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COMPARATIVE MORPHOLOGICAL, ANATOMICAL AND HABITAT STUDIES ON *DACTYLORHIZA ROMANA* (SEB.) SOÓ SUBSP. *ROMANA* AND *DACTYLORHIZA ROMANA* (SEB.) SOÓ SUBSP. *GEORGICA* (KLINGE) SOÓ EX RENZ & TAUB. (ORCHIDACEAE) IN TURKEY

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Abstract

In this study, 2 subspecies; *Dactylorhiza romana* (Seb.) Soó subsp. *romana* and *Dactylorhiza romana* (Seb.) Soó subsp. *georgica* (Klinge) Soó ex Renz & Taub. mainly distributed in Turkey were investigated in terms of morphological, anatomical characters and habitat properties. Plant and soil samples of *D. romana* subsp. *romana* were taken from 9 localities and *D. romana* subsp. *georgica* from 7 localities in Turkey. We observed that some of morphological characters such as; underground part length, tuber length, the longest leaf width, and bract length had different properties between both subspecies. Cross sections of the leaves, stems, tubers and roots were taken by free-hand and stained with Sartur solution and Safranin. The same procedure was performed for the surface sections of leaves and stem. Stomatal and epidermal cells, starch granules dimensions, lengths of raphides, stomata index, and cuticular thickness were measured. Anatomical characters of leaves such as cuticle thickness and stomata index displayed between two subspecies. Although *D. romana* subsp. *georgica* grows in high altitudes, *D. romana* subsp. *romana* prefers lower altitudes. The most common habitat of *D. romana* is meadow, macchie and forest lands. We obtained that both of them had similar soil properties, while the differencies came out by habitat properties. Morphological and habitat characters were compared with independent-T test.

Introduction

Dactylorhiza Neck. ex Nevski, belongs to Orchidaceae, differs by its fingerlike tuberoids from the other genera of family (Renz & Taubenheim, 1984). It comprises between 12 and 75 species worldwide (Devos *et al*., 2006). According to Inda *et al*., (2010) the great variation in the number of species defined in this genus can be explained by three factors: (1) *Dactylorhiza* species present a bewildering degree of morphological variation, (2) *Dactylorhiza* constitutes a complex of species in which hybridization and polyploidization, (3) Divergent underlying species' concepts have been applied by different authors. Thus, some researchers accept morphologically distinct allotetraploid forms as different species, whereas others consider that well-known, geographically or ecologically separated allotetraploids should be interpreted as subspecies. During recent decades, numerous molecular studies have been conducted to analyse the phylogeny and evolution of *Dactylorhiza* or to determine molecular support for different recently published taxonomic decisions (Inda *et al*., 2010). According to Devos *et al*., (2003), most *Dactylorhiza* species are diploid (2n=40) or tetraploid (2n=80) and the European species can be sorted into six groups on the basis of morphological and cytological data: (1) the *D. sambucina* group, comprising diploid and triploid species, (2) the *D. incarnata* group, comprising diploid species and sometimes called the ''diploid Marsh-Orchids'', (3) the *D. majalis* group, or the ''tetraploid Marsh-Orchids'', comprising only tetraploid species, (4) the *D. maculata* group, or the ''spotted Marsh-Orchids'', comprising both diploid and tetraploid species, (5) the *D. iberica* group, comprising one diploid species, and (6) the *D. aristata* group, comprising also one diploid species. *Dactylorhiza romana* (Seb.) Soó, belongs to *D. sambucina* group (Pedersen, 2006; Shipunov *et al*., 2004) and was investigated in this study.

Dactylorhiza (*Orchidaceae*), is mainly distributed in Europe, Mediterranean area and Asia (Shipunov *et al*., 2004; Daşkın, 2007). 13 species from Turkey is recorded (Renz & Taubenheim, 1984; Daşkın, 2007).

Description: Plants are 15 to 40 cm. Base of stem is composed of 2-3 large brownish sheaths, enclosing the numerous linear to lanceolate leaves. Spike is shortly cylindrical. Bracts are herbaceous, exceeding the violet-purple, dull red or yellow (sometimes whitish) flowers. Sepals are oblong with the size of 13 x 5 mm; lateral sepals are ± reflexed. Petals are obliquely ovate, with 10 × 4-7 mm sized dimensions. Labellum ± stretches forwards with the shape of ovate to broadly ovate, and 15 mm broad, is shortly 3-lobed in front, sometimes entire, without dots or lines. Spur is 10-25 mm.

Taxanomy of *D. romana*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Asparagales
Family	:	Orchidaceae
Subfamily	:	Orchidiodeae
Tribe	:	Orchideae
Subtribe	:	Orchidinae
Genus	:	*Dactylorhiza*
Species	:	*romana*

D. romana has two subspecies (*D. romana* subsp. *romana* and *D. romana* subsp. *georgica*) in Flora of Turkey (Renz & Taubenheim, 1984). Both subspecies are collected in Turkey for their medicinal purpose and used in food and hot-drink industry worldwide (Sezik, 2002) (Table 1).

Table 1. According to "Flora of Turkey" the differences between *D. romana* subsp. *romana* and *D. romana* subsp. *georgica* (Renz & Taubenheim, 1984).

Taxon name	*D. romana* subsp. *romana*	*D. romana* subsp. *georgica*
Morphology	Flowers are red to yellow	Flowers are mainly yellow
	Flowers are rather large	Flowers are rather small
	Labellum is usually broader than long	Labellum is usually longer than broad
	Labellum is up to 15 mm broad	Labellum is up to 8 mm broad
	Spur is cylindrical	Spur is narrowly cylindrical
	Spur is longer than ovary	Spur is equaling or shorter than ovary
		Spur is (8-) 10-12 mm
	Spur is (13-)17-20(-25) mm	Spur is ± straight, directed horizontally or
	Spur is curved ± steeply upwards	slightly upwards
Flowering period	April-June	(April) May-June
Altitude	0-1200 m	20-2000 m
Habitat	Limestones slopes in macchie and *Quercus* scrub, mixed and coniferous forests	Coniferous forest, forest margins, Quercus scrub, alpine meadows
Distribution	Mediterranean area, Crimea	Caucasia, North of Iran, Turkestan
Phytogeographical region	Mediterranean	Euxine

D. romana is an orchid in Turkey, commonly known as Salep, Çam kökü, Elçik. Tubers have been traditionally used by Turkish people for treating different ailments (such as; aphrodisiac, tonic, anti-constipant) and as food (for ice-cream or as hot drink) (Baytop, 1994; Baytop, 1999). "Salep" is a hot drink made in Turkey and is made from the powder of tubers of orchids. The "salep" powder obtained from the tubers of *D. romana* is famous with its high quality content, however uncontrolled collection from nature may cause extinction and alarming need of its conservation (Sezik, 2002; Tecimen *et al*., 2009).

Chemical constituents of *D. romana* tubers are musilage (61,05%), starch (0.45%), reduction sugar (4.5%), saccharose (0.44%), total N (0.74%), water (10.96%), residue on ignition (5.98%) (Sezik, 1967).

Even though *D. romana* subsp. *romana* and *D. romana* subsp. *georgica* are not present in "The Red Book of Turkey" a book that lists the species under risk (Ekim *et al*., 2000). Since the species is faced to be over collection and annihilated because of persistent collection an attempt had to be started. We anticipate this study encourage researchers and practitioners for re-production experiments. Within the current study we intended to determine habitat, morphological and anatomical properties of the *D. romana* subsp. *romana* and *D. romana* subsp. *georgica* in detail so as to provide base knowledge for further studies and give courage to the future researchers.

Material and Methods

Plant and soil samples of *D. romana* subsp. *romana* were taken from 9 localities and *D. romana* subsp. *georgica* from 7 localities between 2007 and 2009 in Turkey (Table 2, Figs. 1, 2). Samples are stored in the Faculty of Forestry Herbarium of Istanbul University (ISTO No: 35107-35119). Moreover, habitat properties of 122 records from previous studies and Herbarium records of EGE, ISTE and ISTF were collected and reviewed (Altıok & Behçet, 2005; Cansaran, 2002; Çelik, 2006; Demirelma, 2006; Eminağaoğlu & Anşin, 2004; Fırat, 2002; Güler, 2005; İşler, 2005; Kandemir, 2000; Karaer, 1994; Korkmaz, 1994; Koyuncu, 1999; Körüklü, 1997; Kutbay, 1993; Ocak & Tokur, 2000; Peşmen & Güner, 1976; Sahranç, 2001; Serin & Ertuğrul, 1999; Varol, 2004; Yüzbaşıoğlu, 2004; Kreutz, 2009).

Table 2. Site properties and locality of sampling points *in Dactylorhiza romana* subsp. *romana* and *Dactylorhiza romana* subsp. *Georgica*.

Sample				Altitude (m)	Slope (%)	Aspect	Locality
Sampling points	Taxon	Plant	Soil				
1	subsp. *georgica*	5	X	1251	0	0	Kastamonu - Taşköprü
2	subsp. *georgica*	-	X	1620	70	NE	Yozgat – Akdağmağdeni
3	subsp. *georgica*	-	X	1688	55	NW	Yozgat - Akdağmağdeni
4	subsp. *georgica*	10	X	1202	0	0	Kastamonu Taşköprü
5	subsp. *georgica*	-	X	1245	32	NE	Kastamonu Taşköprü
6	subsp. *georgica*	7	X	1066	10	W	Kastamonu Taşköprü Elekdağı
7	subsp. *georgica*	3	X	1399	45	S	Yozgat - Akdağmadeni
8	subsp. *romana*	5	X	81	20	N	Çanakkale Biga
9	subsp. *romana*	5	X	703	100	NW	Aydın - Nazilli
10	subsp. *romana*	3	X	772	80	SW	Aydın - Karacasu
11	subsp. *romana*	15	X	55	8	W	Çanakkale Ayvacık
12	subsp. *romana*	13	X	674	12	N	Çanakkale Ayvacık
13	subsp. *romana*	10	X	550	35	W	Çanakkale Bayramiç
14	subsp. *romana*	-	X	269	40	N	Çanakkale Ezine
15	subsp. *romana*	10	X	1357	60	SW	Muğla Köyceğiz - Sandras
16	subsp. *romana*	9		410	15	S	Kastamonu - Çatalzeytin

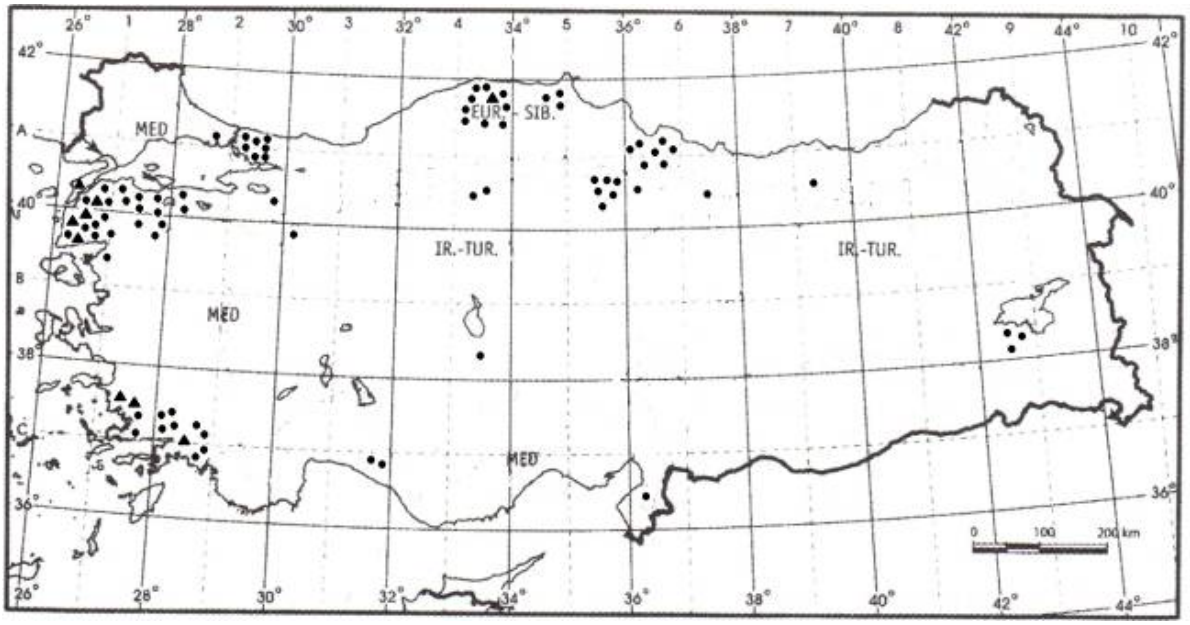

Fig. 1. ● Distribution of *D. romana* subsp. *romana* records; ▲: Distribution of collected samples of *D. romana* subsp. *romana.*

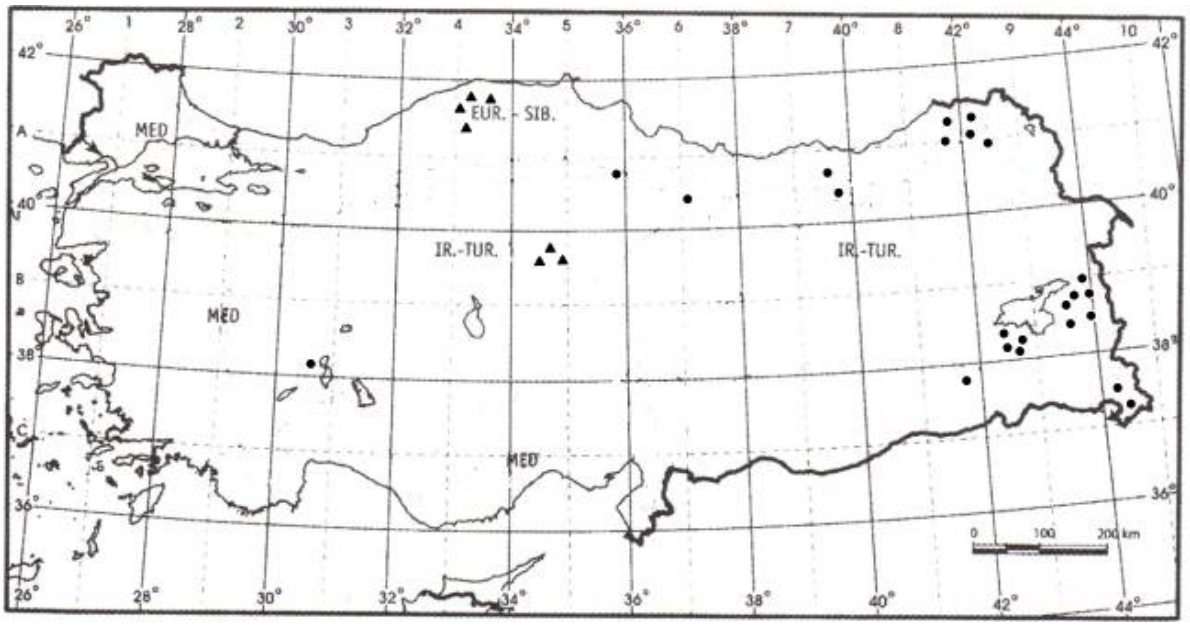

Fig. 2. ●Distribution of *D. romana* subsp. *georgica* records; ▲: Distribution of collected samples of *D. romana* subsp. *georgica.*

The investigated species of *D. romana* were evaluated within 13 morphological, 50 (20 leaf, 10 stem, 11 tuber and 9 root) anatomical and 18 soil characters and habitat features. We observed that some of morphological characters such as; underground part length, tuber length, longest leaf length, bract length, labellum length[1] and spur length[1] different properties between both subspecies. Permanent microscopic preparations were made of plant material stored in 70% ethanol. Cross sections of the leaves, stems, tubers and roots were taken by free-hand and stained with Sartur solution and Safranin. Cross-sections and surface sections were mounted with entellan (Vardar, 1987; İnce, 1989; Ruzin, 1999). The same procedure was performed for the surface sections of leaves & stem. Size of stomata and epidermal cell, starch granules dimensions, lengths of raphides, stomata index and cuticular thickness were measured. The well-staining sections were photographed on Leica DFC295 color camera type, Leica DM2500 light microscope. Anatomical characters of leaves such as cuticle thickness and stomata index displayed between two subspecies.

Soil samples were taken from 0-5 and 5-15 cm depths for investigations of soil properties of *D. romana*. Soil volume weight (g/l), fine soil weight (g/l), skeleton weight (g/l), sand rate (%), silt rate (%), clay rate (%) (Irmak, 1954; Gülçur, 1974), pH (Mc Lean, 1982),

[1] The number of measured samples is limited.

organic carbon (Corg) (%) (Nelson and Sommers, 1982), total nitrogen (Nt) (%) (Jackson 1962, Bremner and Mulvaney 1982) and C:N rates were detected.

The comparison of morphological characteristics of sub-species were made by Mann-Whitney U test. The habitat and soil properties were compared within T test at SPSS pack programme (Kalıpsız, 1981; Özdamar, 2002).

Results and Discussion

Mophological Features: 11 morphological characters with min-max mean and standard deviations of two subspecies of *D. romana* was studied and compared in Table 3. The obvious differences in morphological characters between the two subspecies were listed as underground part length, tuber length, longest leaf width, bract length, labellum length[1] and spur length[1]. The underground part of *D. romana* subsp. *romana* have the ability to go to depth 110 mm. *D. romana* have cylindrical or napiform shaped tuber. *D. romana* subsp. *romana* diverges from *D. romana* subsp. *georgica* with 23,28 mm length tuber. Leaf numbers were the same (4-11) in both subspecies. The longest leaf width of in *D. romana* subsp. *romana* was larger than *D. romana* subsp. *georgica*.

D. romana subsp. *romana* flowers are rather large, with a bract length of 12-35 mm (min-max), whereas *D. romana* subsp. *georgica* flowers are smaller, with 15-24 mm bract length (min-max). Six characters (flower color and size, labellum width, spur shape, proportion of spur/ovary and spur position) were used with respect to morphological discrimination of subspecies according to "Flora of Turkey" (Renz & Taubenheim, 1984). The first character is the width of labellum: it is 6-20 mm in *D. romana* subsp. *romana* and 4-8 mm in *D. romana* subsp. *georgica.* The other character is the proportion of spur/ovary. The spur was observed as longer than the ovary in *D. romana* subsp. *romana.* The length of spur is 7-23 mm in *D. romana* subsp. *romana.* While Renz and Taubenheim (1984) declared that length of spur is minimum 13 mm stated in Flora of Turkey we found it as 7 mm.

Table 3. Morphological Characters of *Dactylorhiza romana*.

	Dactylorhiza romana* subsp. *romana					***Dactylorhiza romana* subsp. *georgica***				
	N	**Min**	**Max**	**Mean**	**Std. Dev.**	**N**	**Min**	**Max**	**Mean**	**Std. Dev.**
Length (mm)	70	170	385	266.46 ± 5.80a	48.51	25	201	346	276.92 ± 7.39a	36.96
Underground part (mm)	70	42	110	71.71 ± 1.87a	15.64	25	45	78	56.64 ± 1.71b	8.53
Tuber width (mm)	69	5	25	14.29 ± 0.46a	3.82	25	9	22	13.84 ± 0.57a	2.87
Tuber length (mm)	69	12	35	23.28 ± 0.55a	4.55	25	13	35	20.88 ± 1.03b	5.15
Leaf number	70	4	11	7.00 a	1.51	25	4	11	7.00 a	1.64
Width of longest leaf (mm)	70	5	14	9.23 ± 0.24a	6.95	25	6	13	8.08 ± 0.36b	1.78
Length of longest leaf(mm)	70	75	195	109.09 ± 2.66a	22.29	25	62	132	102.84 ± 3.44a	17.18
Width of shortest leaf(mm)*	56	4	14	6.96 ± 0.27a	2.04	20	5	10	7.45 ± 0.33a	1.47
Length of shortest leaf(mm)	56	28	155	75.54 ± 3.10a	23.21	20	55	105	82.85 ± 3.46a	15.47
Flower number	59	4	16	7.90 ± 0.29a	2.26	20	5	10	7.50 ± 0.41a	1.85
Bract length (mm)	54	12	35	22.81 ± 0.67a	5.47	25	15	24	19.6 ± 0.39b	1.94

* The distribution was not normal, therefore a non-parametric method, Mann-Whitney was used.

Anatomical features

Leaf surface: It was observed that *D. romana* subsp. *romana* and *D. romana* subsp. *georgic*a had no hair in their surface sections. Additionally, stomata cells on abaxial in both subspecies were tetracytic and anomocytic type. Shape of stomata cells were more or less circular. Stomata cell size was 54.98 × 59.76 µm (subsp. *romana*) and 56.66 x 62.08 µm (subsp. *georgica*). Stomata index of the abaxial surface were 32.75 % in *D. romana* subsp. *romana* and 29.62 % in *D. romana* subsp. *georgica*. Shape of the abaxial epidermal cells in *D. romana* subsp. *romana* was narrowly rectangular, while these cells were widely rectangular in *D. romana* subsp. *georgica*. The epidermal cells were placed parellel to the midrib on abaxial. Shape of adaxial epidermal cells in *D. romana* subsp. *romana* were polygonal and in *D. romana* subsp. *georgica* hexagonal. In normal conditions, stomata cells are observed mostly on abaxial, however both subpspecies had rare stomata cells on adaxial. The size of epidermal cells (width-length) in *D. romana* subsp. *romana* and *D. romana* subsp. *georgica* were 94.58 × 152.08 µm, 108.75 × 102.5 µm (subsp. *georgica*) on the adaxial leaf surface and 54.16 × 122.5 µm (subsp. *romama*), 61.67 × 90.42 µm (subsp. *georgica*) on the abaxial leaf surface respectively (Fig. 3 c,d,e,f).

Cross-section: The epidermal cells on both leaf surfaces were covered by cuticle and this layer of both subspecies was thicker on abaxial than on adaxial. Cuticle layer of *D. romana* subsp. *romana* was lyriate on the midrib of both abaxial and adaxial and smooth close to the margins, whereas it was striate on adaxial in *D. romana* subsp. *georgica*. Cuticular thickness of *D. romana* subsp. *romana* was measured as 11.25 µm on the abaxial leaf surface and 7.5 µm on the adaxial leaf surface, while the thickness dimensions were 12.63 µm and 10.52 µm in *D. romana* subsp. *georgica* respectively. Shapes of adaxial epidermal cells of both subspecies were elongated-rectangular, whereas squared and rounded on abaxial epidermal cells of *D. romana* subsp. *romana* and small-squared on abaxial epidermal cells of *D. romana* subsp. *georgica*. The homogenous chlorenchyma layer was consisted of thin-walled, rounded cells. Spongy parenchymatic cells were 6-9 layered in *D. romana* subsp. *romana*, and 7-10 layered in *D. romana* subsp. *georgica*. Parenchymatic cells of midrib were 12-14 layered in *D. romana* subsp. *romana*, and 12-15 layered in *D. romana* subsp. *georgica*. Both subspecies had many lacunas, accumulated on both sides of midrib and close to the adaxial surface. Mesophyll tissue had plenty of raphides. Collateral vascular bundles were comprised of xylem, phloem and sclerenchyma cells (Fig. 3 a,b).

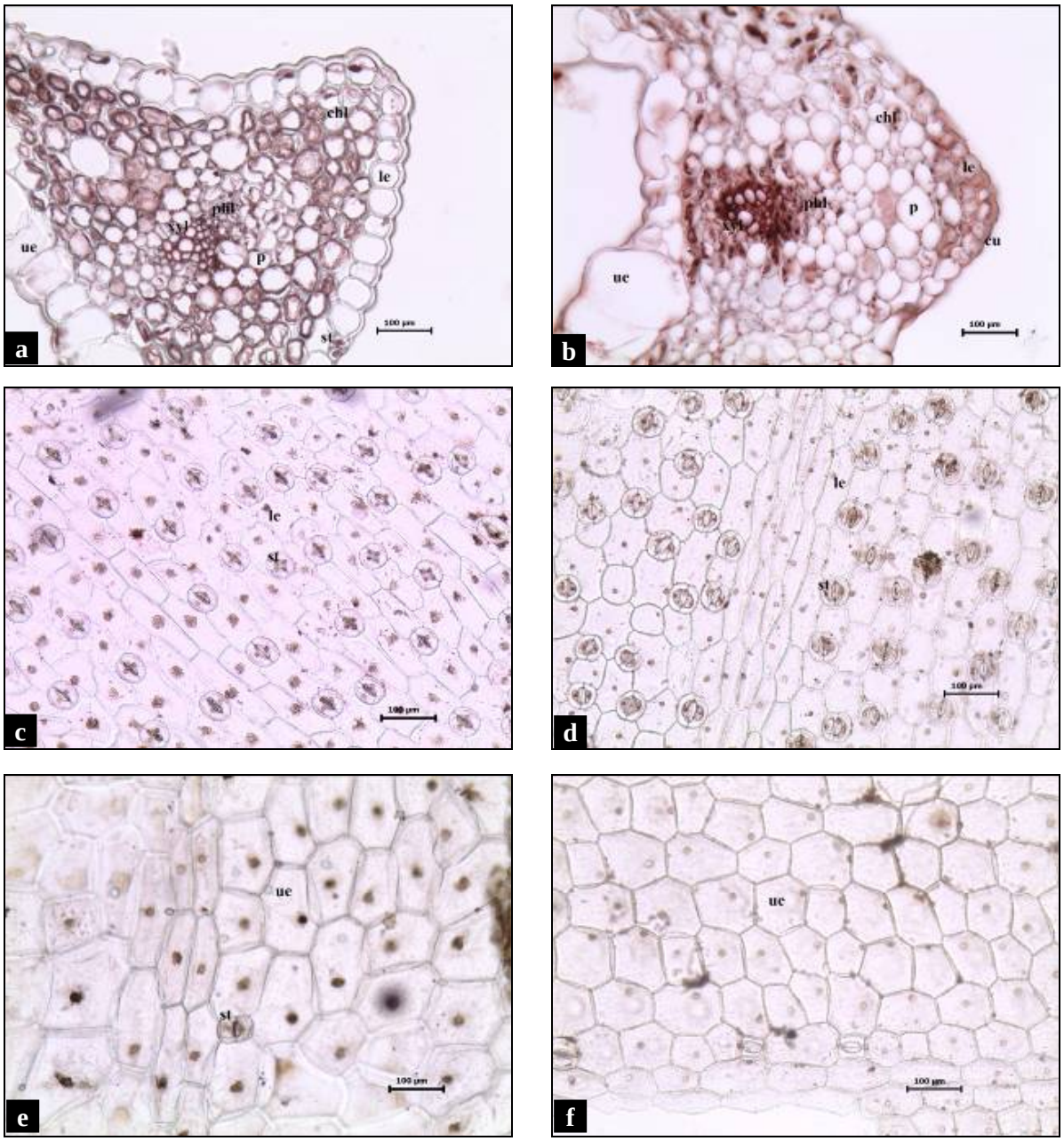

Fig. 3. *D. romana* subsp. *romana*: Transverse section of the leaf (a); abaxial surface of leaf (c); adaxial surface of leaf (e); *D. romana* subsp. *georgica*: Transverse section of the leaf (b); abaxial surface of leaf (d); adaxial surface of leaf (f); cu: cuticle, chl: chlorenchyma, ue: upper epidermis, le: lower epidermis, p: parenchyma, xyl: xylem, phl: phloem, st: stomata, la: lacuna.

Stem: Both subspecies' surface sections of stem lacked hairs and had smooth and thin cuticle. Smooth and thin cuticle were observed at the stem surface. The epidermal cells appear as elongated rectangular shaped at surface sections of the taxa. Stem was angular and its cross section consisted of epidermis, cortex, abaxial ground tissue, vascular bundles and adaxial ground tissue. The epidermal cells were uniseriate, small and ovate shaped. Stomata were rarely detected. Cortex was composed of 3-4 layered, thin-walled, ovate-rounded parenchymatic cells. Shapes of intercellular spaces were trigonal to polygonal. Only *D. romana* subsp. *georgica* had lacunas in cortex. Ground tissue comprised of two different paranchymatic pithes. Abaxial ground tissue cells were 4-6 layered in subsp. *romana* and 5-7 layered in subsp. *georgica*. These cells are thick walled and lignified where they were observed as thicker in *D. romana* subsp. *georgica* than in *D. romana* subsp. *romana*. On the other hand, adaxial ground tissue cells characterized with their thin wall and very large rounded-ovate shape were counted as 13-15 layered in *D. romana* subsp. *romana* and 9-10 layered in *D. romana* subsp. *georgica*. Raphide bundles were more dense in adaxial ground tissue cells. Vascular bundles were collateral type and located just below the lignified abaxial ground tissue. Smaller vascular bundles, located in abaxial ground tissue, were detected between two large vascular bundles in *D. romana* subsp. *romana*. Vascular bundles were surrounded by 1-2 thick walled lignified tissue in *D. romana* subsp. *georgica*. Abaxial ground tissue of *D. romana* subsp. *georgica* were arranged in smaller (Fig. 4 a, b, c, d).

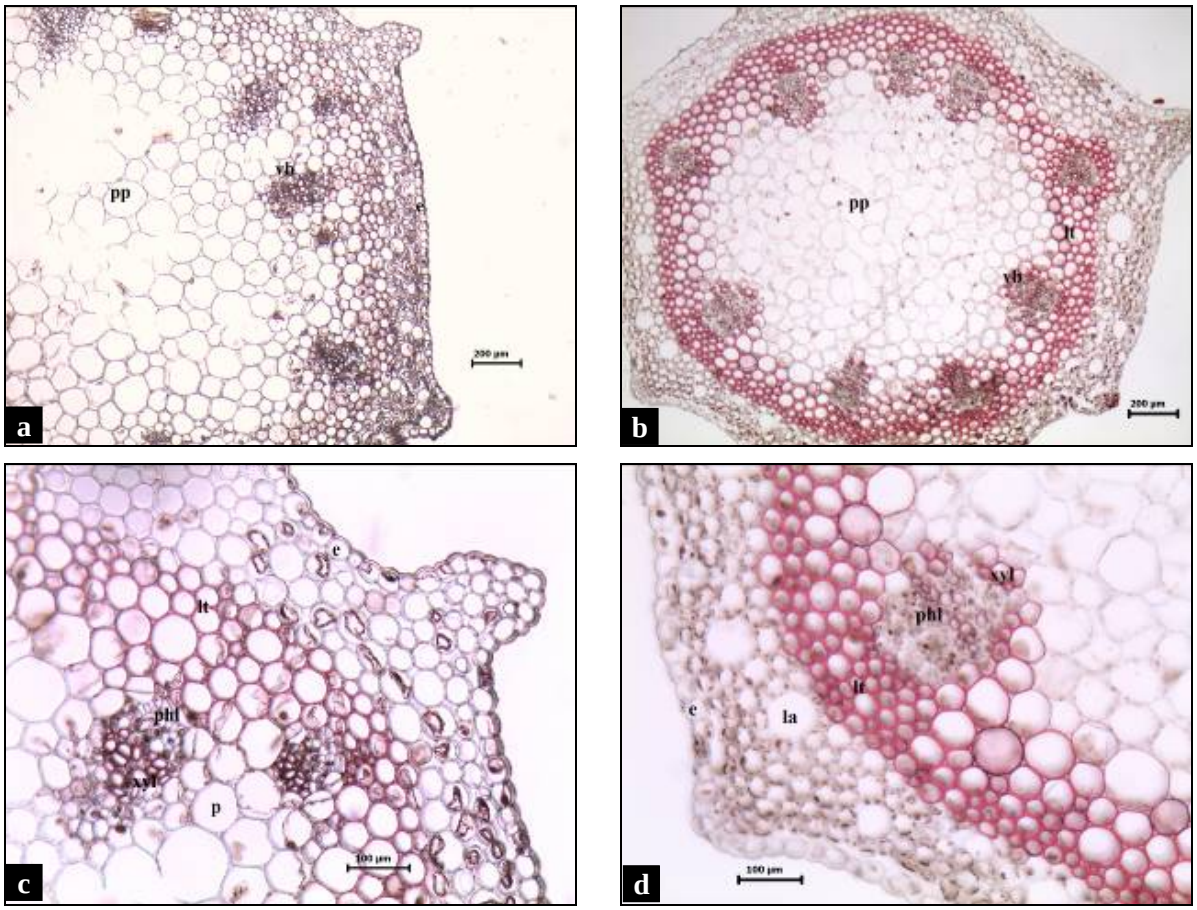

Fig. 4. Transverse section of the stem, *D. romana* subsp. *romana* (a, c); *D. romana* subsp. *georgica* (b, d); e: epidermis, lt: lignified tissue, p: parenchyma, xyl: xylem, phl: phloem.

Tuber: Hairs at tubers were unicellular. Tuber exodermis were composed of 3-4 layered, rectangular, small and thin walled cells. These cells were observed more flat in subsp. *georgica*. Tuber ground tissue was comprised of mucilage and parenchymatous cells. The parenchymatous cells were large, polygonal, lacked of intercellular spaces and had starch grains and raphide bundles. The size of parenchymatous cells were larger in subsp. *romana* than in subsp. *georgica*. Starch grain size was 15.05 x 15.00 µm (width-length) in subsp. *romana* and 16.75 x 20.83 µm (width-length) in subsp. *georgica*. The maximum raphide length was measured as 50.05 µm in *D. romana* subsp. *romana* and 70.84 µm in *D. romana* subsp. *georgica*.

In both subspecies single-layered tissue constituted tuber endodermis. Pericycle and endodermis were single layered. The arrangement of vascular bundles were tetraarch and were embedded in the ground tissue and named as meristeles. They are surrounded by endodermis and pericycle (Fig. 5 a,b,c,d).

Root: The epidermis and endodermis of the root are uniseriate in both subspecies. Epidermal cells were squared or rectangular shaped and thin walled while exodermal cells were ovate, rectangular shaped, elongated and regularly thin walled with 1-2 layers. Anticlinal walls of exodermal cells were smooth. Hairs at roots were unicellular. The cortex is composed of 8 to 10 layered numerous large parenchymatic cells. The shape of cortical cells ranged from globular to oval and their walls were thin. The parancymatic cells located far from center were smaller than the ones close to the center. In the cortex, scattered idioblastic cells contained numerous raphides. Fungal pelotons and hyphae were present in the all cortical cells. Endodermal cell walls have casparian strips. Pericycle was uniseriate, and composed of variously isodiametric shaped, thin-walled cells. Compressed tissue size smaller than endodermis. The vascular bundles were arranged regularly as 5-arched. The vascular elements were embedded in the parenchyma at the periphery of the central cylinder. Xylem had 3 to 5 vesseltubes. Phloem elements were elliptical to rounded patches. The pith was made of parenchymatous cells. Pith cells were composed of thick-walled polygonal and round shaped cells. In addition, intercellular spaces were absent (Fig. 6 a,b,c,d). The subspecies exhibit similarity in terms of root anatomy.

Some anatomical features revealed significant difference between 2 subspecies such as stomata index, size of epidermal cells, cuticle type, cuticle thickness, chlorencyma cell layer in leaf, ground tissue cell rows in stem, lacuna in stem cortex, vascular bundles were surrounded lignified tissue in stem tuber. Aybeke *et al*., (2010) had put forward the anatomical features of the species *D. romana* subsp. *romana.* The stoma index of leaf surface was detected at lower value in our study. While cross section investigations of the leaf we found that cuticle thickness was declared as 10.2 µm -10.2 µm (abaxial-adaxial) we found those values as 11.25 µm- 7.5 µm. Even though stomata cells were observed on abaxial surfaces we detected stomata cells on adaxial surfaces of both subspecies.

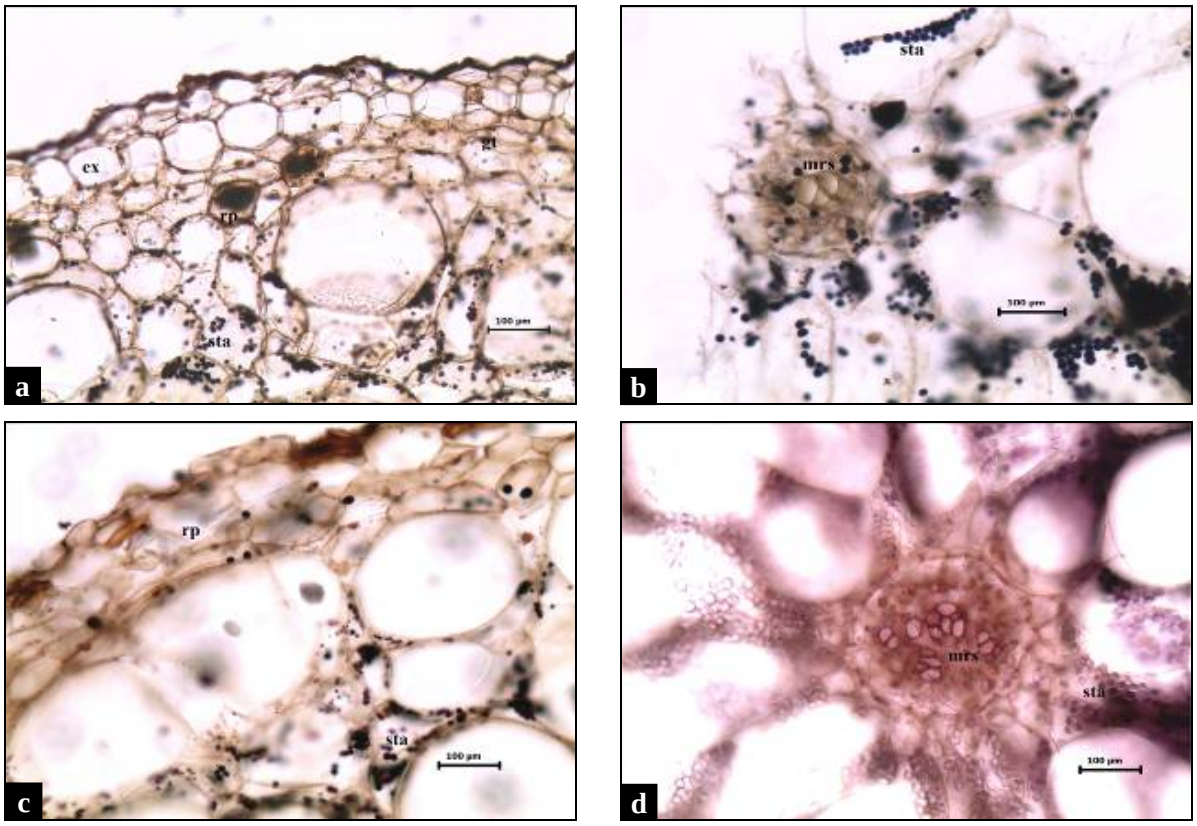

Fig. 5. Transverse section of the tuber, *D. romana* subsp. *romana* (a, b); *D. romana* subsp. *georgica* (c, d); vel: velamen, ex: exodermis, gt: ground tissue, mrs: meristele, rp: raphide, sta: starch.

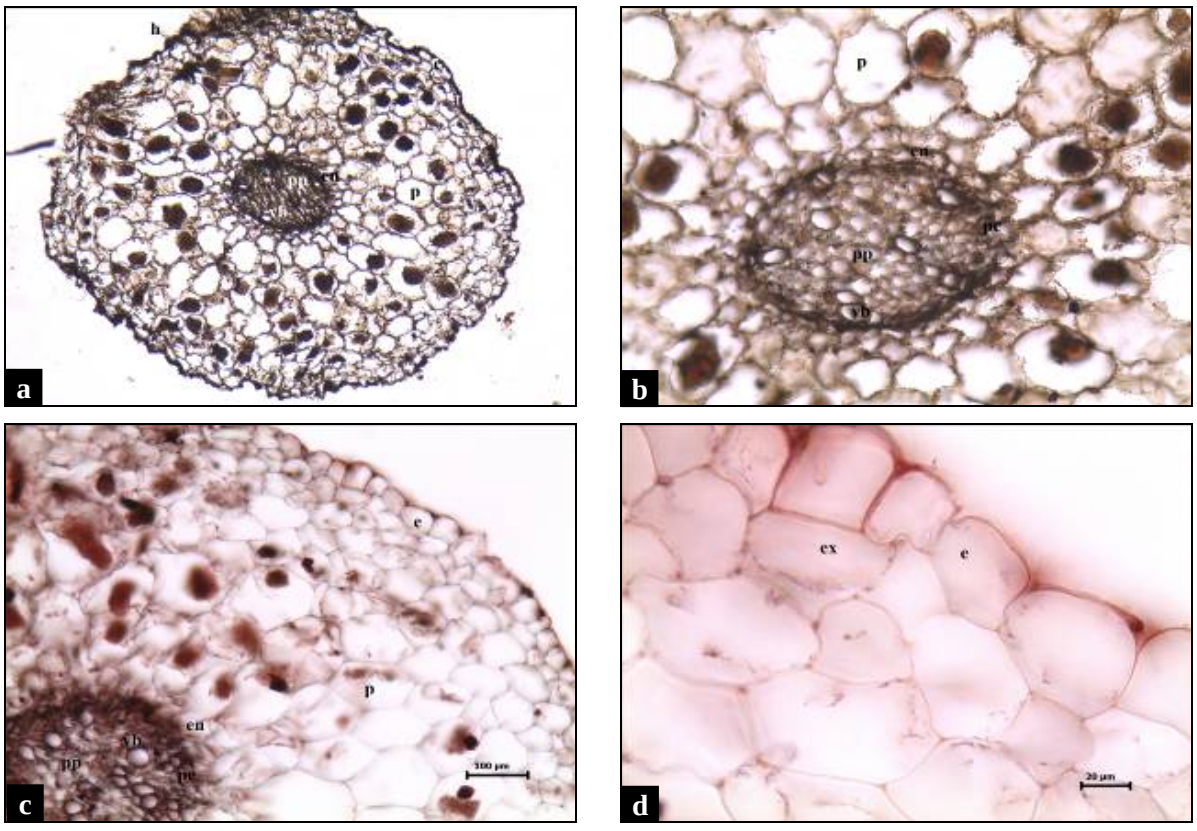

Fig. 6. Transverse section of the root, *D. romana* subsp. *romana* (a, b); *D. romana* subsp. *georgica* (c, d); e: epidermis, ex: exodermis, p: parenchyma, en: endodermis, pe: pericycle, vb: vascular bundle; pp: parenchymatous pith.

Ecological features: *D. romana* subsp. *romana* was recorded at 98 locations as given at the previous studies and 9 records were added to the current literature data within this study. According to available data the distribution of the species are at Amasya, Antalya, Aydın, Balıkesir, Bilecik, Bitlis, Bursa, Çanakkale, Çankırı, Gümüşhane, İstanbul, Kastamonu, Konya, Muğla, Samsun, Sinop, Tokat, Hatay provinces (Fig. 1). The other sub-species were recorded at 24 locations according to previous studies and 7 more locations were added by this study resulting 31 locations totally. According to all data in literature *D. romana* subsp. *georgica* is distributed at Artvin, Bitlis, Gümüşhane, Hakkari, Isparta, Kastamonu, Siirt, Tokat, Trabzon, Van and Yozgat provinces.

According to altitudinal distribution *D. romana* subsp. *romana* is located between 55 – 1900 m asl while *D. romana* subsp. *georgica* showed its altitudinal distribution between 700–2100 m asl whereas *D. romana* subsp. *romana* demonstrated a wider altitudinal range. The records in literature exposed that subsp. *romana* showed negative while subsp. *georgica* showed positive relation with increasing altitude (Fig. 7a). There is a higher relation between existences of subsp. *georgica* at percentage and altitude with R^2 0.57. While subsp. *romana* is disappeared after the elevation 1600 m, subsp. *georgica* has its highest distribution and also disappeared at elevation below 700 m. Species generally preferred forest lands for their main distribution (Fig. 7b) whereas subsp. *romana* was also met under olive farm lands while subsp. *georgica* could only be seen at abandoned agricultural lands.

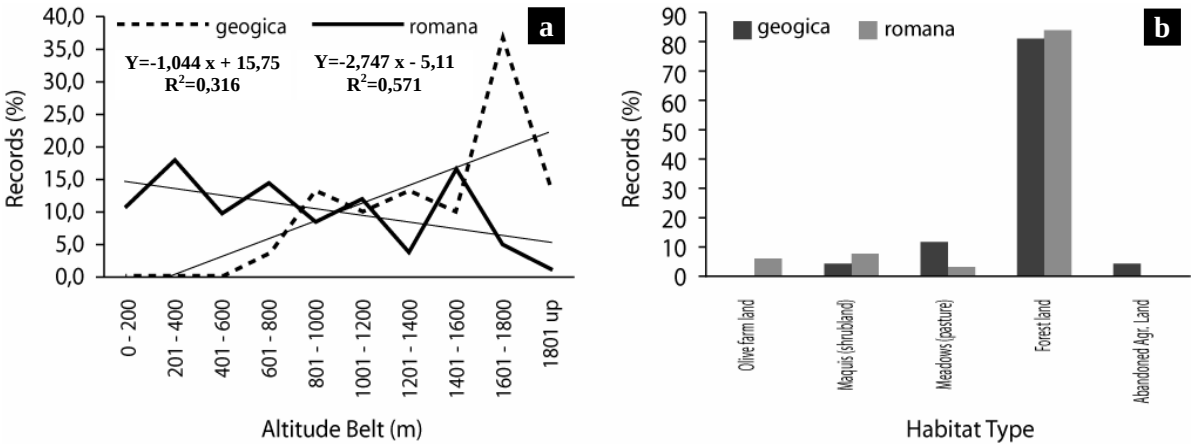

Fig. 7. Record numbers of *Dactylorhiza romana* subsp. *romana* and subsp. *georgica* % values a: according to altitude; b: habitat selections.

Statistically no significant differences could be detected between soil properties of sub-species. The soils are generally stony and have light soils with coarse texture (Çepel, 1988) where the sand rate of the soils is 52.06% (Table 4). However there no statistically significant difference could be detected at pH of soils for 0-5 cm depth the pH of subsp. *romana* was 0.4 pounds higher than subsp. *georgica* (Table 4). Both subspecies prefer soils with high organic matter content. That should be attributed to the forest habitat selection of the subspecies since forest lands offer high organic matter content (Fisher & Binkley, 2000).

Table 4. Soil properties of sampling points *in Dactylorhiza romana* subsp. *romana* and *Dactylorhiza romana* subsp. *georgica* (T testi p <0.05).

Soil properties		*Dactylorhiza romana* subsp. *romana*					*Dactylorhiza romana* subsp. *georgica*				
		N	Min	Max	Mean	Std. dev.	N	Min	Max	Mean	Std. Dev.
0-5 cm	Volume weight (g/l)	8	819.20	1280.70	1031.05 ± 58.84a	166.43	7	588.60	1160.30	900.06 ±81.99a	216.93
	Fine soil weight(g/l)	8	573.00	977.70	722.38 ± 43.37a	122.67	7	516.50	904.40	668.23 ±62.20a	164.57
	Stone weight (g/l)	8	76.80	574.30	308.68 ± 53.31a	150.78	7	15.50	643.80	231.83 ± 2.66a	245.14
	Sand (%)	8	52.06	81.63	71.18 ± 3.50a	9.90	7	56.47	70.31	64.35 ± 2.27a	6.02
	Silt (%)	8	5.36	28.51	14.17 ± 2.70a	7.65	7	10.94	28.43	19.06 ± 2.03a	5.38
	Clay (%)	8	9.72	19.43	14.65 ± 1.15a	3.26	7	10.63	23.80	16.59 ± 1.86a	4.92
	pH	8	5.46	7.13	6.20 ± 0.21a	0.61	7	4.80	6.68	5.82 ± 0.24a	0.62
	Corg (%)	8	1.64	5.15	3.14 ± 0.43a	1.22	7	1.59	6.00	3.67 ± 0.60a	1.58
	Nt (%)	8	0.02	0.21	0.11 ± 0.03a	0.08	7	0.04	0.27	0.12 ± 0.03a	0.07
	C//N	8	15.68	71.00	42.34 ± 8.12a	22.96	7	13.97	66.42	38.32 ± 7.35a	19.44
5-15 cm	Volume weight (g/l)	8	938.20	1395.80	1202.78 ± 57.60a	162.93	7	795.60	1263.10	1138.57 ±61.11a	161.67
	Fine soil weight(g/l)	8	652.30	1082.00	830.73 ± 51.58	145.90	7	514.00	992.10	781.81 ± 59.28a	156.83
	Stone weight (g/l)	8	155.70	658.40	371.94 ± 56.03a	158.48	7	152.70	644.50	356.76 ± 60.82a	160.92
	Sand (%)	8	50.18	79.79	68.82 ± 3.79a	10.72	7	63.14	70.45	66.57 ± 1.09a	2.89
	Silt (%)	8	5.34	28.33	15.00 ± 3.25a	9.18	7	10.81	21.86	16.78 ± 1.73a	4.59
	Clay (%)	8	9.42	21.49	16.19 ± 1.40a	3.95	7	12.55	22.40	16.65 ± 1.49a	3.94
	pH	8	5.27	7.44	6.23 ± 0.24a	0.67	7	4.82	6.53	5.85 ± 0.24a	0.65
	Corg (%)	8	0.71	2.84	1.60 ± 0.23a	0.64	7	0.00	7.57	2.03 ± 0.99a	2.62
	Nt (%)	8	0.00	0.06	0.03 ± 0.01a	0.02	7	0.01	0.11	0.04 ± 0.01a	0.03
	C//N	8	20.97	190.54	74.62 ± 18.40a	52.05	7	1.48	169.31	52.54 ± 20.89a	55.27

We can conclude that there are morphological, anatomical and ecological differences between two subspecies. The underground part length, tuber length, longest leaf width, and bract length, labellum length and spur length characters revealed differences in respect to morphological characteristics. The stomata index, size of epidermal cells, cuticle type, cuticle thickness, chlorencyma cell layer in leaf, ground tissue cell rows in stem, lacuna in stem cortex features were differing between the subspecies and additionally vascular bundles were surrounded lignified tissue in stem tuber. Those features are subsidiary specifications at diagnosis of subspecies. Even though habitat and soil characteristics demonstrate similarity their altitudinal and horizontal spreading characteristics show differences from each other. Whilst the presence of *D. romana* subsp. *georgica* increase with increasing altitude the *D. romana* subsp. *romana* disappears.

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