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Identification and Genetic Diversity of Korean Tomato Cultivars by RAPD Markers

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Cultivated tomato, *Lycopersicum esculentum*, is a very important crop. We selected 36 cultivars and studied them for identification and polymorphism by employing random amplified DNA (RAPD) analysis with 80 oligonucleotide primers. Of the 80 primers, 36 primers (45.0%) were polymorphic. Detection of polymorphism in cultivated tomato opens up the possibility of development of its molecular map by judicious selection of genotypes. Molecular markers can also be used for cultivar identification and protection of the plant breeder's intellectual property rights (plant breeders' rights, PBRs). As an example, DNA polymorphism using OPC-13 primer that did not produce the OPC-13-01 band was only found in Junk Pink and Ailsa Craighp cultivars. OPA-12-03 and OPB-15-07 were fragments specific to the TK-70 cultivar and were absent in other cultivars. DNA polymorphism in cultivated tomato in this study was correlated with a type of inflorescence, although some cultivars had exceptions. These approaches will be useful for developing marker-assisted selection tools for genetic enhancement of the tomato plant for desirable traits.

Key words : Tomato, *Lycopersicum esculentum*, cultivar, random amplified DNA (RAPD)

Introduction

Progress in plant breeding, genetics, and molecular biology combined with an enhanced concern for both ex-situ and in-situ preservation of biodiversity [7]. The progression has greatly increased the responsibility and visibility of those charged with long-term conservation and the use of plant genetic resources [2]. It is very important to define identity, purity, and stability of varieties for breeder's rights protection as well as for an effective seed quality control program [11,13]. Part of the solution to this challenge may lie in the use of powerful, yet relatively simple and inexpensive, molecular techniques to generate information to better organize the useful genetic variation present within a collection or cultivars [5].

Cultivated tomato, *Lycopersicum esculentum* Miller (Solanaceae), is a very important crop due to high value of its fruits for fresh consumption. The tomato is a herbaceous, usually sprawling plant in the Solanaceae or nightshade family that is typically cultivated for the purpose of harvesting its fruit for human consumption. The taxonomy keys, interior of ripe fruit red, fruit diameter, leaves subdivided, internodes short, densely pubescent, and large accresant calyx are a revision to that formulated by Rick *et al.* [18] and published in the Tomato Genetics Cooperative. Genotypic differences within tomato cultivars detected by molecular markers can also be used for cultivar identification and protection of the plant breeder's intellectual property rights (plant breeders' rights, PBRs). Data reported in all the studies dealing with the application of DNA markers in tomato cultivar identification are useful and provide important background information to address the issue of PBRs [4,14].

In this study thirty-six cultivars of tomato from Korea were analyzed for RAPD (random amplified polymorphic DNA) markers. RAPD assay has been useful in determining genetic relationships among closely related species [5]. RAPD analysis is quick, robust, requires minimal preliminary work [12,15].

We expected that the RAPD analyses assess the amount and structure of genetic diversity among cultivars of tomato in Korea. Lee *et al.* [9] studied the relationships of some cultivars of cherry tomato in Korea. However, the genetic diversity and characteristics of many cultivars of tomato in Korea has not been studied [8]. The basic question is it possible to detect the identification of cultivars using RAPD makers.

Materials and Methods

Plant materials and preparation of DNA

A collection of tomato cultivars was showed in Table 1. Total

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Table 1. Characteristics of 36 selected tomato cultivars used in this study

No.	Cultivar	Inflorescence	Type of pistil	Type of fruits	Color of fruit	No. of locule
1	ABC	Determinate	Syn	RO	Pink	3-4
2	Calmart	Determinate	Syn	RO	Pink	3-4
3	Chico III	Determinate	Syn	LC	Pink	2-3
4	430-2-21-2sm	Indeterminate	Syn	SF	CL	8-9
5	Cal-J	Determinate	Syn	RO	Pink	3-4
6	Heinz 1370	Determinate	Uni	SF	Pink	5-6
7	Nova	Determinate	Syn	LC	Pink	3
8	Quick Pick	Determinate	Syn	PS	CL	2-3
9	TK-70	Determinate	Syn	RO	Pink	5
10	Venus	Indeterminate	Syn	SF	Pink	4-5
11	Ven 8	Determinate	Syn	RO	Pink	7-8
12	Walter	Determinate	Syn	RO	Pink	4-5
13	Hongjin 10	Indeterminate	Uni	RO	CL	3-4
14	Hongjin 12	Determinate	Syn	RO	CL	6
15	73-8	Indeterminate	Syn	SF	CL	4-5
16	FTvR-30	Indeterminate	Syn	RO	CL	5-6
17	Ohio MR-12	Indeterminate	Syn	SF	CL	3
18	Extase ABC	Indeterminate	Uni	RO	Pink	2-3
19	June Pink	Indeterminate	Syn	SF	CL	8-10
20	Manarucie	Indeterminate	Uni	RO	Pink	5
21	Money marker ABC	Indeterminate	Uni	RO	Pink	2-3
22	Niagara VF	Indeterminate	Syn	RO	Pink	5
23	Ponderose	Indeterminate	Syn	SF	Pink	9-11
24	Ailsa Craighp	Indeterminate	Uni	RO	Pink	2
25	Ace 55VE	Determinate	Syn	RO	Pink	6
26	Divisoria	Determinate	Syn	LC	Pink	4
27	Earliana	Indeterminate	Syn	SF	CL	5-7
28	GCR 26	Indeterminate	Syn	RO	Pink	2-3
29	JB-T-400	Indeterminate	Syn	SF	Pink	4-5
30	Jubi	Indeterminate	Syn	RO	Pink	5-6
31	New Yorker	Determinate	Syn	RO	Pink	4-6
32	NFR-1	Indeterminate	Syn	SF	Pink	5-7
33	Out door	Indeterminate	Uni	SF	CL	2
34	Plex	Indeterminate	Syn	RO	Pink	3-4
35	Sioux	Determinate	Syn	SF	Pink	7-9
36	Yangja	Determinate	Syn	RO	CL	5-6

Syn: Syncarpous, Uni: Unicarpellate, RO: Round, LC: Lentgthened cylindrical, SF: Slightly flattened, CL: Colorless.

genomic DNA was extracted from approximately 1.0 g of young leaves using the plant DNA Zol Kit (Life Technologies Inc., GrandIsland, New York, U.S.A.) according to the manufacturer's protocol. DNA was suspended in TE buffer and stored at -20°C.

RAPD analysis

RAPD analysis was performed using 80 different 10-base oligonucleotide primers (OPA-01~20, OPB-01~20, OPC-01~20, and OPD-01~20, series) purchased from Operon Technologies (Alameda, CA). A mixture contained 20 ng of template DNA, 0.5 pmol of a random primer, 10x PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), 3.0 mM $MgCl_2$, 2.5 μM each dNTP, and 1.0 U *Taq* DNA polymerase. Amplification was performed in a Gene Amp PCR System 9700 (Perkin Elmer-Applied Biosystems), which was programmed for initially denatured at 94°C for 30 sec, followed by 40 cycles of 40 sec of denaturation at 94°C, 40 sec of annealing at 40°C and 60 sec of primer extension at 72°C, then finally incubated at 72°C for 7 min. The amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light using Polaroid 667 film. A 100 bp ladder DNA marker (Pharmacia) was used in the end of gels for the estimation of fragment size. Because RAPD markers had a disadvantage of reproduction, all experiments were done twice.

Statistical analyses

All RAPD bands were scored manually and only unambiguously scored bands were used in the analyses. In addition, replicate cultivars were assayed in separate experiments to verify repeatability of results. Because RAPD bands are dominant markers, it was assumed that each band corresponded to a single character with two alleles, presence (1) and absence (0) of the band.

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh *et al.* [22]: the percentage of polymorphic loci (P_p) mean numbers of alleles per locus (A), effective number of alleles per locus (A_E), and gene diversity (H) [16].

A phenetic relationship was constructed by the neighborjoining (NJ) method [19] using the NEIGHBOR program in PHYLIP version 3.57 [6].

Results

The 36 tomato cultivars were studied using 80 oligonecleotide primers for RAPD analysis. From a number of 80 primers, 44 primers did not give any clear-cut bands, or showed only smeared bands. The results with these primers were omitted from the analyses, and RAPD markers amplified 36 primers (45.0%) were analyzed. The number of bands generated by each primer varied from 2 (OPC-10) to 11 (OPC-06), with an average

Table 2. List of decamer oligonucleotide utilized as primers, their sequences, and associated fragments

Primer	Sequence (5′ to 3′)	No. of fragments detected	No. of *P*p
OPA-04	AATCGGGCTG	7	4
OPA-07	GAAACGGGTG	4	4
OPA-08	GTGACGTAGG	7	7
OPA-12	TCGGCGATAG	5	3
OPA-13	CAGCACCCAC	7	4
OPA-18	AGGTGACCGT	10	7
OPA-19	CAAACGTCGG	7	3
OPA-20	GTTGCGATCC	4	1
OPB-01	GTTTCGCTCC	5	1
OPB-02	TGATCCCTGG	3	1
OPB-05	TGCGCCCTTC	8	7
OPB-06	TGCTCTGCCC	6	5
OPB-08	GTCCACACGG	6	1
OPB-10	CTGCTGGGAC	4	2
OPB-15	GGAGGGTGTT	7	3
OPB-16	TTTGCCCGGA	3	1
OPB-17	AGGGAACGAG	6	6
OPB-20	GGACCCTTAC	3	3
OPC-05	GATGACCGCC	8	2
OPC-06	GAACGGACTC	11	3
OPC-08	TGGACCGGTG	8	4
OPC-10	TGTCTGGGTG	2	0
OPC-11	AAAGCTGCGG	7	0
OPC-13	AAGCCTCGTC	4	2
OPC-14	TGCGTGCTTG	6	1
OPC-15	GACGGATCAG	7	1
OPC-18	TGAGTGGGTG	9	3
OPC-19	GTTGCCAGCC	9	6
OPC-20	ACTTCGCCAC	10	0
OPD-02	CGACCCAACC	10	2
OPD-07	TTGGCACGGG	4	0
OPD-08	GTGTGCCCCA	10	1
OPD-09	CTCTGGAGAC	5	3
OPD-11	AGCGCCATTG	4	1
OPD-13	GGGGTGACGA	4	1
OPD-20	ACCCGGTCAC	8	1
Total		228	94

of 6.3 bands per primer. The size of the amplified DNA fragments varied from 340 bp to 3.0 kbp. Totally 228 DNA fragments (bands) were found among 36 cultivars. Among of these 228 bands, 92 (40.4%) bands were polymorphic. In a simple measure of intracultivars variability by the percentage of polymorphic bands, the Earliana accession exhibited the lowest variation (8.6%). Otherwise the TK-70 cultivar showed the highest (37.4%) (Table 3). Statistical analysis showed that mean number of alleles per locus (A) ranged from 1.086 to 1.374 with a mean of 1.201, while the effective number of alleles per locus (A_E) ranged from 1.056 to 1.335. The phenotypic frequency of each band was calculated and used for estimating genetic diversity (H) with in cultivars. The cultivated tomato cultivars maintained a low level of genetic diversity for polymorphic primers. The total H was 0.085 across cultivars. Shannon's index of phenotypic diversity (I) of Korean tomato cultivars ranged from 0.048 to 0.204 with a mean of 0.123.

The amplification products observed of 36 cultivars using primer OPC-11 (Fig. 1), exhibited the monomorphic pattern of lack of DNA polymorphism in cultivated tomato. On the other hand OPA-08, OPA-12, and OPB-17 exhibited the useful patterns of polymorphism in cultivars.

Table 3. Measurements of genetic variation for cultivars of 36 selected tomato cultivars

Cultivar	Np	Pp	A	A_E	H	I
ABC	60	27.3	1.270	1.215	0.117	0.168
Calmart	58	26.1	1.261	1.216	0.116	0.165
Chico III	59	26.7	1.266	1.211	0.114	0.164
430-2-21-2sm	70	31.5	1.315	1.256	0.137	0.197
Cal-J	72	32.4	1.324	1.267	0.143	0.204
Heinz 1370	42	18.9	1.189	1.131	0.075	0.110
Nova	57	25.7	1.257	1.226	0.119	0.168
Quick Pick	79	35.6	1.356	1.311	0.164	0.231
TK-70	83	37.4	1.374	1.335	0.175	0.246
Venus	42	18.9	1.189	1.134	0.078	0.114
Ven 8	48	21.6	1.216	1.153	0.090	0.131
Walter	44	19.8	1.198	1.156	0.085	0.122
Hongjin 10	52	23.4	1.234	1.166	0.097	0.142
Hongjin 12	55	24.8	1.248	1.215	0.113	0.161
73-8	61	27.5	1.275	1.196	0.110	0.161
FTvR-30	47	21.2	1.212	1.190	0.099	0.140
Ohio MR-12	49	22.1	1.221	1.180	0.097	0.138
Extase ABC	54	24.3	1.243	1.192	0.104	0.150
June Pink	33	14.9	1.149	1.109	0.061	0.088
Manarucie	40	18.0	1.180	1.132	0.074	0.107
Money marker ABC	41	18.5	1.185	1.126	0.072	0.107
Niagara VF	36	16.2	1.162	1.119	0.066	0.097
Ponderose	26	11.7	1.117	1.083	0.049	0.071
Ailsa Craighp	38	17.1	1.171	1.118	0.067	0.099
Ace 55VE	38	17.1	1.171	1.121	0.068	0.100
Divisoria	34	15.3	1.153	1.099	0.058	0.086
Earliana	19	8.6	1.086	1.056	0.032	0.048
GCR 26	21	9.5	1.095	1.059	0.035	0.053
JB-T-400	34	15.3	1.153	1.103	0.060	0.088
Jubi	32	14.4	1.144	1.097	0.056	0.083
New Yorker	24	10.8	1.108	1.082	0.045	0.065
NFR-1	38	17.1	1.171	1.114	0.066	0.097
Out door	31	14.0	1.140	1.093	0.054	0.080
Plex	29	13.1	1.131	1.094	0.053	0.077
Sioux	28	12.6	1.126	1.078	0.046	0.069
Yangja	34	15.3	1.153	1.106	0.060	0.089
Mean	44.7	20.1	1.201	1.154	0.085	0.123

The number of polymorphic loci (Np), percentage of polymorphism (Pp), mean number of alleles per locus (A), effective number of alleles per locus (A_E), gene diversity (H), and Shannon's information index (I).

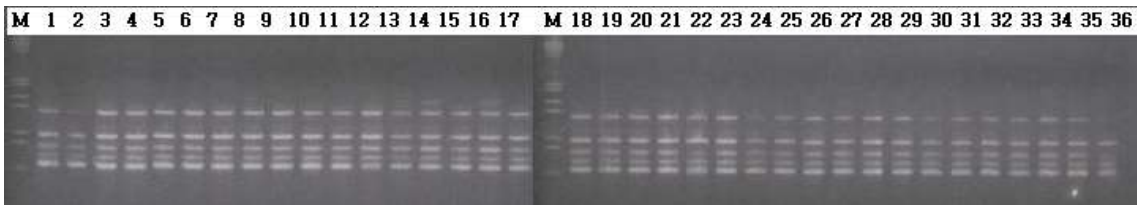

Fig. 1. DNA bands obtained from a collection of 36 tomato cultivars amplified with OPC-11 primer. M: Molecular weight of standard.

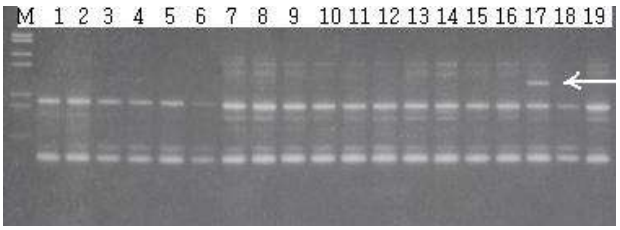

Fig. 2. Ideogram of cultivar-specific DNA fragments obtained by RAPD OPA-12 primer. The arrow indicates the unique band that was produced a 1500 bp DNA fragment in the Ohio MR-12.

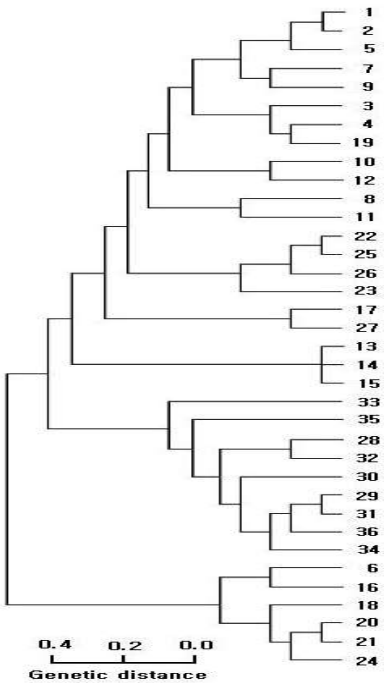

Fig. 3. Dendrogram of the 36 Korean tomato cultivars listed in Table 1, based on RAPD analysis.

As an example, the presence of DNA polymorphism using OPC-13 primer that did not produced the OPC-13-01 band only in Junk Pink and Ailsa Craighp cultivars.

OPA-20-03 and OPB-15-07 fragments are specific for TK-70 cultivar, whereas no products were detected in individuals from other cultivars. OPA-12-01 was specific for Ohio MR-12 cultivar (Fig. 2), OPB-08-03 and OPD-08-06 for Niagara VF, OPC-08-02 for Ponderose, and OPC-14-04 for Ven 8. These specific DNA fragments seemed to be useful to discriminate among cultivars and were used to develop the SCAR (sequence-characterized amplified region) markers.

Some cultivars could not be differentiated and twelve out of 34 nodes in the dendrogram were supported by bootstrap values of less than 50% (Fig. 3). However, three cultivars, Honggjin 10, Honggjin 12, and 73-8 were not separated by RARD primers in this study.

Discussion

Among 228 bands were obtained, a number of 92 (40.4%) bands of 36 tomato cultivars were polymorphic. The levels of genetic variation found within Korean cultivars of tomato were low in relation to the mean value of results in same species. Some workers reported similar results in cultivated tomato [10,14]. The lack of polymorphism has also been reported in other self-pollinated crops such as soybean, potato, and ground nut [7,11,20]. Such insufficient variability in DNA polymorphism makes it difficult to construct a genetic map, if using populations derived from across between cultivars [2]. A low level DNA polymorphism in cultivated tomato was also reported using AFLP (amplified fragment length polymorphisms) and SSR (simple sequence repeats) primer pairs [14].

RAPDs are a convenient and effective marker system for tomato species identification [2]. The RAPD analysis was chosen for the present study since the procedures involved were simple, not requiring probes or Southern-blot hybridization, as in the case of RFLP (restriction fragment length polymorphism). The amplification is reproducible by sticky adhering to the specific protocols though RAPD marker system has certain disadvantages such as reproducibility [21].

Out of the 228 bands, only seven fragments are specific for one cultivar. The efficiency to find a RAPD marker useful for purity determination was 3.1%. This value is very low than those of in pepper, tomato hybrids, and Sonanaceous species [1,17]. It is proposed that similar lineages from parents and bulks are used to our experiments.

The 36 tomato cultivars were chosen for the present study

a broad spectrum of variation for several morphological and agricultural traits. The characteristics of 36 cultivars that show greater diversity for inflorescence, type of pistil, color of fruits, and numbers of locules for tomato are given in Table 1. For example the demonstration of DNA polymorphism in many cultivated tomato in this study is correlated with type of inflorescence, although some cultivars have exceptions. The observation of polymorphism could be attributed in selection of cultivars with diverse characteristics including hybridization lines, as well as to specific primers used in the RAPD analysis. These bands will be useful for developing mapping cultivars. However, dendrograms obtained with RAPD marker systems did not indicate group of cultivars according to their morphological types. Thus, associating polymorphic DNA fragments with different traits requires further study using the selected trait-specific cultivars and their progenies.

In conclusion, the present study indicated the presence of DNA polymorphism in cultivated tomato using RAPDs. This open up a possibility for developing a molecular genetic map that will lead to the application of marker-assisted selection tools in genetic enhancement of cultivated tomato.

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초록 : 한국 내 토마토 재재종의 RAPD에 의한 동정과 유전적 다양성

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재배종 토마토(*Lycopersicum esculentum*)는 중요 작물의 하나이다. 36 재배종에 대해 80개 RAPD (random amplified polymorphic DNA) 마커로 동정과 다형성을 조사하였다. 80개 마커 중 36개(45.0%)는 다형성을 나타내었다. 재배종 토마토에서 다형성의 탐지는 유익한 유전자형의 선택에 의한 분자 지도 발전 가능성을 제공할 수 있다. 분자 마커는 역시 식물 육종 지적 권리(PBRs)의 동정과 보호를 위해 사용될 수 있다. 한 예로써 OPC-13 시발체를 사용한 DNA 다형은 Junk Pink와 Ailsa Craighp 품종은 OPC-13-01 밴드가 결여되어 있다. OPA-12-03와 OPB-15-07는 TK-70 품종에 특이 마커로 다른 품종에는 없다. 본 연구의 재배종 토마토에서 DNA 다형은 일부 예외는 있지만 개화 타입과 관련이 있다. 이런 접근은 바람직한 토마토 품종 육성에 유전적 정보를 높이는데 유익할 것으로 사료된다.