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Morphological and anatomical features of seeds of Turkish *Romulea* taxa (Iridaceae) and their taxonomic significance

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Abstract – This paper reports on the assessment of morphological (macro and micro) and anatomical characters of seeds of *Romulea* taxa distributed in Turkey with the use of one-way analysis of variance, cluster analysis and principal component analysis. Morphological characteristics such as size, shape, color and surface of seeds were examined with the use of light and scanning electron microscopes. Thicknesses of testa and phytomelan layer, sizes of embryo in seeds were studied anatomically. The outcomes revealed that taxa were similar in some aspects such as color and shape of seeds. However, seed size, thickness of testa and phytomelan layer, shape of the epidermal cells in testa and sizes of embryo were different among taxa, and have taxonomic value in the distinction of these taxa from each other. In addition, the seed surfaces were more or less different for the examined taxa on an interspecific level. Consequently, seed morphology and anatomy with a few exceptions demonstrated diversity and they had taxonomic importance in terms of distinguishing among species.

Keywords: Anatomy, morphology, phytomelan, *Romulea*, scanning electron microscopy, seed, Turkey

Introduction

Romulea Maratti belongs to the subfamily Crocoideae (Ixioideae) in the family Iridaceae. The Ixioideae is the biggest subfamily of Iridaceae, with more than 800 species of 30 genera (Isik and Donmez 2007). The Ixoideae is a consistent subfamily in terms of morphologic and anatomic features (Rudall and Goldblatt 1991, Ozdemir et al. 2011). The *Romulea* genus originated from South Africa, and spreads over a wide area, including the Mediterranean zone, has more than 90 species in a range of distribution areas. The genus *Romulea* is represented by 7 taxa in Turkey, all of which are listed in table 1 (Marais 1984, Erol and Kucuker 2003).

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Tab. 1. Examined taxa and their origin.

No	Taxa	Locality (Origin)
R1	*R. bulbocodium* (L.) Seb. & Mauri var. *bulbocodium*	Izmir, Çeşmealti
R2	*R. bulbocodium* (L.) Seb. & Mauri var. *crocea* (Boiss. & Heldr.) Baker	Antalya, Manavgat
R3	*R. bulbocodium* (L.) Seb. & Mauri var. *leichtliniana* (Heldr. ex Hal.) Bég.	Hatay, Samandag
R4	*R. tempskyana* Freyn	Antalya, Falezler
R5	*R. ramiflora* Ten. subsp. *ramiflora*	Balikesir, Patria
R6	*R. linaresii* Parl. subsp. *graeca* Bég.	Istanbul, Kurtköy
R7	*R. columnae* Seb. & Mauri subsp. *columnae*	Izmir, Yamanlar

In the classification of *Romulea* species sexual parts such as anther, filament and stigma are often used to separate the taxa from each other. However, classification of *Romulea* taxa in Turkey as well as in Sicily and South-West Europe is difficult because of the frequent emergence of gynodioecism, which is the presence of only hermaphrodite or female flowers in a population. Namely, if there are only female flowers on the plants, *Romulea* taxa look morphologically similar (Davis 1984, Moret et al. 1992, Moret et al. 1993). This situation leads to some problems in their classification from time to time, thus additional characters that will support the existing identification are needed in the classification of Turkish *Romulea* taxa.

Seeds and small fruits have typical morphological features, such as shape, colour, dimension, microstructure (including ultrastructure) and they may provide valuable contributions to plant taxonomy. In a practical sense, many significant data gathered from seed or fruit epidermal surface analyses are acquired with scanning electron microscope (SEM). These surface features include micromorphological characteristics, such as shape of the cells, seed surface ornamentation and epicuticular protrusions. These features demonstrate a large diversity from subspecies to family level and they are not readily changed under the influence of habitat conditions (Barthlott 1981). However, anatomical features are usually as helpful as morphological features for plant diagnostics, and they often are useful in the separation of closely related taxa (Karamian et al. 2012, Akalin-Urusak and Kizilarslan 2013).

The significance of morphology and anatomy in the classification of taxa in the Iridaceae has been revealed by various studies (Marais 1984, Erol and Kucuker 2003, Isik and Donmez 2007). Accordingly, the main objective of this study is to investigate morphological and anatomical features of the seeds of the *Romulea* species native to Turkey with light microscope (LM) and SEM in order to discover differences and specificities of the seed morphology and anatomy of examined taxa with the use of statistical analysis. Exhaustive description of seed structures of the examined species are offered in this paper for the first time.

Materials and methods

Plant material

Seeds of 7 taxa, which had been gathered from native populations in Turkey and cultivated at the Alfred Heilbronn Botanical Garden in Istanbul University, were utilized for

morphological and anatomical observations. Investigations were performed with 10 ripe seeds for each taxon. The taxa examined and their origins are given in table 1.

Morphological and anatomical analyses

Macromorphological features such as the shape, size, raphe presence or absence and colour of the seeds were examined using an Olympus ZS51 stereomicroscope and Kameram Imaging Software. For micromorphological observations of seed surface ornamentation, specimens were prepared for electron microscopy by mounting with silver adhesive on the stub, coated with gold, and analysed with a JEOL Neoscope-5000 scanning electron microscope.

For anatomical features such as testa thickness, phytomelan layer thickness, shape of epidermal cells and embryo size, cross sections were taken with a fully automatic microtome (Thermo Shonda Met Finesse) from the middle of the seeds. Afterwards, they were passed through a variety of alcohol and xylene series, and stained with haematoxylin (Harris-RRSP67-E) in a staining device (ASC 720 Medite), and were covered with Entellan for anatomical structures to be examined (Inceer et al. 2010). Anatomical characters were observed using an Olympus CX21FS1 microscope and Kameram Imaging Software.

The terminology of morphological and anatomical characters was carried out in accordance with Stearn (1985).

Statistical analyses

Data of studied parameters were performed with the SPSS computer program. The Duncan multiple-range test was utilized to determine the statistical significance of differences among the values obtained for different taxa. Grouping of taxa was carried out utilizing the clustering analysis method (UPGMA) in accordance with quantitative characters in Tab. 2. In addition, principal component analysis (PCA) ordination and similarity matrix were performed based on some morphological and anatomical characters of seeds such as seed dimensions, testa thickness, phytomelan layer thickness and embryo sizes.

Results

Seed size, raphe and shape characters of taxa were evaluated macromorphologically and differences among the studied taxa were observed (Tab. 2). In seed dimensions, R6 and R7 had the greatest differences among the examined species. Their values range from 2.04 mm to 4.54 mm for length and from 2.88 mm to 3.67 mm for width, respectively. Colour of seeds in all cases is orange, but colour tones are different in R1 (darker). However, colour of seeds is determined to be unimportant in the distinction of the taxa of *Romulea* examined. The shape of seeds in these taxa indicates major differences. Likewise, the ratio values of the seed sizes show variations, which vary between 0.60 and 1.28. Seeds are ovate in R1 (0.60), R2 (1.28), R4 (0.72), R5 (1.10), R6 (0.70), and elliptical in R3 (1.25), R7 (1.23). However, the raphes of the taxa also differ. The raphe is very prominent in R4 and R7, compared to other species (Fig. 1).

Seed surfaces of the studied taxa exhibited diversity of surface ornamentation (Fig. 2 and Tab. 2). The seed surface ornamentation, which is a useful character in the separation of

Tab. 2. Morphological and anatomical features in seeds of the Turkish *Romulea* taxa. Results represent mean values ± standard deviation; means with different letters are significant at p = 0.05 level (Duncan's multiple-range test), for taxa abbreviations see Tab. 1; L – length, W – width.

Taxa	Colour	Shape	Seed size		Ratio (L/W)	Seed surface ornamentation	Testa thickness (µm)	Phytomelan layer thickness (µm)	Testa epidermal cells	Embryo sizes	
			L (mm)	W (mm)						L (µm)	W (µm)
R1	Dark orange	Ovate-Transverse	2.14± 0.15de	3.53± 0.11c	0.60	Reticulate	218.84± 10.70ab	54.20± 5.81d	Undulated	298.38± 11.32d	224.47± 15.35cd
R2	Orange	Ovate-Late	3.87± 0.12a	3.02± 0.21de	1.28	Reticulate-Foveate	213.05± 8.64b	86.01± 6.37b	Crushed	317.91± 7.55c	289.25± 11.65b
R3	Orange	Elliptical-Late	3.04± 0.28c	2.43± 0.08e	1.25	Reticulate-Areolate	81.93± 10.56e	54.11± 10.69cd	Crushed	175.98± 35.99f	156.82± 21.24e
R4	Orange	Ovate-Latissime	2.51± 0.17d	3.48± 0.04b	0.72	Reticulate	238.31± 5.58a	77.03± 11.38bc	Crushed	246.04± 34.08e	196.41± 18.63d
R5	Orange	Ovate-Late	3.56± 0.08b	3.21± 0.12d	1.10	Reticulate-Areolate	183.21± 14.63c	56.01± 9.41cd	Crushed	388.80± 11.79a	328.60± 12.42a
R6	Orange	Ovate-Depresse	2.04± 0.16e	2.88± 0.19de	0.70	Reticulate	135.79± 19.25d	99.32± 3.54a	Crushed	351.44± 17.43b	281.12± 33.49bc
R7	Orange	Elliptical	4.54± 0.49a	3.67± 0.38a	1.23	Alveolate	156.53± 20.91cd	63.63± 2.93c	Flat	296.63± 12.68d	245.33± 10.66c

taxa, varies from slightly (R3) or intensively (R2) reticulate to alveolate (R7). However, seeds of R5 have a vaguely reticulate-areolate sculpture, while there are elevated protrusions on the seed surface of R1 which is distinguished by these protrusions from R4 and R6 (Fig. 2). On the other hand, the seed surface patterns of R4 and R6 are not significant in terms of the separation of taxa (Tab. 2).

The anatomical characteristics of the seeds of Turkish *Romulea* are given in table 2. Accordingly, the mean values of testa thickness vary from 81.93 µm to 238.31 µm, and this character varied significantly among the examined taxa. A thick testa is noted in R4, whereas the testa in R3 is thin (Tab. 2). In other respects, shapes of epidermal cells of testa differ in the studied taxa (Tab. 2), which have elevated-flat, crushed and undulating cells in unequal or

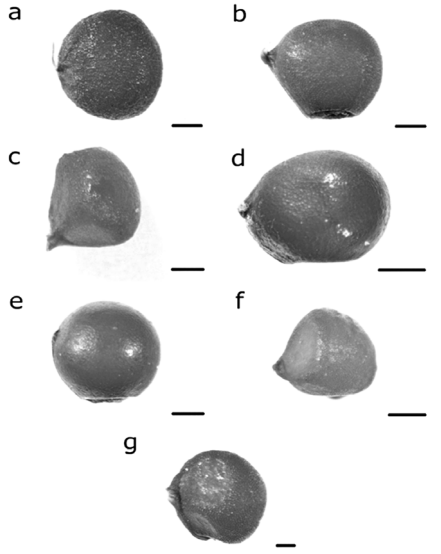

Fig. 1. Turkish *Romulea* seeds; a: R1, b: R2, c: R3, d: R4, e: R5, f: R6, g: R7; for taxa abbreviations see Tab. 1; scale bars = 1 mm.

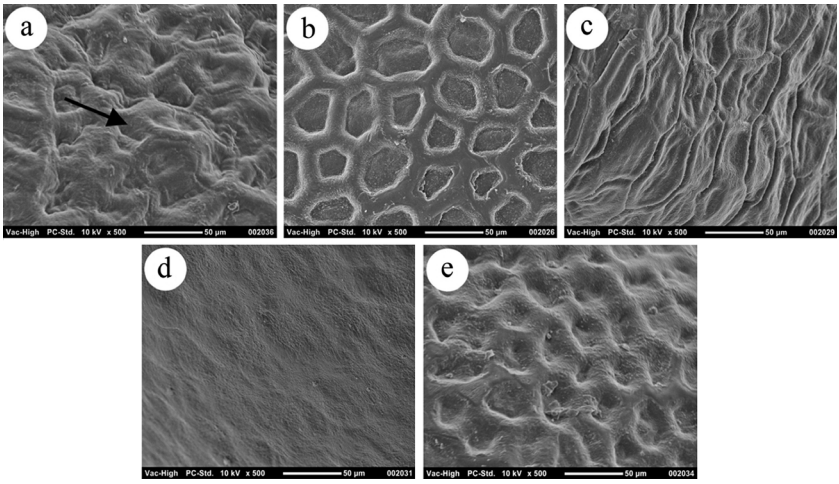

Fig. 2. Seed surfaces of *Romulea* taxa; a: R1; Reticulate, b: R2; Reticulate-Foveate, c: R3; Reticulate-Areolate, d: R5; slightly Reticulate-Areolate, e: R7; Alveolate; for taxa abbreviations see Tab. 1. The arrow shows protrusion on the seed surface.

equal forms, and thick or thin walls in cross sections (Fig. 3). In addition to these findings, embryo sizes of the seeds have been shown to range from 175.98–388.80 µm to 156.82–328.60 µm, and embryos in seeds formed oval (R4 and R5), globular (R2 and R3) and prolonged (R1, R6 and R7) in shapes (Fig. 3).

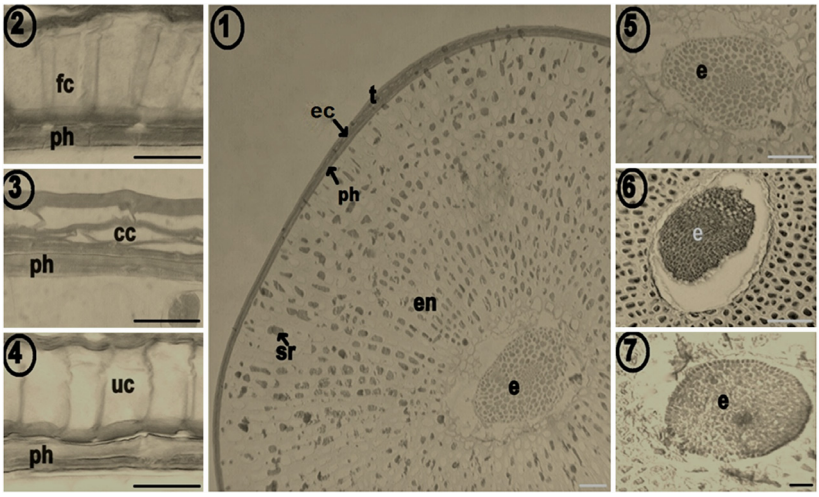

Fig. 3. Cross section structures of Turkish *Romulea* seeds; 1: overall appearance; anticlinal cell types: 2: flat cell (R7), 3: crushed cell (R2, R3, R4, R5 and R6), 4: undulated cell (R1), types of embryo, 5: oval (R4 and R5), 6: prolonged (R1, R6 and R7), 7: globular (R2 and R3); for taxa abbreviations see Tab. 1; e – embryo, en – endosperm, sr – storage reserve, t – testa, ec – epidermal cells of testa, fc – flat cell, ph – phytomelan layer, cc – crushed cell, uc – undulated cell; white scale bars = 100 μm, black scale bars = 50 μm.

An unweighted pair group method with arithmetic mean (UPGMA) dissimilarity clustering dendrogram for Turkish *Romulea* taxa according to the examined quantitative characters in Tab. 2 is given in Fig. 4. Accordingly, branches in the dendrogram are divided into two main clads and sub-clads. R7 composes the first main branch; other taxa make up the second main branch. Also, R7 differs clearly from other taxa in terms of characters deter-

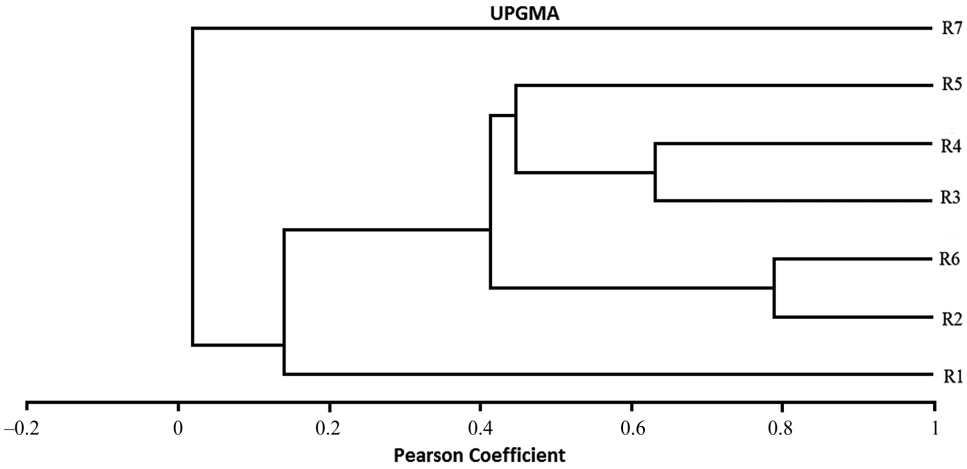

Fig. 4. UPGMA clustering of *Romulea* taxa in Turkey based on morphological and anatomical characters of the seeds; for taxa abbreviations see Tab. 1.

mined in Tab. 2. Furthermore, PCA ordination and similarity matrix based on some morphological and anatomical characters of seeds such as seed dimensions, testa thickness, phytomelan layer thickness and embryo sizes are presented in Fig. 5 and Tab. 3. Among the studied taxa, the closest and the most distant species are determined. According to these results, R6 and R2 are the most closely related species (percentage similarity: 0.789), R6 and R7 are the most distantly related species (percentage similarity: –0.169) (Tab. 3 and Fig. 5). In addition, the cumulative variance value of principal components achieved 63.14 % (axis 1: 32.29%, axis 2: 30.85%).

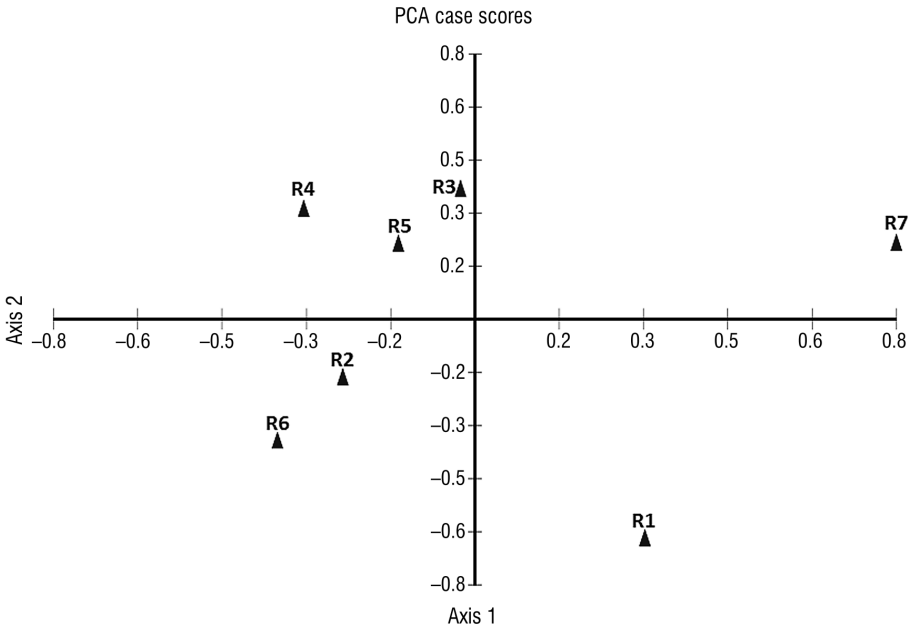

Fig. 5. Principal component analysis of *Romulea* taxa in Turkey based on morphological and anatomical characters of the seeds; for taxa abbreviations see Tab. 1.

Tab. 3. Similarity matrix among the Turkish *Romulea* taxa based on some morphological and anatomical characters of seeds; for taxa abbreviations see Tab. 1.

Taxa	R1	R2	R3	R4	R5	R6	R7
R1	1	–	–	–	–	–	–
R2	0.310	1	–	–	–	–	–
R3	0.070	0.514	1	–	–	–	–
R4	–0.044	0.383	0.633	1	–	–	–
R5	0.070	0.514	0.514	0.383	1	–	–
R6	0.292	0.789	0.310	0.450	0.310	1	–
R7	0.070	0.029	0.271	–0.118	0.029	–0.169	1

Discussion

The morphological (macro and micro) and anatomical characters of seeds frequently indicate major differences among taxa of the same genus or family, and these differences provide a significant contribution to delimiting various taxonomic levels of taxa and to the establishment of taxonomic relationships. Although reliability and sustainability of seed properties in classification are known (Dahlgren and Clifford 1982, Grilli Caiola et al. 2010), no researches appeared to have been performed so far concerning seed morphology and anatomy of *Romulea* taxa in Turkey.

In this investigation, macromorphological features such as size, shape and raphe characters of seeds of *Romulea* taxa in Turkey have indicated the presence of variations among the examined taxa. Although it is reported as being only globular in the Flora of Turkey and Aegean Islands by Marais (1984), seed shape in almost all of the examined *Romulea* taxa varied (excluding seed shapes of R2 and R5) (Tab. 2). The seed sizes of *Romulea* taxa are quite variable. The broadest seeds are found in R7, and the smallest seeds in R6. Both of these measurements are considerably different from those obtained from other examined taxa. These variations in the seed shape are compatible with the diagnostic characters in Flora of Turkey (Davis 1984) for the *Romulea* species. In contrast to the findings of Marais (1984), the seed colour of Turkish *Romulea* taxa has been found to be orange and its tones rather than brown, and the colour of seed has no use taxonomically. One of the remarkable characters is also the presence of raphes on seeds, and their size. The raphes of R4 and R7 are more pronounced than on other examined taxa, and there is no prominent raphe in R1 (Fig. 1). At the same time, the sizes of seeds having a raphe are generally smaller than those do not have a marked raphe. This result is attributable to the principle that wind-dispersed seeds should be in small size and should bear raphe-like structures (Tebbitt 2005).

The seed morphology has not been sufficiently utilized in the taxonomy of Iridaceae family (Erol et al. 2006). Results obtained from this research indicate that some macromorphological features, particularly the shapes and sizes of seeds of Romulea taxa reveal as distinctive characters in terms of classification, because of their diversity among taxa, so they may be used as supporting characters in taxonomic studies.

Seed surface structures have been utilized for some purposes such as the solution of systematic problems, interpretation of evolutionary interactions, and illumination of the adaptive features of the seed surface (Heywood 1971, Sulaiman 1995). In this investigation, the surface patterns range from slightly (R3) or intensively (R2) reticulate to alveolate (R7) in the examined taxa. R5 has very weak reticulate-areolate surface and the seed surface of R1 has visible raised protrusions (Fig. 2). And so, the surface patterns of seed coats are found helpful in taxonomy for some *Romulea*. Nonetheless, variations in the obtained results indicate that the seed surface characters support the frequently used characters in the systematics of the genus (according to Davis 1984) (except for R4 and R6), and can contribute to classification of Turkish *Romulea* species.

The testa thicknesses and epidermal cell types of the seeds can provide precious data about phylogenetic classification of flowering plants (Corner 1976). In this investigation, the differences in anatomical features such as testa thickness, phytomelan thickness, shape of epidermal cells of testa and embryo size of the seeds of Turkish *Romulea* have been identified. The most distant species are R3 (81.93 μm) and R4 (238.31 μm), while the other species examined are located between 81.93 μm and 238.31 μm. Likewise, the epidermal

cells of testa indicate difference in Turkish *Romulea* species. They form different shapes such as undulating (R1), polygonal crushed (R2–R6) and flat (R7) in the cross sections of the seeds of the examined taxa (Tab. 2). Accordingly, epidermal cells can be used in the separation of R1 and R7 from other taxa, and can be of some use in the taxonomy of examined taxa. In this regard, the results obtained are consistent with a previous similar study in Iridaceae (Grilli Caiola et al. 2010).

The taxonomic significance of phytomelan-covered seeds was explained by Huber (1969), and the presence-absence and thickness (if any) of phytomelan layer are of notable taxonomic significance in the distinction of taxa in the Iridaceae family (Dahlgren and Clifford 1982). The thickness of the phytomelan layer in Turkish *Romulea* species ranged from 54.11 μm to 99.32 μm and changed significantly in examined *Romulea* species (Tab. 2). This finding attributed as the thickness of phytomelan layer can be useful in the classification of Turkish *Romulea* species because of differentiated thickness in almost all of the examined taxa in this investigation (Tab. 2).

The embryos of the Turkish *Romulea* taxa exhibit a wide variety in terms of shapes and sizes (Tab. 2). While the embryo in R2 and R3 is usually swollen and well arranged, the embryo in other examined taxa is more or less elongated (as if not well developed).The anatomical findings of this investigation are compatible with those of Grilli Cailo et al. (2010), who have made a study with similar variables including anatomical features on some *Crocus* seeds.

One of the most important characters which distinguish R1, R4 and R6 from each other is the colour and length of filaments (Davis 1984). However, it is impossible to separate the taxa from each other in the case of gynodioecism in one of the populations of these taxa since there will be only female flowers. In this investigation, seed shape, size and presence of raphe on seeds morphologically; thickness of phytomelan layer, embryo size anatomically are applicable characters in distinguishing these taxa.

UPGMA of cluster analysis was used to evaluate the morphological and anatomical features of the seeds among the analyzed taxa. In this dendrogram, branches that are markedly dissimilar to other taxa were created for R1 and R7. This finding can be attributed to R1 and R7 being different from the other analyzed taxa in terms of the studied characters. Generally, the branches contained nearly related taxa are compatible with the traditional taxonomic rank of Turkey's *Romulea* species. In other words, the morphological and anatomical features of the seeds supported the characters used in the distribution of *Romulea* species in the flora of Turkey (according to Davis 1984).

Principal component analysis can be helpful in giving data about the variability of quantitative characters. The obtained cumulative variance values of principal components show that the studied characters in Turkish *Romulea* taxa can be of use in explaining the differences among the taxa because of high variance value. In addition, the morphological and anatomical features of the seeds chosen for PCA to evaluate the characteristics that are significant in description change among the analyzed specimens (Tab. 3). Likelihood ratios among the taxa were determined. The closest relationship was seen between R6 and R2, additionally the most distant relationship was found between R6 and R7. These results indicate that a similarity matrix can give information about the classification of Turkish *Romulea* taxa if there is gynodioecism in a population.

In conclusion, this investigation supports the use of seed surface, shape, size and the cross section of seed such as testa and phytomelan layer thickness, shape of testa epidermal cells and embryo sizes as the distinctive characters in the classification within the *Romulea* taxa in Turkey.

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