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# ADDITION TO CHARACTERS OF ENDEMIC *AUBRIETA CANESCENS* SUBSP. *CANESCENS* BORNM. (BRASSICACEAE) FROM TURKEY

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**Abstract**

Investigation on the taxonomical, morphological, anatomical, karyological and ecological characteristics of the endemic *Aubrieta canescens* ssp. *canescens* Bornm. growing in Turkey was carried out. Data were obtained at the flowering and fruiting times of populations. The sampling was made from various locations in each year from 2012 to 2014. In morphological studies, description of the endemic taxon stated in the Flora of Turkey revised in the light of detailed evaluations of many specimens, and features exclusive to the taxon. Additionally, images were taken about the seed's surface of different taxa by using Scanning Electron Microscope micromorphologically. Structures of the stem, root and leaf cross-sections were examined in anatomical studies. In karyological studies, the chromosome number of the taxon was found as 2n = 16 (x = 8) and consisted karyotype formula of taxon as five median, two submedian, one subtelocentric and one telocentric chromosome pairs. In general, the physical features of the soil and live together taxa of the examined taxon were investigated ecologically. This study is the first one where morphological (macro and micro), anatomical, karyological and ecological features of endemic *Aubrieta canescens* subsp. *canescens* was carried out. According to IUCN criteria, risk category of the taxon was also identified.

## Introduction

*Brassicaceae,* which has approximately 365 genus and 3250 species widespread around the world, is known as a large family having economic importance (Simpson 2006, Tekin *et al.* 2013). Turkey including 61 genera and 653 species is one of the most diverse places (Al Shehbaz *et al.* 2007). This family is easily diagnosed, however, no extensively recognized classification system is not present yet (Koch *et al.* 2003, Khosravi *et al.* 2009).

The *Aubrieta* Adans. genus (Brassicaceae) includes perennial herbaceous plants represented by about 20 species between Middle Asia and South Europe (Gustavsson 1986, Phitos 2002), and cultivated in gardens of Europe today (Davis 1965). Turkey is the leading location where the genus has the most diversity. In Turkey, c. 10 *Aubrieta* taxa of which 6 are endemic (Guner *et al.* 2012). High endemism ratio (as 60%) indicates that Turkey is one of the gene centers of the *Aubrieta* genus.

In the literature survey, it has been encountered some investigations on *Aubrieta* species such as morphology and cytology of the some Bulgarian species (Ančev and Goranova 2009), palynology of *A. pinardii* (Inceoglu and Karamustafa 1977) and molecular data such as plastid matK and chloroplast gene *ndh*F of *A. deltoidea* (Koch *et al.* 2001, Beilstein *et al.* 2006) to elucidate the phylogenetic relationships within Brassicaceae. Apart from these, it was not possible to find out any study directly related to the genus. Due to the scarcity of data on the species of this genus multidisciplinary study needs to be studied.

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*Aubrieta canescens* ssp. *canescens* Bornm. (Acc) is one of the 10 taxa included in the *Aubrieta* genus in Turkey. It is an endemic taxon from Southern Anatolian. The taxon is found on stony slopes and rocky areas. In this study, morphological (macro and micro), anatomical, karyological, ecological features and IUCN risk category of this taxon in Turkey have been investigated for the first time.

## Materials and Methods

Plant samples collected from various fields in 2012-2014 and their vouchers specimens were listed in Table 1 and their locations are shown in Fig. 1a. The plant samples were identified in consultation of the Flora of Turkey and stored in the ISTF (Istanbul University Science Faculty Herbarium). At the same time, Acc samples presented in various herbaria (ISTF, ISTE, ISTO, AKDU, KATO, OGU, KNYA) in Turkey were also examined (more than a hundred individuals in total). Macromorphological observations such as leaf, fruit and seed features were examined using an Olympus ZS51 stereomicroscope and Kameram Imaging Software. For micromorphological observations, seeds obtained from samples were prepared for electron microscopy by mounting to table with silver adhesive, coated with gold and analysed with JEOL Neoscope-5000 scanning electron microscope. The terminologies utilized are mainly that of Stearn (1985).

**Table 1. Origin of the examined Acc.**

| Specimens No. | Voucher | Origin |
|---|---|---|
| I | ISTF41066 | Icel, Mut |
| II | ISTF41065 | Antalya, Tahtali d. |
| III | ISTF41067 | Konya, Beysehir |
| IV | ISTF41068 | Isparta, Dedegol |
| V | ISTF41069 | Kutahya, Murat d. |

For anatomical investigation, cross sections were taken by a fully automatic microtome (Thermo Shonda Met Finesse) from root, stem and leaf. Afterwards, they were passed through a variety of alcohol and xylene series, and stained with haematoxylin or safranine in a staining device (ASC 720 Medite), and were covered with Entellan in order to examine anatomical structures (Karaismailoglu 2015). Anatomical characters were observed by Olympus CX21FS1 microscope and Kameram Imaging Software.

For karyological examinations, obtained root meristems from germinating seeds of the least five plants were utilized. Root tips were treated with a-monobromonaphthalene at 4°C for 12 hrs. After, they were fixed with Carnoy for 24 hrs at 25°C. Later, the roots were hydrolyzed with 1N HCl for18 min at 60°C. The chromosomes were stained with acetic orcein. Permanent slides were made with Entellan and photographs were taken. Chromosomes were categorized following Levan *et al.* (1964).

The soil specimens collected from indicated areas in Table 1 and Fig.1a were gathered from five locations closest to the spread areas. The physical analyses were performed in accordance with Chapman and Pratt (1961), and Atasagun *et al.* (2013). Besides, chemical analyses were made following the method of Shirdam *et al.* (2008) with some modifications. First of all, the soil samples were homogenized and 0.5 g sample was measured. After, they were inserted DAP 60 teflon vessels and added 10 ml nitric acid to each vessel. Later, they were placed in Berghof MWS-4 microwave oven, filtered and measured by comparison with standard solutions in ICP-OES.

## Results and Discussion

Morphological characters are generally compatible with features mentioned in the flora of Turkey (Davis 1965). Accordingly, perennial, stems usually are 5 - 10 cm prostrate or rarely ascending (Fig. 1b). Leaves are very variable, entire to weakly dentate, or with 1 - 2 deep teeth on each, or no dentate (Fig. 1c, d, e). Sepals are saccate, 5 - 11 mm. Petals are violet, 11 - 19 mm. Style is 3 - 6 mm. Fruits are 9 - 11 mm × 2 - 5 mm, usually inflated or terete, with a sparse to dense indumentums of stellate hairs (Fig. 1f, g). Seeds usually are ovate or globular and its surface covers with mucilage plaques (Fig. 1h, i). Flowering time: April - August, fruiting time: May - September.

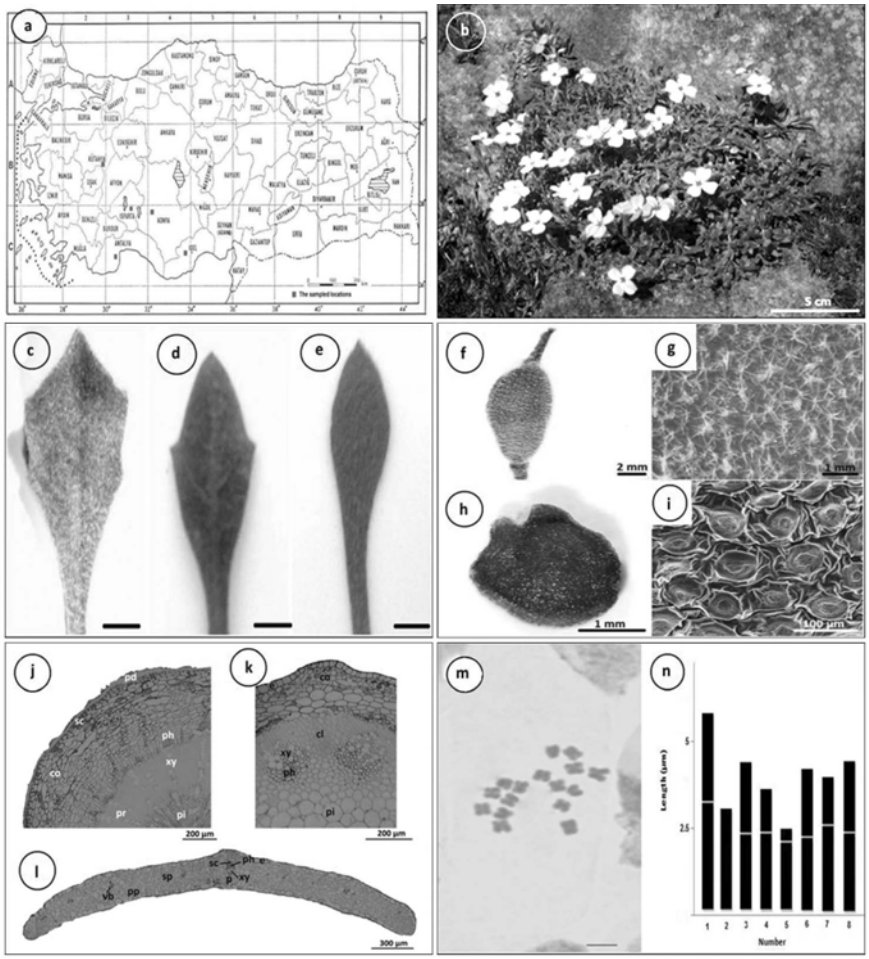


Fig. 1. a: Map showing the sampling areas; b: The general appearance of *Aubrieta canescens* subsp. *canescens* (cm: centimeter), leaf types of the *Aubrieta canescens* subsp. *canescens;* c: Containing bidentate on each surface of leaves; d: Containing monodentate on each surface of leaves; e: Dentateless leaves (scale bars: 1 cm), fruit and seed surface structures; f: General appearance of the fruit; g: Stellate hairs on fruit surface, h: General appearance of the seed, i: mucilage plates on seed surface, cross sections of the various structure; j: root, k: stem; l: leaf (pd: periderm, sc: Sclerenchyma; co: cortex, ph: phloem, xy: xylem, pr: pith ray, pi: pith, e: epidermis, cl: collenchyma, p: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle), somatic chromosomes of the Acc; m: metaphase chromosomes (Scale bar: 10 µm), n: ideogram.

*Aubrieta* is taxonomically a typical genus and repeated field trips were required to determine the status this taxon (Cullen 1965). In line with this information, description of Acc which had made with very narrow-scope in Flora of Turkey (Davis 1965) intensive field studies were in detail and tested the previous description. New data about some morphological features, flowering and fruiting time, and habitat of the plant were carried out. Accordingly, Acc leaves were found to have a wide variety so that they found 1 - 2 deep or weakly teeth on each or no dentate whereas they were reported only 1 - 2 toothed in Flora of Turkey (Davis 1965). In addition, length of fruits which was noted to vary from 9 to 10 mm in Flora of Turkey (Davis 1965) is found between 9 and 11 mm, and this feature being value in terms of taxonomy is updated. Shape of the seeds which are usually ovate or globular and their surfaces cover with mucilage plaques have described for the first time in this investigation. Habitat, flowering and fruiting time of taxon are also re-explained with some differences such as having dry and stony habitats and flowering time: April - August, and fruiting time: May - September.

**Table 2. Measurement of somatic chromosomes in Acc.**

| Number | Short arm (s) (μm) | Long arm (l) (μm) | Total length (μm) | Arm rato (l/s) | Relative length (%) | Centromeric index | Chromosome type |
|---|---|---|---|---|---|---|---|
| 1 | 2.51 | 3.04 | 5.55 | 1.21 | 16.36 | 7.4 | m |
| 2 | - | 2.87 | 2.87 | - | 8.46 | - | t |
| 3 | 2.02 | 2.13 | 4.15 | 1.05 | 12.24 | 5.95 | m |
| 4 | 1.22 | 2.17 | 3.39 | 1.77 | 9.99 | 3.59 | sm |
| 5 | 0.34 | 1.91 | 2.25 | 5.61 | 6.64 | 1.01 | st |
| 6 | 1.98 | 2.05 | 4.03 | 1.03 | 11.89 | 5.83 | m |
| 7 | 1.36 | 2.43 | 3.79 | 1.78 | 11.17 | 4.09 | sm |
| 8 | 2.02 | 2.21 | 4.23 | 1.09 | 12.48 | 6.08 | m |

On the outer surface of the Acc root was located a multi-layered periderm. Sclerenchyma clusters were located under periderm. The cortex was formed by multilayer parenchymatic cells, their diameters ranged from 95 to 145 μm. Endodermis layer was not very clear. There was a discernible cambium between xylem and phloem. The most covering space in the roots was secondary xylem. Pith rays were composed of broad parenchymatic cells (Fig. 1j).

A cross-section taken from the stem, vaguely angular structure was found and plated with uniseriate epidermis. There were a few cortex layers under epidermis. Ovoid shaped collenchymas were located next to epidermis. Parenchyma cells were 30 - 50 μm × 15 - 25 μm in dimensions. Xylem and phloem elements were not very obvious. The type of transmission bundle was open collateral. The diameters of vessel members ranged from 150 - 180 μm. In the inner layer was located the essence composed from large parenchymatic cells (Fig. 1k).

In the abaxial and adaxial surfaces of the leaf were seen to have single layer epidermis cells. The leaf was bifacial. There was palisade parenchyma on both sides of the leaves, so leaves were isolateral types. The mesophyll was distinguished as a 5 - 8 layered spongy in thickness 95 - 120 μm and a 2 - 4 layered palisade parenchyma in thickness 30 - 75 μm. Leaves had collateral vascular bundles which contained phloem, xylem and sclerenchymatous structure. Vascular bundles were enclosed by parenchymatic cells (Fig. 1l).

Metcalfe and Chalk (1957) mentioned anatomical features and their uses in taxonomy in the Cruciferae family. For this purpose; the root, stem and leaf anatomy of Acc are given in this study for the first time. The roots include a thin periderm layer in the outermost; they have large pith rays which occur with primary xylem components and sclerenchymatic structures. Sclerenchymatic cells in the roots are buried in cortex parenchyma. Acc has collenchyma cells

next to a thick epidermis layer (15 - 35 µm) in the stem and exhibits similar images with the some *Alyssum* and *Erysimum* species (Orcan 1997, Orcan and Binzet 2003, Cansaran *et al.* 2007). The leaf is isobilateral. In the cross sections of the leaf, palisade parenchyma in the mesophyll layer covers more space than sponge parenchyma which is seen reductions. The leaf has anisocytic stomata which also called as Cruciferae type owing to widely available in this family (Cansaran *et al.* 2007). The anatomical features of all taxa belonging to genus in parallel with Acc are important in the establishment of the evolutionary relationships among taxa, and in the questioning of the usability of anatomical properties in terms of taxonomy.

**Table 3. Soil analysis of Acc populations.**

| Texture | I | II | III | IV | V |
|---|---|---|---|---|---|
| | Sandy-loam | Clay-loam | Sandy-loam | Sandy-loam | Sandy-loam |
| Silt (%) | 10.72 | 16.49 | 14.54 | 12.35 | 19.46 |
| Clay (%) | 19.43 | 45.43 | 24.27 | 19.11 | 12.74 |
| Sand (%) | 65.85 | 32.63 | 54.58 | 65.81 | 64.17 |
| $CaCO_3$ (%) | 1.89 | 2.66 | 3.17 | 1.08 | 1.44 |
| Organic matter (%) | 2.11 | 2.79 | 3.44 | 1.65 | 2.19 |
| pH | 7.11 | 6.72 | 6.64 | 6.93 | 6.57 |
| Ca (ppm) | 1.61 | 0.87 | 1.44 | 0.98 | 1.76 |
| Mg " | 84.43 | 46.49 | 22.14 | 98.15 | 3.45 |
| Fe " | 41.13 | 11.94 | 75.65 | 41.07 | 66.19 |
| S " | 3.45 | 7.98 | 1.61 | 0.89 | 11.64 |
| P " | 44.43 | 21.49 | 10.04 | 8.41 | 3.17 |
| K " | 163.19 | 198.22 | 79.34 | 119.24 | 53.08 |
| Na " | 14.13 | 19.54 | 9.87 | 15.36 | 11.47 |
| Zn " | 3.4 | 4.9 | 11.43 | 7.11 | 6.53 |
| Mn " | 6.56 | 1.09 | 0.98 | 1.95 | 3.46 |
| Ni " | 1.17 | 1.75 | 1.09 | 0.43 | 0.61 |
| Mo " | 0.21 | 0.36 | 0.57 | 0.21 | 0.63 |
| Cu " | 0.46 | 0.63 | 0.76 | 1.76 | 1.14 |

The karyogram and ideogram and types of chromosomes of Acc were studied in detail (Table 2 and Fig. 1 m, n). The somatic chromosomes of the Acc were distributed in diploid cells, determined as 2n = 2x = 16 (Fig. 1 m). Also, the basic chromosome number was identified as x = 8. Accordingly, the karyotype formula of Acc was defined with 4 m (metacentric), 2 sm (submetacentric), 1 st (subtelocentric) and 1t (telocentric) chromosome types. The lengths of chromosomes ranged from 2.25 to 5.55 µm. At the same time, the overall length of the haploid chromosome was 30.26 µm (Table 2).

The chromosome number, morphology and ploidy level of the Acc have been worked out for the first time in this investigation. Chromosome number was determined as 2n = 16. Warwick and Al-Shehbaz (2006) reported that the basic chromosome number of the *Aubrieta* genus is x = 8. In this study, basic chromosome number of Acc was also found as x = 8. In addition, karyotype formula of the taxon was fixed as n = 8 = 4 m + 2 sm + 1 st + 1t.

*A. canescens* subsp. *canescens* usually grows in a dry habitat at an altitude of 1,060 - 2,400 m, and is a significant member of rocky or stony areas. Generally, it is found together with *Hypecoum*

*pendulum* L., *Arabis caucasica* Willd. subsp. *brevifolia* (DC.) Cullen, *Barbarea verna* (Mill.) Asch, *Hesperis kotschyi* Boiss., *Polygala anatolica*, *Telephium imperati* L. subsp. *orientale* (Boiss.) Nyman, *Geranium tuberosum* L. subsp. *tuberosum*, *Centaurea pichleri* Boiss. subsp. *pichleri*, *Xeranthemum annuum* L., *Alkanna orientalis* (L.) Boiss. var. *orientalis*, *Verbascum chionophyllum* Hub.-Mor, *Hordeum murinum* L. subsp. *glaucum* (Steud.) Tzvelev.

Chemical and physical assays of the soil specimens collected from sampling locations in the native distribution field of Acc are shown in Table 3. Physical analysis in the soils taken from different populations of the Acc showed that this taxon usually preferred slight acidic soils and it existed in sandy-loam and clay-loam soils. In addition, chemical analysis of the same soil samples indicated that Acc was not very picky in terms of organic matter. In terms of the examined elements, the soil is generally poor in magnesium (%), phosphorus (%) and calcium (%), however, it is more ordinary in normal conditions in terms of the other examined elements.

According to the IUCN categories and criteria (2001), this taxon has been determined as Least Concern (LC) so that it is partially common and abundant. Although the number of the individuals in these populations is not high, the range of populations is wide, therefore this group is not threatened or in danger of extinction in the sense of IUCN categories. However, due to the low number of individuals in populations, it tends to be included in these categories.

With this investigation, the classification of the mentioned endemic taxon in Turkey which constituted with insufficient in the sense of morphological characters, without flowers or mature fruit, and a few specimens according to the Flora of Turkey, was questioned. The description of the examined taxon was completed. With the intension of solving the problems in the classification of the taxon, some of the taxonomical characters of the genus were revealed. With further studies on the other taxa of the genus, the phylogenetic relationships amongst the taxa can be dissolved, and the classification of the genus can be reconstructed using micro morphological, anatomical, cytological and ecological methods, besides the morphological characters alone.

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