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Karyology of *Prunella grandiflora* and *Prunella vulgaris* from Türkiye

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Türkiye'den *Prunella grandiflora* ve *Prunella vulgaris*'in karyolojisi

Abstract: *Prunella* L. species are distributed in very different habitats since they can adapt to variable ecological conditions. These conditions cause variations in morphological characters. It is very important to support morphological and taxonomic characters with genetic data. So far, various diploid numbers have been reported in the chromosomal records of Turkish *Prunella* species. The situation is particularly complex in *P. vulgaris* L. and *P. grandiflora* (L.) Turra species. The aim of this study is to eliminate the complexity by presenting karyological data of these species. The chromosome number and chromosome lengths of *P. grandiflora* and *P. vulgaris* were reported for the first time from Turkey. The diploid and basic numbers were detected as $2n = 28$ and $x = 7$ by ploidy levels of $4x$ in *P. grandiflora* and *P. vulgaris*. As a result, the karyology of the genus *Prunella* was evaluated by comparing previous and present results. The listed data provided important contributions to the cytotaxonomy of the genus *Prunella*: (i) the basic number was x = 7, (ii) the most common diploid number was $2n = 28$, and (iii) high polyploidy rates. The polyploidy probably played an important role in the speciation of the genus.

Key words: basic number, karyotype, polyploidy, *Prunella*

Özet: *Prunella* L. türleri değişken ekolojik koşullara uyum sağlayabildikleri için çok farklı habitatlarda dağılım göstermektedir. Bu koşullar morfolojik karakterlerde çeşitliliğe neden olmaktadır. Morfolojik ve taksonomik karakterlerin genetik verilerle desteklenmesi çok önemlidir. Şimdiye kadar Türkiye *Prunella* türlerinin kromozom raporlarında çeşitli diploid sayılar bildirilmiştir. Özellikle *P. vulgaris* L. ve *P. grandiflora* (L.) Turra türlerinde durum karmaşıktır. Bu çalışmanın amacı, bu türlerin karyolojik verilerini sunarak karmaşıklığı ortadan kaldırmaktır. *P. grandiflora* ve *P. vulgaris*'in kromozom sayısı ve kromozom uzunlukları Türkiye'den ilk kez rapor edilmiştir. Diploid ve temel sayılar *P. grandiflora* ve *P. vulgaris*'te $2n = 28$ ve $4x$ ploidi seviyesi ile $x = 7$ olarak tespit edilmiştir. Sonuç olarak, *Prunella* cinsinin karyolojisi önceki ve mevcut sonuçları karşılaştırarak değerlendirildi. Listelenen veriler *Prunella* cinsinin sitotaksonomisine önemli katkılar sağladı: (i) temel sayı $x = 7$'dir, (ii) en yaygın diploid sayı $2n = 28$'dir ve (iii) yüksek poliploidi oranları. Poliploidi muhtemelen cinsin türleşmesinde önemli bir rol oynamıştır.

Anahtar Kelimeler: temel sayı, karyotip, poliploidi, *Prunella*

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1. Introduction

The genus *Prunella* L. (Lamiaceae) contains eight species (12 taxa) and five hybrids distributed in Europe, Asia, North America, and Northwest Africa (Harley et al., 2004; Govaerts, 2023). In Türkiye, there are 4 species growing, and there are no endemic species (Dirmenci, 2012; Çelenk et al., 2024). Since the species can adapt to variable ecological conditions, they are distributed in very different habitats (from low altitudes such as sea levels to high altitudes such as alpine zones). Variable ecological conditions cause variable morphological features in structures such as inflorescence, leaves, and indumentum. Herbaceous, square stem, not aromatic, rhizomatous; leaves simple to pinnatifid; flowers blue, purple, cream, or white; inflorescences ovoid to an oblong spike; 2-lipped corolla and calyx; stamens 4, lower pair longer are important morphological and taxonomic characters (Edmonson, 1982; Harley et al., 2004).

It is very important to support morphological and taxonomic characters with genetic data. The cytotaxonomy contributes to the solution of taxonomic problems by using cytogenetic data such as the number of chromosomes (basic and diploid), the sizes of chromosomes or their parts (long arm, short arm, total chromosome, relative and total haploid lengths), karyotype formula and karyotype asymmetry (intrachromosomal and interchromosomal) (Peruzzi and Eroğlu, 2013; Eroğlu, 2015; Martin et al., 2022). In order to reveal systematic and evolutionary relationships more clearly, the morphological characteristics of the genus *Prunella* should be supported by cytotaxonomic data.

In the genus *Prunella*, the most frequent chromosome number is $2n = 4x = 28$. The diploid numbers of the taxa belonging to the genus *Prunella* vary from 14 to 32, such as $2n = 14$, 21, 24, 28, 30, and 32. The species are diploid ($2x = 14$), triploid ($3x = 21$), and poliploid ($4x = 24$, 28, and 32) (Hruby, 1932; Böcher, 1949; Fernandes and Leitão, 1984; Malik et al., 2017; Javadi and Safikhani, 2023; Mirzadeh Vaghefi and Jalili, 2023).

So far, various diploid numbers have been reported in the chromosomal reports of Turkish *Prunella* species. The

situation is particularly complex in *P. vulgaris* L. and *P. grandiflora* (L.) Turra species. The aim of the present study was to present the karyological data of these species to eliminate the complexity.

2. Materials and Method

2.1. Plant samples

The *Prunella* species were collected from natural habitats in Türkiye. The distribution map was generated by Google Maps (Fig. 1). The habitat and flower photos of the species are given in Figure 2. The plant samples were deposited in Balıkesir University, Necatibey Faculty of Education, Department of Biology Education. The collection information is given below.

Prunella grandiflora: Türkiye, Balıkesir, Edremit, Kazdağı, Kartalçimeni hill, 1750 m, 28.07.2007, Dirmenci 3473. ***Prunella vulgaris***: Türkiye, Adana: 10 km from Saimbeyli to Himmetli, 800 m, 20.07.2007, Dirmenci 3569 & Akçiçek.

2.2. Cytogenetic procedure

Prunella seeds were germinated between moist filter papers at room temperature (germination). Germinated root tips were treated with saturated water solution of α - bromonaphtalene overnight at 4ºC (pretreatment), fixed in ethanol–glacial acetic acid solution (3:1) (fixation), hydrolyzed with 1N HCl at room temperature (hydrolysis), squashed in 45% acetic acid (preparation), and stained with 2% aceto-orcein (staining).

Permanent slides were made with the standard liquid nitrogen method. Slides were dried for 24 h at room temperature and mounted in Depex (Martin et al., 2023).

At least 10 metaphase plates were analyzed to detect diploid chromosome numbers by Software KaryoType.

3. Results

Figure 3 represents the small metaphase chromosomes of *Prunella* species. The chromosome numbers ($2n = 28$) of *Prunella grandiflora* and *P. vulgaris* are confirmed. The chromosome numbers and total chromosome lengths are reported for the first time from Türkiye. Due to the small size of chromosomes and the indistinct centromere, only total chromosome lengths were measured without long and short arm lengths.

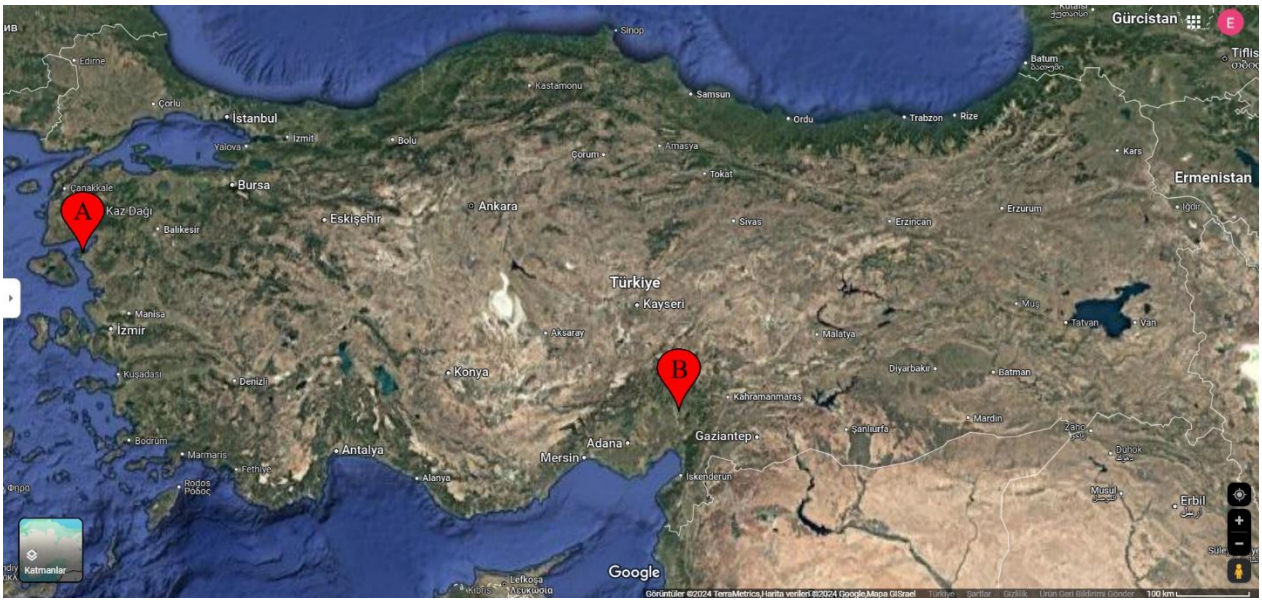

Figure 1. Distribution map of the studied species in Turkey. (A) *Prunella grandiflora* and (B) *Prunella vulgaris*

Figure 2. Habitat and flowers of *Prunella* species. (A) *Prunella grandiflora* and (B) *Prunella vulgaris*

3. Results

Figure 3 represents the small metaphase chromosomes of *Prunella* species. The chromosome numbers (2*n* = 28) of *Prunella grandiflora* and *P. vulgaris* are confirmed. The chromosome numbers and total chromosome lengths are reported for the first time from Türkiye. Due to the small size of chromosomes and the indistinct centromere, only total chromosome lengths were measured without long and short arm lengths.

In *Prunella grandiflora*, the total haploid length (THL) and mean haploid length (MHL) are 12.59 and 0.90 µm, respectively (Table 1). The chromosomes are quite small, and chromosome lengths range from 0.38 to 1.41 µm. (Table 2). In *P. vulgaris*, the total haploid length (THL) and mean haploid length (MHL) are 12.98 and 0.93 µm, respectively (Table 1). The chromosomes are quite small, and chromosome lengths range from 0.42 to 1.33 µm (Table 2).

4. Discussions

The chromosome number and chromosome lengths of *Prunella grandiflora* and *P. vulgaris* were reported for the first time from Türkiye. The different chromosome numbers for these species (especially *P. vulgaris*) were presented in the literature, which were 2*n* = 14, 21, 24, 28, 30, and 32 (Hruby, 1932; Böcher, 1949; Fernandes and Leitão, 1984; Malik et al., 2017; Javadi and Safikhani, 2023; Mirzadeh Vaghefi and Jalili, 2023). The most common somatic number in *Prunella* species was 2*n* = 28 (Probatova et al., 1991; Vitek et al., 1992; Melnikov, 2019), which was also presented in this study. It was reported that the number 2*n* = 32 proposed by Hruby (1932) was incorrect because the chromosome examination methods at that time did not allow a correct distinction between short and long chromosomes, and long chromosomes could be counted excessively (Böcher, 1949). However, presented all diploid numbers cannot be ignored.

In the genus *Prunella*, the basic chromosome number was *x* = 7 (Böcher, 1949; Magulaev, 1979; Melnikov, 2019). However, *x* = 6 and 8 cannot be ignored. The basic number was detected as *x* =7 by ploidy levels of 4*x* in *P. grandiflora* and *P. vulgaris*. Magulaev (1979) reported the diploid (2*n*= 2*x* = 14), triploid (2*n* = 3*x* = 21), and tetraploid (2*n* = 4*x* = 28) plants in the Caucasian population and suggested that the polyploidy as the main factor in the widespread occurrence of *P. vulgaris*. There is a positive correlation between polyploidy and environmental adaptation, and the polyploidy is one of the main mechanisms effective in the evolution process of plants. The polyploid organisms generally exhibit higher vitality and superior characteristics than their diploid relatives (Eroğlu, 2022).

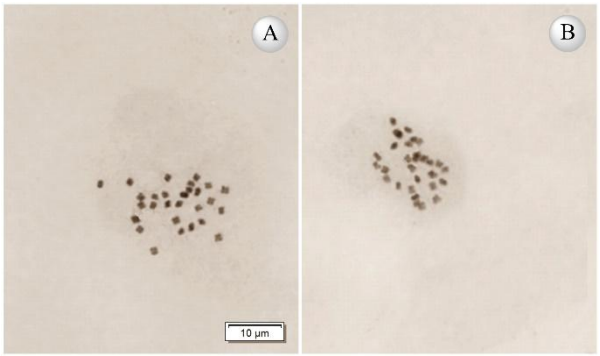

Figure 3. Metaphase chromosomes of *Prunella grandiflora* (A) and *Prunella vulgaris* (B). Scale bar 10 µm

Table 1. The karyological parameters of *Prunella* species

Parameters	*P. grandiflora*	*P. vulgaris*
Basic number (*x*)	7	7
Diploid number (2*n*)	28	28
Ploidy level	4*x*	4*x*
Total haploid length (µm)	12.59	12.98
Mean haploid length (µm)	0.90	0.93

Table 2. Total chromosome lengths of *Prunella* species

P. grandiflora		*P. vulgaris*	
Chromosome	**Length (µm)**	**Chromosome**	**Length (µm)**
1	1.41	1	1.33
2	1.32	2	1.28
3	1.24	3	1.25
4	1.19	4	1.16
5	1.13	5	1.13
6	1.02	6	1.07
7	0.95	7	0.94
8	0.88	8	0.92
9	0.79	9	0.83
10	0.71	10	0.78
11	0.63	11	0.72
12	0.51	12	0.65
13	0.43	13	0.50
14	0.38	14	0.42

The karyotype asymmetry is one of the most effective parameters in understanding karyotype evolution (Eroğlu et al., 2019). While the early stages of karyotype evolution are periods in which symmetrical karyotypes are dominant, karyotypes become asymmetrical in the later process (Martin et al., 2023). Intrachromosomal (M_{CA}) and interchromosomal asymmetry (CV_{CL}) values could not be calculated because arm lengths (short and long) could not be measured. There are no records of karyotype asymmetry in the genus *Prunella*, and this remains a general problem or deficiency.

In this study, the karyology of the genus *Prunella* was evaluated by comparing previous and present results. The listed data will provide important contributions to the cytotaxonomy of the genus *Prunella*: (i) the basic number was *x* = 7, (ii) the most common diploid number was 2*n* = 28, and (iii) high polyploidy rates. The polyploidy probably played an important role in the speciation of the genus.

Conflict of Interest

The authors have declared no conflict of interest.

Authors' Contribution

All authors contributed to the study's conception and design. All authors read and approved the final manuscript.

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