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Molecular diversity and phylogentic analysis of *Capsicum annuum* varieties using the nrDNA ITS region

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ABSTRACT

The genus *Capsicum* with its special flavour has been cultivated as a vegetable crop and spread throughout the American, African and Asian tropics. Because the genus *Capsicum* has various species and varieties, the clear species discrimination is always a focal point of research. In this study, analysis of phylogenetic relationship and evolution was been evaluated based on the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA). The ITS1-5.8S-ITS2 region was successfully amplified from 26 *Capsicum annuum* varieties and 4 *C. eximium* varieties using ITS universal primers. Results showed that the ITS1 region ranged from 244 bp to 251 bp, and (G + C) content (%) ranged from 38.89% to 58.33%; the 5.8S region ranged from 147 bp to 166 bp, and (G + C) content (%) ranged from 36.59% to 45.58%; and the ITS2 region ranged from 220 bp to 225 bp, and (G + C) content (%) ranged from 53.39% to 62.95%. According to the sequence alignment result, the highest dissimilarity rate among *C. annuum* varieties appeared between Coletti 2 and E41 EnZa, while among *Capsicum eximium* varieties, the highest dissimilarity rate appeared between Dangjo Gochu-1 and E49.9531-1. In the phylogenetic tree, *C. annuum* and *C. eximium* varieties formed one independent group by themselves, sharing only 79% identity with each other. Two subgroups were further separated in the *C. annuum* variety group. This result suggests that ITS region is efficient in the *Capsicum* phylogeny analysis. This work helps us further understand the phylogenetic relationship of *Capsicum* species.

1. Introduction

Paprika (*Capsicum* spp.) is an important spice and vegetable crop in China, Korea, Japan, Mexico, Turkey, Spain and USA, where several types of this crop exhibiting considerable morphological variations are grown (Paran et al., 1998). There are 20–30 species in the genus *Capsicum*, of which five are domesticated including the species *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinensis* Jacq, *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz et Pav and approximately 22 are wild and endemic to the American tropics (Portis et al., 2007). Among the domesticated species, *C. annuum* is the most widespread popular and important species around the world. However, just in the *C. annuum*, there are many different varieties, such as large-fruited bell fruits, small pungent types, chilies and other types differing in flower and fruit colour, shape, size and taste. To understand the evolutionary relationships among *Capsicum* species and within *Capsicum* species, the relationships between some morphological or biochemical character and genetic variation, researchers have attempted many investigations (Pickersgill, 1988; Loaiza-Figueroa et al., 1989; Collera-Zúñiga et al., 2005). However, the level of polymorphism for morphological characteristics in genotypes is sometimes so limited that it is inadequate to allow varietal identification and purity determination (Ilbi, 2003).

Nowadays, molecular identification of PCR-based methods sharing more advantages compared to traditional identification is believed to be a reliable alternative tool for accurate authentication (Joshi et al., 2004). To understand the phylogenetic relationships among paprika cultivars, the effectiveness of PCR-based DNA markers has also been evaluated based on the polymorphism, such as randomly amplified polymorphic DNA (RAPD),

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Table 1
Specimen voucher, colour, shape and size of pepper fruit, virus resistance, and plant vigour of *Capsicum annuum* varieties used in this study and their accession numbers in NCBI GenBank database.

Number	Varieties	Specimen voucher	Colour	Shape	Size (mm)	Virus resistance	Plant vigour	Accession number
Caspicum annuum								
1	Dangjo Gochu	P26	Green					JQ885413
2	E49.9524	P10	Red	Mini sweet conical	Dia 25-30	HR Tm:0	S	JQ885414
3	Nokwang	P9	Green					JQ885415
4	E49.9526	P8	Yellow	Mini sweet conical	Dia 25-30	HR Tm:0	S	JQ885416
5	E49.9531	P7	Orange	Mini sweet conical	Dia 25-30	HR Tm:0	S	JQ885417
6	Cupra 1	P6	Red	Blocky	Dia 75-80	HR Tm:0-2	S	JQ885418
7	R.Z.208	P1	Yellow	Blocky		HR Tm:0-2		JQ885419
8	Coletti 1	P2	Yellow	Blocky	Dia 80-85	HR Tm:0-3	MS	JQ885420
9	Scirocco 1	P3	Red	Blocky	Dia 80	HR Tm:0-3	S	JQ885421
10	Magno	P27	Orange	Blocky	Dia 85+	HR Tm:0-2	MS	JQ885422
11	Veyron	P17	Red	Blocky		HR Tm:0-3		JQ885423
12	Boogie	P19	Orange	Blocky		HR Tm:0-2		JQ885424
13	Orange glory	P18	Orange	Blocky		HR Tm:0-3		JQ885425
14	Fiesta 1	P28	Yellow	Blocky	Dia 75-80	HR Tm:0-2	MS	JQ885426
15	Scirocco 2	P4	Red	Blocky	Dia 80	HR Tm:0-3	S	JQ885427
16	E41 EnZa	P5	Yellow	Blocky		HR Tm:0-3		JQ885428
17	Piment	P22	–					JQ885429
18	Fiesta 2	P23	Yellow	Blocky	Dia 75-80	HR Tm:0-2	MS	JQ885430
19	Rudongfu	P24	Orange					JQ885431
20	Maratos	P25	Red	Blocky	Dia 80+	HR Tm:0-2	S	JQ885432
21	Ferrari	P21	Red	Blocky	Dia 75-85	HR Tm:0-2	S	JQ885433
22	Coletti 2	P13	Yellow	Blocky	Dia 80-85	HR Tm:0-3	MS	JQ885434
23	Derby	P20	Yellow	Blocky		HR Tm:0-2		JQ885435
24	Cupra 2	P16	Red	Blocky	Dia 75-80	HR Tm:0-2	S	JQ885436
25	Fieho	P11	Yellow	Blocky		HR Tm:0-2		JQ885437
26	Mazzona	P12	Orange	Blocky	Dia 80	HR Tm:0-2	S	JQ885438
Capsicum eximium								
27	Dangjo Gochu-1	P1	Green					JQ885439
28	E49.9524-1	P2	Red	Mini sweet conical	Dia 25-30	HR Tm:0	S	JQ885440
29	E49.9531-1	P5	Orange	Mini sweet conical	Dia 25-30	HR Tm:0	S	JQ885442
30	Cupra	P6	Red	Blocky	Dia 75-80	HR Tm:0-2	S	JQ885443

Non-filled means indeterminate. In size (mm) column, 'Dia' indicates diameter. In Virus resistance column, 'HR' and 'Tm' level indicate the resistant degree (high resistance) and resistant virus types. Tm:0, 1, 2, and 3 mean that paprika variety is resistant to pepper mild mottle virus, tobacco mild green mosaic virus, tobacco mosaic virus, and tomato mosaic virus, respectively. In Plant vigour column, 'S' and 'MS' indicate strong and mediate strong, respectively.

restriction fragment length polymorphism (RFLP), and/or inter-simple sequence repeat (ISSR) (Prince et al., 1992, 1995; Lefebvre et al., 1993; Paran et al., 1998; Kumar et al., 2001, 2007; Lefebvre et al., 2001; Thul et al., 2012). These studies demonstrated that the level of variation among domesticated peppers is sometimes lower than that among wild peppers, and that the variation among the large-fruited peppers is limited compared with that among the domesticated pungent peppers (Paran et al., 1998). In addition, much *Capsicum* breeding has been carried out mostly concerning *C. annuum* (Pickersgill, 1997), and the breeding populations have been involved extensively that particularly hybrid varieties increasingly trend to narrower genetic variation. Consequently, the discrimination of varieties from each other and understanding of their genetic diversity within populations becomes more difficult and more urgent.

Applying DNA barcoding for species identification has been considered as a forensic tool in many plant species (Schindel and Miller, 2005). Since the concept of applying DNA barcoding for the identification of global species was first proposed, considerable debate has been achieved. According to the Barcode of Life data systems (http://www.boldsystems.org), there have been more than 1,100,000 DNA barcode records of 95,000 species as of February 2011. In NCBI GenBank database (http://www.ncbi.nlm.nih.gov), there have been over 180,000 DNA barcode records of plants as of February 2011. Among these DNA barcodes, the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) comprising the ITS1 intergenic spacer, 5.8S ribosomal RNA (rRNA), and the ITS2 intergenic spacers (ITS1-5.8S-ITS2), is the most commonly used region (Coleman, 2003; Sun and Hong, 2011; Sun et al. 2011). Because of its high level of variation and species discrimination, the ITS region has been considered as a marker suitable for taxonomic classification over a wide range of levels (Coleman, 2003). In the present study, we used the nrDNA ITS region as a more efficient and more stable approach for paprika variety identification and examined the level of variation among different varieties of *C. annuum*. We also evaluated the relative effectiveness of this DNA marker in revealing variation among closely related cultivars. This work would provide a more convenient, more rapid approach for receiving more variable genetic information among *C. annuum* varieties.

2. Materials and methods

2.1. Plant materials

Thirty paprika varieties (26 belonging to *C. annuum* and 4 belonging to *C. eximium*) existing different morphological characteristics investigated in this study were provided by Department of Horticulture, Kangwon National University, Korea. The *C. annuum* materials covering all varieties of this species in Korea and *C. eximium* materials, were grown under hydroponic systems in automatic control plastic greenhouse. When the seedlings grew up large enough, they were moved to the main cultivation area of the greenhouse. Fresh mature leaves were sampled from these paprika varieties and immediately stored in liquid nitrogen condition. Their specimens and relevant information listed here have been deposited in the National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). The NCBI GenBank