

## Discovery of New Diploid *Perilla* Species in Korea

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### Abstract

Recently diploid ( $2n=20$ ) wild type *Perilla* species was reported from Japan. We collected several wild type *Perilla* in Jeju island, Korea. Two collections, Jeju-3 and Jeju-17, could be classified as *P. citriodora* and *P. hirtella*, respectively. Their bract color and type of serration at leaf base are agreed with the classification. Collections and their corresponding species shared specific RAPD markers which distinguished them from other species.

### Media summary

In this paper we report new wild type *Perilla* species in Korea

### Key Words

*Perilla*, *frutescens*, *citriodora*, *hirtella*

### Introduction

*Perilla* (*Perilla frutescens* Brit.) is widely cultivated as an oil seed and vegetable crop in Korea and other Asian countries. The species includes two varieties that are differentiated on the basis of their morphology and dual uses. *Perilla frutescens* var. *frutescens* is used as an oil crop (Ren in chinese, Deulggae in Korean and Egoma in Japanese). The other is *P. frutescens* var. *crispa*, a Chinese medicine or vegetable crop (Zisu in chinese, Cha-jo-ki in Korean and Shiso in Japanese). Since *P. frutescens* has been extensively cultivated and used in East Asia, the origin of *Perilla* crops has generally been considered to be East Asia (Makino 1961, Li 1969, Nitta 2001), although the wild ancestor of *P. frutescens* has not yet been identified. These two crops have the same chromosome number,  $2n = 40$  (Yamane 1950, Honda et al. 1994), and are cross-fertile by artificial pollination.

In this study, we report two new wild type *Perilla* species in Korea and characterized their morphological and molecular biological characteristics.

### Methods

Twenty wild type *Perilla* germplasm were collected from Jeju island, Korea in 2002. Their seed were planted in a green house on June 1 in 2003 at National Yeongnam Agricultural Experiment Station, RDA, Korea. DNA was extracted from fresh leaf of each collection and used for RAPD analysis. At flowering time, morphological characteristics were observed. Root tips were used to observe chromosomes and pretreated with 8-hydroxy quinoline for three hours following fixing overnight. Specimens were softened for 30 seconds in 60 centi-degree of 1N HCl and stained with 0.1% of aceto-carmin. Specimens were observed under microscope (Zeiss Axioplan) and photographed by the Sony 3CCD color video camera (CCD-IRIS) aided with software KS400 (Ver. 2.0, Kontron Elektronik).

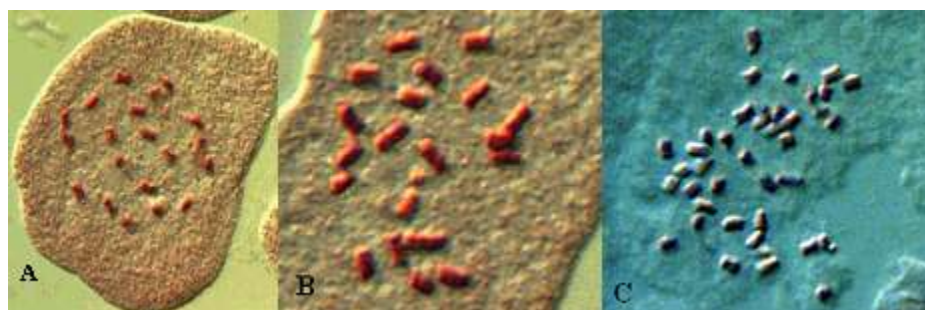
### Results

Among 20 wild type collections, only two collections Jeju-3 and Jeju-17 were identified as *P. hirtella* and *P. citriodora*, respectively. Chromosome number of Jeju-3 and Jeju-17 were 20 but were 40 in other collections of other *Perilla* species (Figure 1). Though the karyotyping was impossible because of small

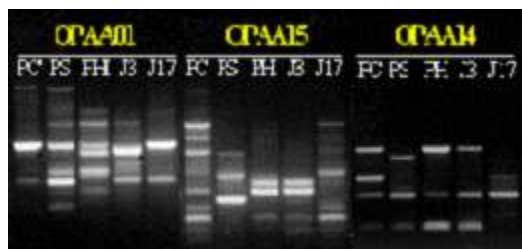
chromosome of *Perilla*, the size of chromosomes among different species appeared to be similar. We think *Perilla* as allo-tetraploid because many characteristics follow Mendel's laws, no quadrivalent pairing has been reported yet. Phenotype was clearly distinguished in different species and the characteristics agreed with previous reports (Nitta 2001). Jeju-17 shows a white bract colour that is a unique characteristic of *P. citriodora* (Table 1). Distinct serration is an inherent characteristic of *P. hirtella* which is shown in Jeju-3 but is absent in Jeju-17 and *P. citriodora*. Jeju-3 and Jeju-17 showed unique RAPD markers which were same as in *P. hirtella* and *P. citriodora*, respectively (Figure 2).

**Table 1. Taxonomic characteristics of three *Perilla* species and two wild type collections.**

Character	<i>P. hirtella</i>	<i>P. citriodora</i>	<i>P. frutescens</i>	Collected 3	Collected 17
Serration at leaf base	distinct	?indistinct	indistinct	distinct	?indistinct
Bract color	?green	?white	?green	?green	?white
Chromosome number (2n)	20	20	40	20	20



**Figure 1. Aceto-carmin stained metaphase chromosome of *Perilla* species. A; Jeju-3, B; Jeju-17, C; *P. frutescens*.**



**Figure 2. RAPD pattern of two wild type Jeju-3 (J3) and Jeju-17 (J17) and three diploid species *P. citriodora* (PC), *P. setoyensis* (PS) and *P. hirtella* (PH). Jeju-3 and Jeju-17 (CPAA11) showed same markers with *P. hirtella* and *P. citriodora*, respectively.**

## Conclusion

With this result it is suggested that the *Perilla frutescens* is not diploid but tetraploid. Diploid species *P. citriodora* and *P. hirtella* are possibly the ancestor of *Perilla* species.

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