

A novel random amplified polymorphic DNA-based strategy for genetic diversity analysis and identification of tomatoes

X. Cao, Z. Wu, R. Zhou, F.L. Jiang and Z.E. Yang

Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in East China, Ministry of Agriculture, College of Horticulture, Nanjing Agricultural University, Nanjing, China

Corresponding author: Z. Wu
E-mail: wzh@njau.edu.cn

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ABSTRACT. Cultivar identification diagrams (CIDs) provide a rapid and efficient approach for identifying cultivars based on random amplified polymorphic DNA (RAPD) markers. In this paper, 64 tomato cultivars were identified using a CID. Using RAPD profiles, clustering analysis was performed to analyze genetic diversity. The results showed that 8 RAPD primers could completely separate the 64 cultivars according to the obtained polymorphic bands; a CID of the 64 tomato cultivars was then constructed. As verification of the CID validity, 8 randomly selected cultivars were investigated and proven to be well distinguished. In addition, 33 DNA bands were obtained, 20 (60.6%) of which were polymorphic. Genetic distances were calculated with a range of 0.032 to 1.402. Clustering analysis showed that the 64 tomato cultivars were divided into 4 groups with a similarity coefficient of 0.40. Using this novel strategy, with the same RAPD data, both CID and clustering analysis can simultaneously determine tomato cultivars and their genetic diversity.

Key words: Tomato; Random amplified polymorphic DNA; Cultivar identification diagram (CID); Cultivar identification; Genetic diversity analysis

INTRODUCTION

The tomato, the annual or perennial herbage of *Lycopersicon* of Solanaceae, originates from Peru and Ecuador in South America and is cultivated worldwide. With extensive adaptability, tomato plants can grow from 45° south latitude to 65° north latitude. Germplasm resources of tomatoes are plentiful, including one cultivated species and 12 wild relatives (Rick et al., 1990; Peralta et al., 2006). Following the introduction, breeding, and domestication of the tomato, more and more cultivars have been developed. Subsequently, the cultivar chaos phenomenon became an issue, which brings with it difficulties for cultivar application and research. Therefore, establishing an effective strategy to distinguish tomato cultivars is essential.

Traditional approaches for tomato cultivar identification are usually based on morphological and physiological characteristics; however, this procedure is easily influenced by the surrounding environment and is a time-consuming practice (Baird et al., 1996). In addition, when considering the differentiation of closely related species, this can be an extremely difficult work. Therefore, to establish an effective strategy to identify tomato germplasm is very important. Furthermore, the tomato has become an excellent model system for studying genetic engineering, plant tissue culture, and molecular genetics. The identification of tomato cultivars provides a basis for tomato germplasm and molecular biology research, which have great theoretical significance and practical value.

Molecular markers are powerful tools for assessing genetic diversity and identifying cultivars. Many DNA-based marker systems, such as random amplified polymorphic DNA (RAPD), simple sequence repeat, amplified fragment length polymorphism, sequence characterized amplified region, and sequence-related amplified polymorphism have been used in this discipline (Palombi and Damiano, 2002; Lee et al., 2004; Fang et al., 2006; Comlekcioglu et al., 2010; Aruga et al., 2012). Among these, RAPD has proven to be a particularly useful means for cultivar analysis and genetic diversity detection (Williams et al., 1990) because it has the advantage of speed and simplicity. However, to our knowledge, traditional analytic methods, such as clustering analysis, have many deficiencies because they are based on many different primer designs, and the output result is only in a digital form generated by software calculations. In other words, the traditional method requires much work and cannot provide polymorphic primers and markers to distinguish one cultivar from another.

Cultivar identification diagrams (CIDs) by RAPD were developed in the last 3 years, and it has so far been used for the identification of sweet orange, pear, grapevine, loquat, and apple cultivars by Fang's group (Lin et al., 2011; Zhang et al., 2011; Zhao et al., 2011; Sun et al., 2012; Wang et al., 2012). As a novel strategy for cultivar identification using RAPD markers, CID overcomes the defects of the traditional analytic method, requiring fewer primers for RAPD-polymerase chain reaction (PCR) amplification, and the results are output in diagram form. However, the CID strategy cannot be used to efficiently analyze the genetic diversity of plant cultivars.

In order to overcome the deficiencies of existing methods, we used the same RAPD profiles for clustering analysis and CID by RAPD to simultaneously identify tomato cultivars and their genetic diversity. This novel strategy provides a more rapid, powerful, and efficient means of identifying tomato cultivars and their genetic diversity than previously reported.

MATERIAL AND METHODS

Plant material

A total of 64 cultivars were used in this study (Table 1). Of the 64 cultivars, 58 were introduced from the Tomato Genetic Resource Center, University of California, and 6 tomato cultivars were from the People's Republic of China; all 64 tomato cultivars were assessed with RAPD markers (Table 2).

Table 1. Sources of tomato cultivars used in this experiment.

No.	Accessions	Species	Origin ^a	No.	Accessions	Species	Origin ^a
1	LA4031	<i>S. lycopersicum</i>	TGRC	33	LA0490	<i>S. lycopersicum</i>	TGRC
2	LA4032	<i>S. lycopersicum</i>	TGRC	34	LA1222	<i>S. lycopersicum</i>	TGRC
3	LA4037	<i>S. lycopersicum</i>	TGRC	35	LA1598	<i>S. pimpinellifolium</i>	TGRC
4	LA4038	<i>S. lycopersicum</i>	TGRC	36	LA1627	<i>S. galapagense</i>	TGRC
5	LA4040	<i>S. lycopersicum</i>	TGRC	37	LA1698	<i>S. lycopersicum</i>	TGRC
6	LA4043	<i>S. lycopersicum</i>	TGRC	38	LA1777	<i>S. habrochaites</i>	TGRC
7	LA4044	<i>S. lycopersicum</i>	TGRC	39	LA2093	<i>S. pimpinellifolium</i>	TGRC
8	LA4047	<i>S. lycopersicum</i>	TGRC	40	LA2413	<i>S. lycopersicum</i>	TGRC
9	LA4054	<i>S. lycopersicum</i>	TGRC	41	LA2661	<i>S. lycopersicum</i>	TGRC
10	LA4055	<i>S. lycopersicum</i>	TGRC	42	LA2662	<i>S. lycopersicum</i>	TGRC
11	LA4063	<i>S. lycopersicum</i>	TGRC	43	LA2683	<i>S. lycopersicum</i>	TGRC
12	LA4064	<i>S. lycopersicum</i>	TGRC	44	LA2706	<i>S. lycopersicum</i>	TGRC
13	LA4065	<i>S. lycopersicum</i>	TGRC	45	LA2838A	<i>S. lycopersicum</i>	TGRC
14	LA4071	<i>S. lycopersicum</i>	TGRC	46	LA3120	<i>S. lycopersicum</i>	TGRC
15	LA4081	<i>S. lycopersicum</i>	TGRC	47	LA3183	<i>S. lycopersicum</i>	TGRC
16	LA4089	<i>S. lycopersicum</i>	TGRC	48	LA3320	<i>S. lycopersicum</i>	TGRC
17	LA4091	<i>S. lycopersicum</i>	TGRC	49	LA3473	<i>S. lycopersicum</i>	TGRC
18	LA4099	<i>S. lycopersicum</i>	TGRC	50	LA3475	<i>S. lycopersicum</i>	TGRC
19	LA4030	<i>S. lycopersicum</i>	TGRC	51	LA3538	<i>S. lycopersicum</i>	TGRC
20	LA4034	<i>S. lycopersicum</i>	TGRC	52	LA3847	<i>S. lycopersicum</i>	TGRC
21	LA4036	<i>S. lycopersicum</i>	TGRC	53	LA3915	<i>S. lycopersicum</i>	TGRC
22	LA4042	<i>S. lycopersicum</i>	TGRC	54	LA3916	<i>S. lycopersicum</i>	TGRC
23	LA4068	<i>S. lycopersicum</i>	TGRC	55	LA3918	<i>S. lycopersicum</i>	TGRC
24	LA4072	<i>S. lycopersicum</i>	TGRC	56	LA3919	<i>S. lycopersicum</i>	TGRC
25	LA4073	<i>S. lycopersicum</i>	TGRC	57	LA3920	<i>S. lycopersicum</i>	TGRC
26	LA4082	<i>S. lycopersicum</i>	TGRC	58	LA4355	<i>S. lycopersicum</i>	TGRC
27	LA4083	<i>S. lycopersicum</i>	TGRC	59	Zhongza No. 9	<i>S. lycopersicum</i>	CH-BJ
28	LA4090	<i>S. lycopersicum</i>	TGRC	60	Zhongza No. 105	<i>S. lycopersicum</i>	CH-BJ
29	LA4096	<i>S. lycopersicum</i>	TGRC	61	Zhongza No. 109	<i>S. lycopersicum</i>	CH-BJ
30	LA4098	<i>S. lycopersicum</i>	TGRC	62	Jiangshu No. 14	<i>S. lycopersicum</i>	CH-JS
31	LA4070	<i>S. lycopersicum</i>	TGRC	63	Hezuo 908	<i>S. lycopersicum</i>	CH-SH
32	LA0483	<i>S. galapagense</i>	TGRC	64	Suhong 2003	<i>S. lycopersicum</i>	CH-JS

^aTGRC, courtesy of the Tomato Genetic Resource Center, University of California; CH-BJ, Beijing, China; CH-JS, Jiangsu, China; CH-SH, Shanghai, China.

Table 2. RAPD primers and annealing temperatures used in this experiment.

Primer	Nucleotide sequence (5'→3')	Annealing temperature (°C)
Y4	GTTTCGCTCCT	44.4
Y22	GGACCCAACCT	42.8
Y27	GTGTGCCCAA	40.4
Y28	GTGTGCCCAT	43.7
Y33	AAGCCTCGTCA	42.8
Y39	AGCGTCCTCCA	42.6
Y42	AGCGTCCTCCC	41.7
Y53	TGGTGCGGTTG	43.7

Genomic DNA isolation

Genomic DNA was isolated from the young leaves of 64 tomato seedlings using the modified cetyl trimethyl ammonium bromide method (Murray and Thompson, 1980; Bousquet et al., 1990). The quality of isolated genomic DNA was checked using a nucleic acid protein detector and then diluted to a final concentration of 30 ng/ μ L with 1X Tris base and ethylenediaminetetraacetic acid buffer and stored at -20°C until use.

RAPD-PCR analysis

First, 60 RAPD 11-mer primers (Yu et al., 2009) were synthesized by Dingguo Changsheng Biotechnology company in Beijing, China. After PCR amplification, only primers with clear bands were selected for the next step. Based on this principle, 8 primers (Table 2) that showed clear and reproducible bands were utilized in cultivar identification. Amplification reactions were carried out in 20- μ L volumes, containing 2.0 μ L 10X buffer, 1.6 μ L 2.5 mM dNTPs, 1.2 μ L 25 mM MgCl_2 , 1.6 μ L 1.0 μ M primer, 0.08 μ L 5 U/ μ L rTaq polymerase Dynazyme, and 30 ng genomic DNA. Amplification was performed in an Autorisierter Thermocycler (Eppendorf, Hamburg, Germany) using the following settings: initial denaturation at 94°C for 5 min; 42 cycles of 2 min for denaturation at 94°C , 1 min at annealing temperature (Table 2), and a 1-min extension at 72°C ; and a final extension for 5 min at 72°C . The PCR amplification products were isolated on 1.5% agarose gels electrophoretically at 100 V for 20 min, using 1X Tris base, acetic acid, and ethylenediaminetetraacetic acid buffer. Then, the gels were stained with 0.5 mg/mL ethidium bromide and photographed under ultraviolet light. DL 2000 plus marker was loaded and served as a standard molecular weight marker. All amplifications were repeated 3 times.

Utilization and verification of the tomato CID

Only the clear and specific bands photographed on the gel prints were selected for cultivar identification. For amplification by 1 primer, when some cultivars had 1 identically sized specific band in the fingerprint, they could be separated singly, while other cultivars sharing the same banding pattern were assigned to the same subgroup. More primers were used for identification until all cultivars could be gradually and completely distinguished by amplifying more specific bands. Based on this, a tomato CID was constructed according to the specific sizes of bands and their related primers.

Eight tomato cultivars that were randomly chosen from 2 different groups were used to verify the feasibility of the CID. If the randomly chosen cultivars could be distinguished accurately and quickly as shown in the complete CID, we would conclude that the method used in this research is a feasible way of identifying tomato cultivars.

Genetic diversity analysis

Polymorphic fragments amplified by primers used in 64 tomato cultivars were scored using a binary matrix: 1 for their presence and 0 for their absence. Genetic distances were calculated using the NTSYS-PC software version 2.11 (Rohlf, 2000). Clustering analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) and the

TREECON program for Windows (version 1.36) neighbor joining and sequential agglomerative hierarchical and nested clustering.

RESULTS

Characterization of RAPD-PCR-amplified products

In order to create a suitable RAPD system with high reproducibility, 11-mer RAPD primers were initially utilized. If the primer that was randomly screened from a stock of 60 11-mer primers could amplify clear fingerprints with polymorphic bands in 64 tomato cultivars, it was selected and further used for tomato cultivar identification. Through screening, 8 primers (Table 2) with high reproducibility and high resolution were obtained, which could completely distinguish all tomato cultivars. The number of derived bands and the polymorphisms revealed by each primer are shown in Table 3. Considering all the primers and amplification products, a total of 33 bands were amplified by 8 primers, 20 of which were polymorphic with an average of 2.5 specific bands per primer. The percentage of polymorphism obtained from the 8 primers was different between cultivars; the maximum ratio was 100% produced by primer Y53, while the minimum value was 50% from primers Y4, Y28, and Y39. On average, the polymorphism percentage of the 8 primers reached 60.6%.

Table 3. Diversity detected by RAPD primers.

Primer	Total number of bands	Number of polymorphic bands	Percentage of polymorphism (%)
Y4	4	2	50.0
Y22	5	3	60.0
Y27	5	3	60.0
Y28	6	3	50.0
Y33	3	2	66.7
Y39	4	2	50.0
Y42	3	2	66.7
Y53	3	3	100.0
Total	33	20	-
Average/primer	4.1	2.5	60.6

Cultivar identification by the CID method based on RAPD

Based on the RAPD-PCR amplification products, 8 primers could distinguish all 64 tomato cultivars. Of these 8 primers, primer Y42 was initially used to detect DNA polymorphisms within all tomato cultivars; the electrophoresis gel image is shown in Figure 1. Primer Y42 generated clear and reproducible band patterns with 2 specific RAPD-PCR bands of 2800 and 1200 bp in all 64 tomato cultivars. Using primer Y42, 64 tomato cultivars were divided into 3 groups: in group 1, the only cultivar (LA4072) with the presence of the 2800-bp band and the absence of the 1200-bp band was distinguished; group 2 consisted of 28 tomato cultivars including LA4031, LA4032, LA4038, LA4043, LA4081, LA4089, LA4073, LA4082, LA4083, LA4090, LA4096, LA4098, LA4070, LA0483, LA0490, LA1222, LA1627, LA1698, LA2093, LA2661, LA2662, LA2683, LA2706, LA2838A, LA3120, LA3183, LA3320, and Jiangshu No. 14, which were differentiated from others by the presence of both the 2800- and 1200-bp bands; and the remaining 35 cultivars in group 3 included LA4037, LA4040,

LA4044, LA4047, LA4054, LA4055, LA4063, LA4064, LA4065, LA4071, LA4091, LA4099, LA4030, LA4034, LA4036, LA4042, LA4068, LA1598, LA1777, LA2413, LA3473, LA3475, LA3538, LA3847, LA3915, LA3916, LA3918, LA3919, LA3920, LA4355, Zhongza No. 9, Zhongza No. 105, Zhongza No. 109, Hezuo 908, and Suhong 2003, which were characterized by the presence of the 1200-bp band and the absence of the 2800-bp band.

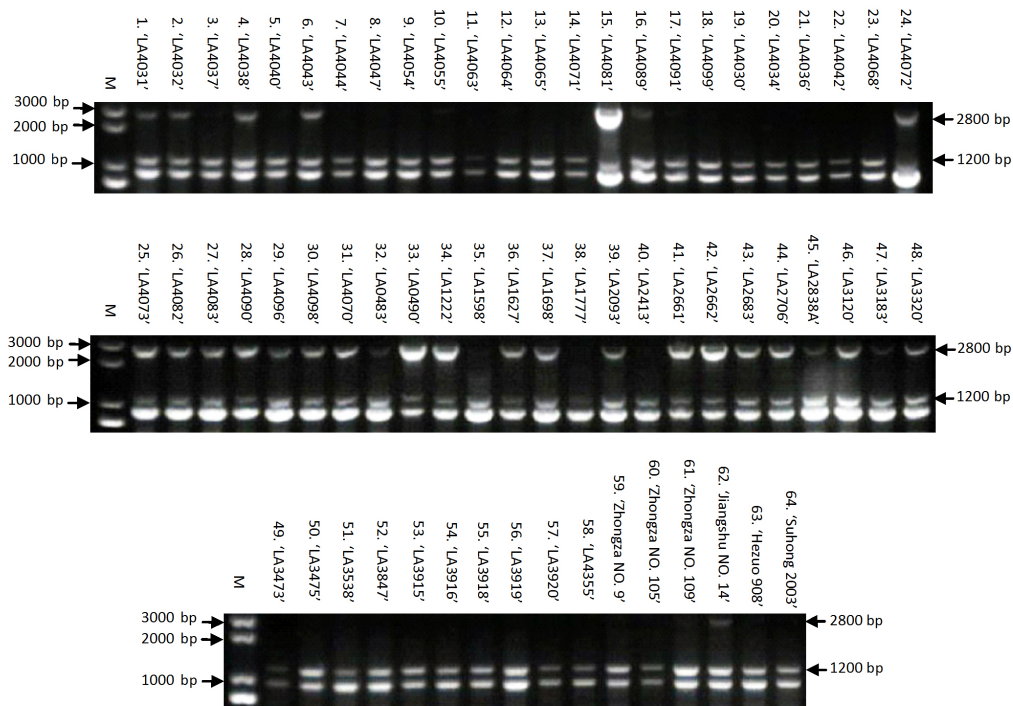


Figure 1. RAPD profile of 64 tomato cultivars obtained with primer Y42. Lanes and accessions of the cultivars are marked at the top. Lane M = DL 2000 plus DNA marker.

Following this method, the other 7 primers and polymorphic bands obtained using them were screened step by step and chosen to identify the tomato cultivars until all cultivars could be completely separated as shown in Figure 2. For example, after the identification by Y42, primer Y4 was used to further separate cultivars from the previously obtained 3 groups. Group 1 included only 1 tomato cultivar; therefore, it was first separated from the others. Group 2, which included 28 tomato cultivars, could be further divided into 4 subgroups using primer Y4; cultivars in subgroup 1, containing LA4032, LA4081, LA4073, LA2093, and Jiangshu No. 14, were identified by Y33, and LA2093 was distinguished from the other 4 cultivars by the presence of an 1100-bp band. Next, using primer Y53, LA4032 did not produce a 1300-bp band and was subsequently separated from the other 3 cultivars. For the remaining cultivars, LA4081 was identified using primer Y28 as it did not express an 1100-bp band, whereas LA4073 and Jiangshu No. 14 were finally differentiated by expression of an 1800-bp band.

Eventually, a tomato CID was constructed using the 8 polymorphic primers, which generated clear and specific sizes of bands using the stepwise identification method described

above (Figure 2); therefore, this can be regarded as an effective and reliable tool for practical tomato cultivar identification.

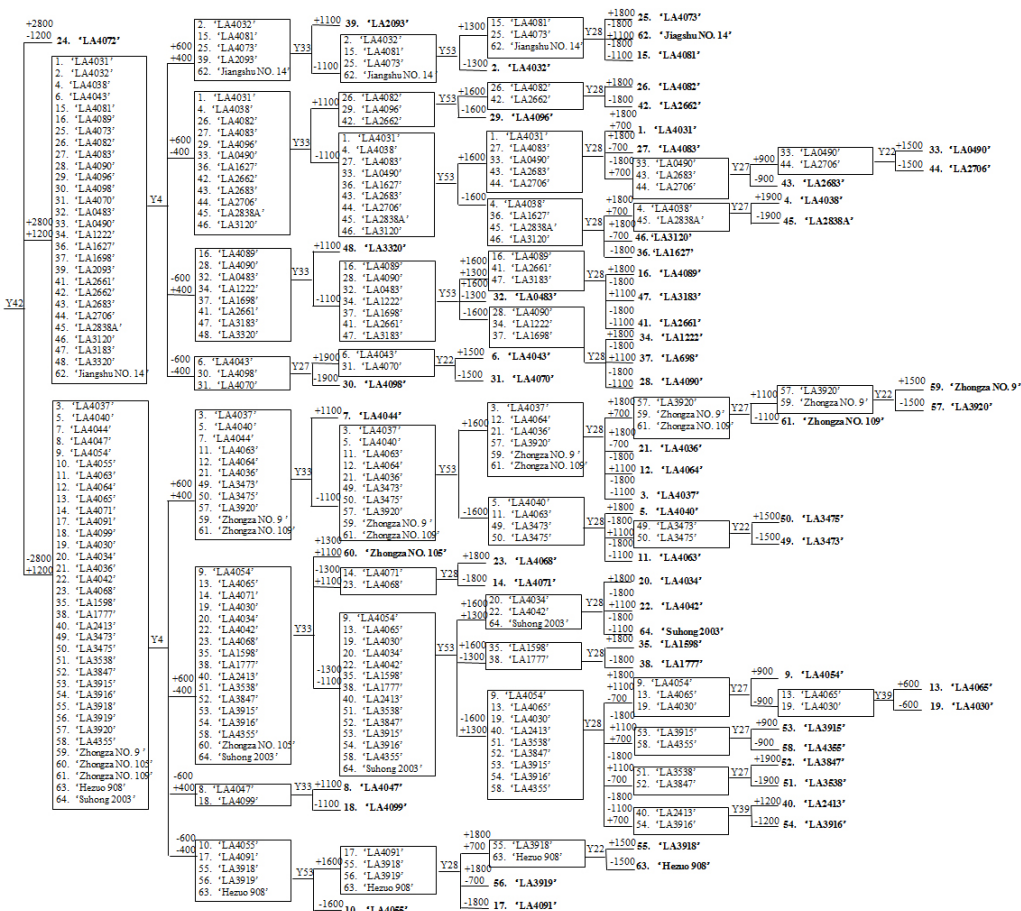


Figure 2. Cultivar identification diagram of tomato cultivars using eight primers. The lane number in the figure represents the size of the band; units are bp. (+) indicates presence of the band; (-) indicates absence of the band. The cultivar names in boldface indicate those that were separated.

Utilization and verification of the CID method based on RAPD

To verify the accuracy and reproducibility of the CID method, 8 of the 64 tomato cultivars were randomly selected. First, 4 cultivars from group 1 (LA4037, LA4038, LA4082, and LA2838A) were tested. From the location of the 4 cultivars in the CID, it was easy to recognize that primers Y42, Y33, and Y27 could well distinguish the 4 cultivars from each other. Electrophoresis images showed that LA4037 was initially separated out from the other 3 cultivars using primer Y42 according to a 2800-bp RAPD-PCR band (Figure 3A). The 3 remaining cultivars were further differentiated by primer Y33, which generated an 1100-bp

band, and LA4082 was separated out (Figure 3B). Lastly, LA4038 and LA2838A were divided by primer Y27 with a specific band of 1900 bp (Figure 3C). In terms of the CID, the other 4 cultivars in group 2 (LA4065, LA4030, LA2413, and Suhong 2003) could be gradually distinguished using primers Y53, Y28, and Y39. In a manner similar to that described above, LA4065, LA4030, LA2413, and Suhong 2003 were effectively separated by the specific 1600-bp (Figure 3D), 700-bp (Figure 3E), and 600-bp (Figure 3F) bands.

The results clearly show that the 8 randomly selected cultivars were verified in excellent agreement with the CID. Following this method, all 64 cultivars could be accurately identified using the CID and 8 polymorphic primers and their product-specific bands, which proved the suitability and efficiency of the CID method for tomato cultivar identification.

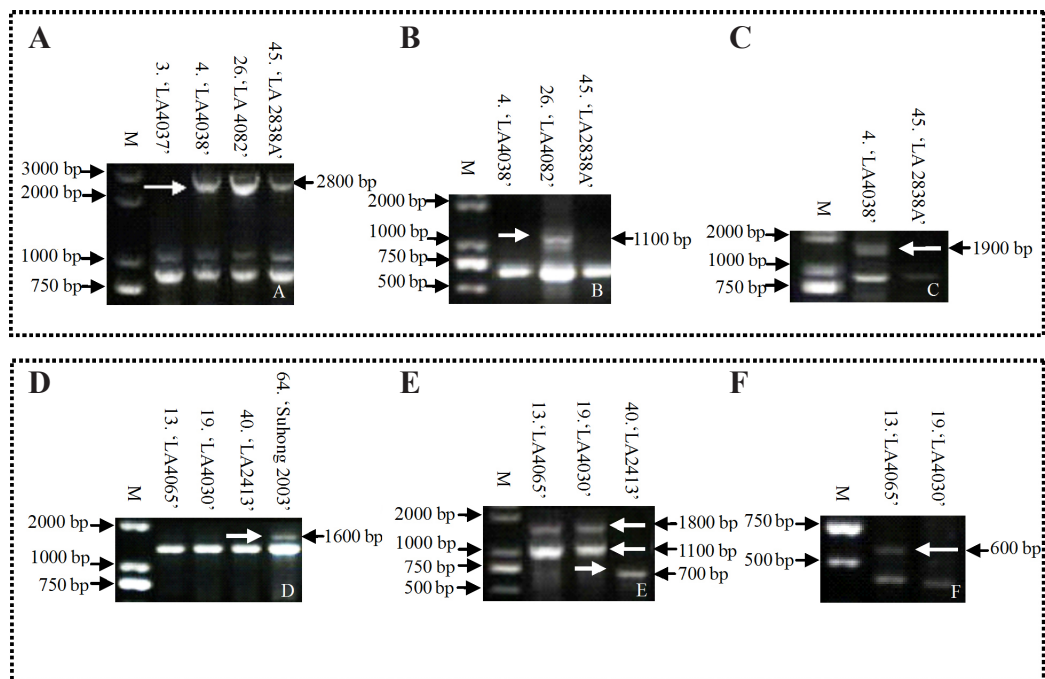


Figure 3. RAPD profiles for randomly selected tomato cultivars by corresponding primers. Lane M = DL2000 plus marker. Group 1 includes A, B, and C. **A.** DNA banding patterns obtained with primer Y42. **B.** DNA banding patterns obtained with primer Y33. **C.** DNA banding patterns obtained with primer Y27. Group 2 includes D, E, and F. **D.** DNA banding patterns obtained with primer Y53. **E.** DNA banding patterns obtained with primer Y28. **F.** DNA banding patterns obtained with primer Y39. The specific bands are indicated by white arrows.

Diversity analysis using clustering analysis based on RAPD

To detect the genetic diversity among tomato cultivars and further verify the accuracy of the CID method described herein, clustering analysis was also established and used. Twenty polymorphic fragments from the 8 primers were scored as follows: 1 for presence or 0 for absence. The obtained data were used to calculate genetic distances using the NTSYS-pc program (Rohlf, 2000; version 2.11). The results indicated that genetic distances between the 64

tomato cultivars ranged from 0.032 to 1.402, with the highest and the lowest values of 1.402 between LA4099 and LA1777 and 0.032 between LA3920 and Zhongza No. 9, respectively. The large genetic distance difference that existed between cultivars reflects the wide range of genetic variations in the 64 tomato cultivars.

A dendrogram based on UPGMA analysis using RAPD markers was constructed (Figure 4), showing that the 64 tomato cultivars formed 4 distinct clusters with a similarity coefficient of 0.40. Cluster I was the largest group and consisted of 50 tomato cultivars, while Cluster II contained 11 cultivars, Cluster III included 2 wild tomato cultivars, LA1598 (35) and LA1777 (38), and Cluster IV included an individual cultivar, LA4096 (29).

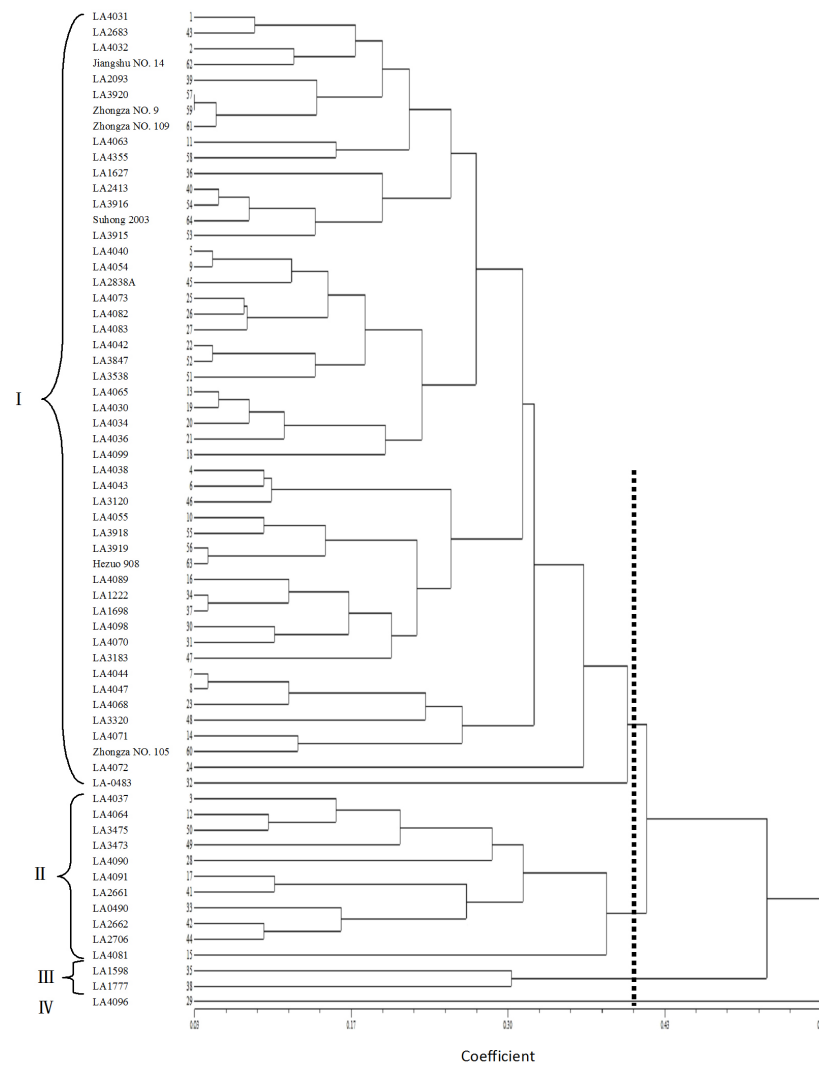


Figure 4. Dendrogram of 64 tomato cultivars based on RAPD marker data and generated from Nei's genetic distance matrix using UPGMA in NTSYSpc 2.11.

DISCUSSION

The molecular characterization of cultivars is essential initial work for breeding, germplasm research, and seed sales in the commercial sector. In recent years, many molecular marker techniques based on DNA fingerprinting have been widely used in cultivar identification (Saker et al., 2006; Chiu et al., 2010) and genetic analyses (Boronnikova et al., 2007; Silvestrini et al., 2008; Bhau et al., 2009; Baysal et al., 2010). Because of the simple, rapid, and highly sensitive nature of the RAPD assay, it is fast becoming the most commonly used method for the molecular characterization of cultivars. In general, the application of RAPD markers in cultivar identification and genetic diversity detection includes the following procedure: first, a large number of primers are required for RAPD-PCR amplification and then electrophoresis; second, polymorphic fragments are scored using a binary matrix; third, genetic distance and clustering analysis with the aid of software are used to analyze genetic diversity; and finally, the results are output as a digital image. However, the identification process is masked in the sense that it is difficult to confirm which primer can be used to distinguish the target cultivar from others. Based on diversity analysis among given cultivars, when new cultivars are added, all primers used in the previous analysis need to be applied to the new cultivar for amplification, and these new data will be used to supplement the original work. In addition, the diversity analysis among all cultivars will be recalculated.

Although RAPD markers have been used for diversity analysis, they are not well managed. CID is a novel strategy for cultivar identification that transforms DNA fingerprinting into diagram information that is marked during the identification process. Therefore, this technique is regarded as one of the most intuitive forms of DNA fingerprinting application. As previously mentioned, CIDs have been successfully used in several fruit crop cultivars, such as sweet orange, pear, grapevine, loquat, and apple (Lin et al., 2011; Zhang et al., 2011; Zhao et al., 2011; Sun et al., 2012; Wang et al., 2012). In this study, the CID approach was first adopted to identify tomato cultivars, and then the entire appraisal process was recorded by CID, which provides information pertaining to the polymorphic primer and specific bands that were used for identification (Figure 2).

As opposed to reported molecular marker-based characterization for cultivar classification, the CID method overcomes the previously described deficiencies. For instance, fewer primers are used in the CID approach. In this paper, only 8 primers were needed to completely separate 64 tomato cultivars, using less time and work than other methods. Also, the CID exhibited better repeatability and reliability. In addition, the most obvious advantage in favor of the CID method is that the evaluation process shows detailed identification information in a diagrammatic format. From the CID of the 64 tomato cultivars, we can easily identify the exact primers that were used to distinguish the target cultivar from others. Furthermore, any randomly selected cultivars from the original 64 cultivars could be distinguished directly by the primers reported in the CID. For example, LA4072 is first separated from other tomato cultivars using primer Y42 in the CID; therefore, to distinguish LA4072, the Y42 primer is sufficient. However, according to genetic distance and clustering analysis, all primers should be used to amplify the cultivars to be detected; then, they can be compared by clustering analysis for further identification. Another advantage of CID is that it is not limited to the existing cultivars; when new tomato cultivars need to be identified, the 8 primers will be initially considered for use. If the 8 primers can completely separate these variants, the process can be directly added into the original CID. If this cannot be done, more primers will be screened

and used for further identification, and then the new data will be used to supplement the CID.

In the research described herein, 8 primers were utilized to amplify 64 tomato cultivars. According to the 8 primers and the specific bands that were subsequently generated, a tomato CID was constructed. In addition, genetic diversity analysis was done using the same RAPD profiles. After RAPD-PCR amplification and electrophoresis, polymorphic fragments were scored as either 0 or 1, and genetic distance and clustering analysis were performed by software. In this study, genetic relationships among cultivars could be clearly clarified by clustering analysis; cultivars with a close genetic relationship fall into the same cluster, otherwise, they fall into different clusters. CIDs can also be used to identify genetic relationships. Usually, it is relatively easy to use CID to distinguish distant genetic relationships; in contrast, however, a close genetic relationship often requires more primers. In this study, clustering analysis (Figure 4) placed LA3920 and Zhongza No. 9 in the same cluster, which suggested that they shared a very close genetic relationship. In the CID (Figure 2), we found that 7 RAPD primers were needed to separate LA3920 from Zhongza No. 9, suggesting that these 2 cultivars shared a similar genetic background. Similarly, to distinguish between LA4065 and LA4030, we also needed to use 7 RAPD primers in the CID (Figure 2), and clustering analysis showed that LA4065 and LA4030 were in the same cluster (Figure 4). These data suggest that there is excellent agreement between the CID and clustering analysis.

CID is an effective strategy for cultivar identification with features including simplicity, speed, and reliability. However, it is not without certain limitations. For instance, when cultivars need to be identified from the constructed CID, we can utilize the specific primers in the CID to distinguish the cultivars with fewer steps. However, for new cultivars that are not included in the CID, existing primers will be used, but if this is not sufficient, more primers will be screened until all cultivars are completely separated. Although CID can be used to estimate the general genetic diversity among cultivars, it cannot provide precise, accurate analysis. In this study, CID could accurately identify different cultivars, but it only offered a general judgment of the genetic diversity among cultivars.

In conclusion, this study proposes a novel strategy using the same RAPD profiles to simultaneously construct a CID and clustering analysis of 64 tomato cultivars. Compared to previously used methods, this novel strategy appears more convenient, efficient, and rapid for systematically understanding cultivar category and genetic diversity among tomatoes.

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