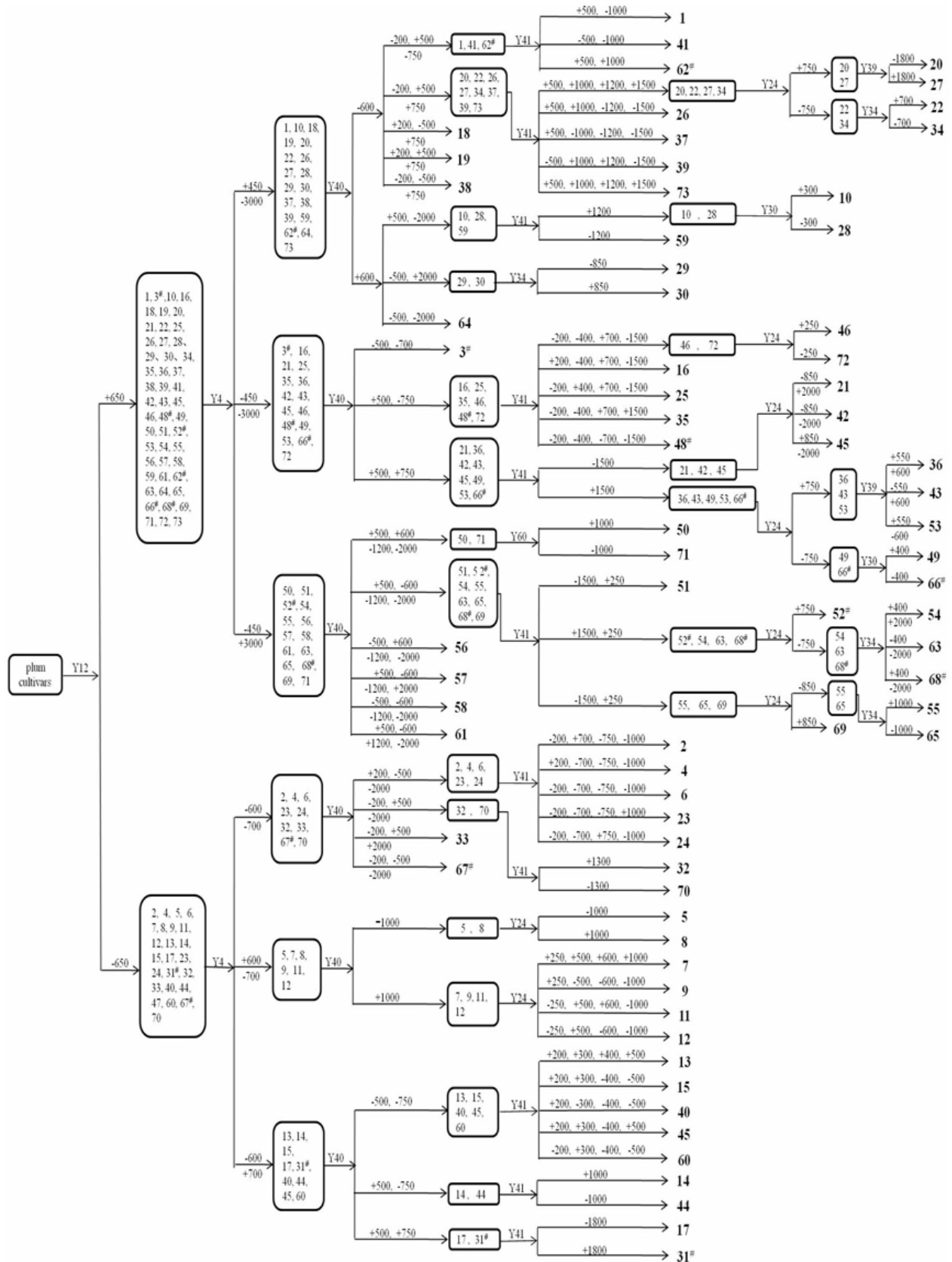
Figure 2. Classification of 73 plum cultivars based on nine 11 nt RAPD primers. The numbers on the branches show the size of the band in bp; (+) band present; (−) band absent; '#' this cultivar was used for validation. Terminal branch numbers in bold indicate the cultivar was uniquely identified.

'Owent' and 'Hubeili', which were clustered into some other group. The genomic sequences of these two groups were amplified by, primer Y4 (Figures 3B and C) that produced a specific ~700 bp band for identification of 'Owent' and 'Hubeili', and one another band (~ 450 bp) was used for identification of 'Conghua-sanhuali' and 'Kaersai'. Figures 3D and E further exemplify how this principle to separate cultivars works.

DISCUSSION

DNA-based molecular markers have acted as versatile tools in various fields like taxonomy, genetic engineering, marker assisted selection (MAS), cultivar identification and variability studies. These classes of markers are found in abundance and are more precise, thus provide an opportunity for direct comparison of genetic materials, as well as not being affected by different environmental conditions or the developmental stage of plants (Reddy et al., 2002). Despite this, DNA markers have not been easily used in genotyping of plants. In fact, the situation is more serious than anticipated, with the question of whether DNA markers can be well and easily used in the identification of plant varieties yielding a negative response from many scientists. No efficient approaches have been developed to use DNA markers easily and efficiently in plant cultivar identification apart from the use of phylogenetic clusters or some fingerprints. Apparently, the clusters formed in phylogenetic trees cannot tell which information can be referable for identification of plant samples, while fingerprinting cannot present all the fingerprints of many cultivars together for identification. These weaknesses could probably be attributed to the fact that no analysis could connect the information of DNA fingerprints with cultivars in an easy, clear and readable way. The new approach developed in this study can use DNA markers efficiently to distinguish the cultivars as desired. It has the advantages of less cost, timeliness and objectivity among others. This strategy can realize the power of DNA markers in plant cultivar identification activities and can use the polymorphic bands of each primer gradually to distinguish every species and individual plant, from which a cultivar identification diagram (CID) can be finally constructed for further use on these cultivars.

Botanical classification of species and cultivars within plum is sometimes controversial, partly because of the ease of interspecific hybridization, which creates numerous intermediate types, and blurs the limits between taxa (Blazek, 2007). This therefore, makes it difficult to avoid the situations of homonym or synonym within the materials, and buttresses the need to identify plum species and cultivars for conservation studies and use of germplasm resources as well as plant variety protection. The ability to distinguish cultivars could be greatly enhanced by using appropriate molecular markers

(Iezzoni and Brettin, 1998; Heinkel et al., 2000). The optimized RAPD was a preferred technique selected to make the identification in this study even more efficient and easy. However, the most important aim of this study was not just how to use RAPD marker to distinguish the 73 plum cultivars, which focuses on the utilization of DNA fingerprints in identifying plant cultivars, but also to develop a new strategy to properly utilize DNA marker in the separation of plum cultivars. This methodology could also be considered as a universal strategy to use in distinguishing cultivars and seed samples in other plant species.

By deployment of the CID strategy, only nine 11 nt RAPD primers were used to distinguish all the 73 selected plum cultivars in this study. The method is very convenient and fast for the user. Although a single RAPD primer cannot distinguish all plum cultivars simultaneously, this method represents a substantial increase in efficiency over previous studies. In addition, it reveals new evidence on the rapid identification of plum cultivars. The informative CID (Figure 2) of the plum cultivars can tell us which primers can be used to separate which plum cultivars. Basically, any two or more plum cultivars can be distinguished by the use of one special primer. For example, the plum cultivars 'Owent' and 'Hubeili' can be distinguished by the use of primer Y4 according to Figure 3B. If the result of PCR amplification shows a special band and the fragment size is about 700 bp, the cultivar can be judged to be 'Owent', otherwise the cultivar is 'Hubeili'. The same principle can be used to distinguish any other two plum cultivars. If more new plum cultivars are released in production, the set of 9 primers selected in this study can be used to run the DNA samples of the new cultivars and the PCR banding patterns can let us know where to position the new cultivars in the CID. If they cannot be separated from the 73 already identified using the 9 primers, additional new primers can be found and used to separate, and then position the cultivars on the CID. By contrast, Shimada et al. (2006) required 20 primers in a RAPDs study to distinguish genetic diversity of 42 plum varieties. It appears that not much work needs to be done and this exercise to generate a larger CID of plum cultivars, which is definitely a significant resource for the plum industry.

Although the method may not accurately reflect genetic relationships among the cultivars, in theory the genetic distance between cultivars separated by the first primer is far greater than the distance between cultivars separated by the last primer. This method is definitely a great addition to plant cultivar identification for cultivar-right-protection and early identification.

This is a first report on using RAPD primers in sequence to identify plum cultivars. In order to verify the reliability of this theory, experimental verification which is an absolute necessity was done and it gave satisfactory results. Therefore, this experiment suggests the possibility of utilizing the DNA markers even in plant
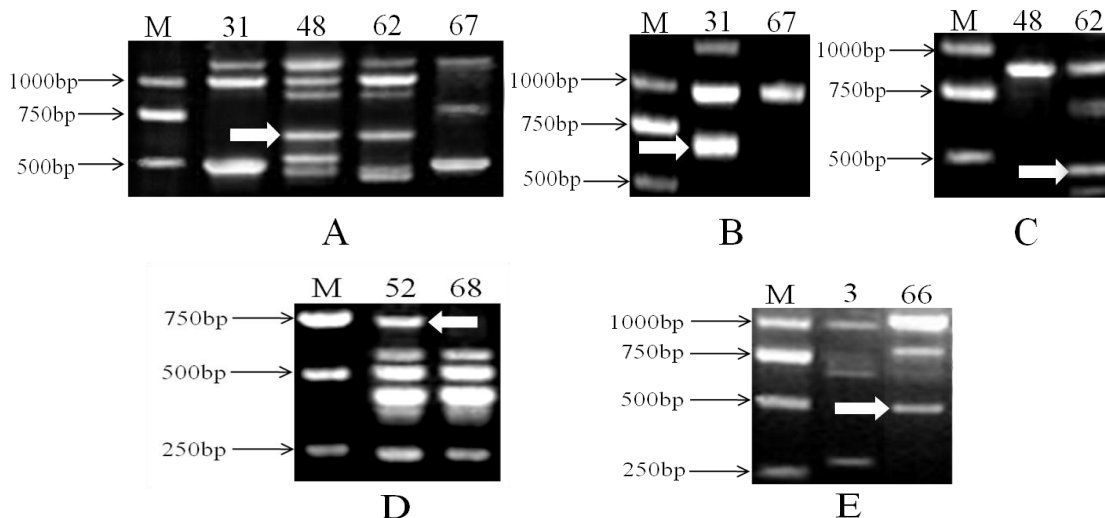
Figure 3. RAPD profiles obtained with RAPD primers. Horizontal arrows indicate the specific bands. The lane numbers correspond to the code in Table 1. M: DNA size marker. A obtained with the Y12 primer, B and C obtained with the Y4 primer, D obtained with the Y24 primer, E obtained with the Y40 primer.

species which have a highly heterozygous genome, without requiring a genetic linkage map and/or any DNA sequence information to distinguish the cultivars. It seems to be an effective technique for convenient development of selection markers in fruit trees. In addition, these polymorphic bands may be developed into special molecular markers for the cultivars identification in future. The amazing results of identification using this new strategy is that a readable and referable CID can be constructed and used in the identification of the related plant species in a manner similar to the use of a periodic table of elements in providing the basic information of chemical elements. We believe that as research on this method progresses, we can use this technique and other molecular makers to develop a table for each species, whether plants or other organisms, which in turn can provide us with the information needed to separate the cultivars as desired. There is also need to test the different markers such as SCAR, SSR and others to ascertain suitability since some markers are sometimes unstable.

In conclusion, this method is rapid, simple, and produces reliable results, since it was possible to demonstrate that a standard set of primers can be used to distinguish between a large number of plum cultivars.

REFERENCES

Blazek J (2007) A survey of thegenetic resources used in plum breeding. Acta Hort. 734:31-45.

Bousquet J, Simon L, Lalonde M (1990). DNA amplification from vegetative and sexual tissues of tree using polymerase chain reaction. Can. J. For Res. 20:254-257.

Cheng ZP, Huang HW (2009). SSR fingerprinting Chinese peach cultivars and landraces (*Prunus persica*) and analysis of their genetic relationships. Sci. Hort. 120:188-193.

Demirsoy L, Demir T, Demirsoy H, Kacar YA, Okumus A (2008). Identification of some sweet cherry cultivars grown in Amasya by RAPD markers. Acta Hort. 795:147-152.

D'Onofrio C, Lorenzis G, de Giordani T, Natali L, Scalabrelli G, Cavallini A (2009). Retrotransposon-based molecular markers in grapevine species and cultivars identification and phylogenetic analysis. Acta Hort. 827:45-52.

Elidemir AY, Uzun I (2009). Assessment of genetic diversity of some important grape cultivars, rootstocks, and wild grapes in Turkey using RAPD markers. Acta Hort. 827:275-278.

Ercisli E, Agar G, Yildirim N, Esitken A, Esitken A, Orhan E (2009). Identification of apricot cultivars in Turkey (*Prunus armeniaca* L.) using RAPD markers. Romanian Biotechnol. Lett. 14: 4582-4588.

Fang J, Qiao Y, Zhang Z, Chao CT (2005). Genotyping fruiting-mei (*Prunus mume* Sieb. Et Zucc) cultivars using AFLP. Hort. Sci. 40:325-328.

Hasnaoui N, Messaoud M, Jemni C, Mokhtar T (2010). Molecular Polymorphisms in Tunisian pomegranate (*Punica granatum* L.) as revealed by RAPD fingerprints. Diversity 2:107-114.

Heinkel R, Hartmann W, Stösser R (2000). On the origin of the plum cultivars "Cacaks Beauty', 'Cacaks Best', 'Cacaks Early' and 'Cacaks Fruitful' as investigated by the inheritance of random amplified polymorphic DNA (RAPD) fragments. Sci. Hort. 83:149-155.

Heinkel R, Hartmann W, Stuttgart-Hohenheim RS (1998). Parental analysis of four 'Cacaks'. Zwetschensorten. Erwerbsobstbau. 40:147-149. (In German)

Iezzoni AF, Brettin TS (1998). Utilization of molecular genetics in cherry. Acta Hort. 498: 53-62.

Javanshah A, Tajabadipour A, Mirzaei S (2007). Identification of a new phenotype (Siah Barg) of pistachio (*Pistacia vera* L.) with shiny-blackish green leaves using RAPD assay. Int. J. Agric. Biol. 9:307-310.

Lu X, Liu L, Gong Y, Zhao L, Song X, Zhu X (2009). Cultivar identification and genetic diversity analysis of broccoli and its related species with RAPD and ISSR markers. Sci. Hort. 122:645-648.

Melgarejo P, Martcnez JJ, HerncLndez Fca, Martcnez R, Legua P, Oncina R, Martcnez-Murcia A (2009). Cultivar identification using 18S-28S rDNA intergenic spacer-RFLP in pomegranate (*Punica granatum* L.). Sci. Hort. 120:500-503.

Murray GC, Thompson WF (1980). Rapid isolation of high molecular weight DNA. Nucl. Acid .Res. 8:4321- 4325.

Papp N, Szilvassy B, Abranko L, Szabo T, Pfeiffer P, Szabo Z, Nyeki J, Ercisli S, Stefanovits-Banyai E, Hegedus A (2010). Main quality attributes and antioxidants in Hungarian sour cherries: identification

of genotypes with enhanced functional properties. Int. J. Food Sci. Technol. 45:395- 402.

Ramming DW, Cociu V (1991). Plum (*Prunus*). In: Moore JV, Ballington JR (eds) Genetic resources of temperate fruit and nut crops. Acta Hort. 290:239-288.

Reddy PM, Saral N, Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its applications in plant breeding. Euphytica 128:9-17.

Rehder A (1954). Manual of cultivated trees and shrubs. Second edition. Dioscorides Press, Portland, Oregon.

Rohrer JR, Ahmad R, Southwick SM, Potter D (2004). Microsatellite analysis of relationships among North American plums. Plant Syst. Evol. 244:69-75.

Salesses G, Grasselly C, Renaud R, Claverie J (1993). Lesporte-greffe des espèces fruitières à noyau du genre Prunus. In: Gallais A, Bannerot H (eds) Améliorationdes espèces cultivées. INRA Editions, Paris. pp. 605-619.

Shimada T, Hamaya H, Haji T, Yamaguchi M, Yoshida M (2006) Genetic diversity of plums characterized by random amplified polymorphic DNA (RAPD) analysis. Euphytica 109:143-147.

Wang Z, Zhang Z, Li H, Gao X, Du G, Tan C (2007). Identification of strawberry cultivars by RAPD and SCAR markers. Acta Hort. Sinica. 34:591-596.

Weinberger JH (1975). Plums, In: J. Janick J. N. Moore (Eds), Advances in fruit breeding. Purdue Univ. Press, West Lafayette, IN, U.S.A. pp. 336-347.

Williams JGK, Kubelik AR, Livak KJ, Rafalski A, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.