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Seed morphology and testa ultrastructure in *Allium stipitatum* complex (Amaryllidaceae; Allioideae) and their systematic significance

Mina KHORASANI[1,*], Shahryar Saeidi MEHRVARZ[1], Shahin ZARRE[2]

[1]Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

[2]Center of Excellence in Phylogeny and Department of Plant Sciences, School of Biology, College of Science, University of Tehran, Tehran, Iran

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Abstract: Characteristic features of seed and testa cells in 13 species of *Allium*, representing its five recognized sections, *Compactoprason*, *Megaloprason*, *Procerallium, Decipientia* and *Pseudoprason* were studied using both stereomicroscope and scanning electron microscope. The identified characters, such as seed shape, alignment and shape of testa cells, features of outer cell walls, verruca type, density of verrucae and granules, seed size as well as cell wall undulations were evaluated statistically. The our findings indicated the significance level of $P \leq 0.01$ for examined characters using the Kruskal–Wallis and ANOVA analyses.Two principal components of factor analysis revealed the following traits to be most important in distinguishing taxa at various taxonomic ranks within the complex (sections, subsections and species), based on 50.94% of total variance and a correlation coefficient of 0.93: shape and sculpturing of periclinal walls, undulation details of anticlinal walls, verruca type, testa cell alignment, and size of seed and testa cells. The general pattern of testa shared by all taxa of studied complex included convex periclinal walls with verrucate surface and undulated anticlinal walls from S- to U-like, but some taxa differed from each other in the details of testa sculpturing. Exceptionally, the monotypic section, *Decipientia* revealed adifferent pattern, showing smooth seed surface composed of polygonal testa cells with tightarrangement, flat periclinal surfacebearingsulcate verrucae, and straight anticlinal walls.

Key words: *Allium stipitatum* complex, seed surface sculpturing, SEM, taxonomy, testa, Iran

1. Introduction

Allium L. (Amaryllidaceae) comprises over 900 species worldwide classified into 15 subgenera and 56 sections (Friesen et al., 2006; Fritsch and Abbasi, 2013). The geographic distribution of the genusis mainly in the Holarctic, from the Mediterranean to central Asia including the Irano–Turanian phytogeographical region as a main diversification center of *Allium* (Matin, 1992; Fritsch and Friesen, 2002). Currently, about 120 *Allium* taxa belonging to seven subgenera and 30 sections are known in Iran (Friesen et al., 2006; Memariani et al., 2012). Taxonomically, *Allium* subg. *Melanocrommyum* (Webb & Berth.) Rouy is a controversial group with about 76 Iranian species and subspecies of the total taxanumber of 140–170 spp. Also, the subgenus encompasses *A. Stipitatum* Regel complex with vague borders at various taxonomic levels (Khorasani et al., 2018a).

Allium stipitatum group consists of plants with basal leaves, inflorescence a dense umbel rich in flowers, long pedicles, pink to purple-violet flowers distributed mainly over western parts of Iran (Khorasani et al., 2018a). The earlier taxonomical works by Wendelbo (1971) placed the members of this group in two sections, *A.* sect. *Megaloprason* Wendelbo, including *A. stipitatum* Regel, *A. altissimum* Regel, *A. chelotum* Wendelbo, *A. giganteum* Regel, *A. jesdianum* Boiss. & Buhse, *A. sarawschanicum* Regel, and *A.* sect. *Regeloprason* Wendelbo, including *A. bakhtiaricum* Regel, based on size and shape of tepals and scape. The subsequent studies on members of complex recorded new taxa and revised the systematic position of some species (Khassanov and Fritsch, 1994; Fritsch, 1996; Mashayekhi et al., 2005; Neshati et al., 2009; Fritsch and Abbasi, 2008; Fritsch et al., 2010).

According to a new classification of *A.* subg. *Melanocrommyum* (Fritsch and Abbasi, 2013), most taxa of *A. stipitatum* complex were assigned to sect. *Procerallium* R.M.Fritsch, including subsections: *Elatae* R.M. Fritsch (with *A. altissimum* and *A. stipitatum*) and *Costatae* R.M.Fritsch (with *A. Orientoiranicum* Neshati, Zarre & R.M.Fritsch, *A. remediorum* R.M.Fritsch, *A.*

* Correspondence: khorasanimm@yahoo.com

pseudohollandicum R.M.Fritsch, *A. kazerouni* Parsa, *A. bakhtiaricum* and *A. jesdianum*) which are distinguished based on scape length, shape of scape base, width of leaves, and size and density of flowers. Also, other morphological and anatomical reports (Khorasani et al., 2018a, 2018b) indicated that following sections: *Compactoprason* R.M.Fritsch (with *A. giganteum*), *Decipientia* (Omelczuk) R.M.Fritsch (with *A. chelotum*), *Pseudoprason* (Wendelbo) K.Perss. & Wendelbo (with *A. hooshidaryae* Mashayekhi, Zarre & R.M.Fritsch), *Megaloprason* Wendelbo (with *A. sarawschanicum* Regel), are closely related to the members of this complex. In addition, shape of perianth, color of filaments, ovary surface, and presence of nectary ducts at base of ovary are some characters used by Fritsch and Abbasi (2013) for delimitation of species and sections within the complex.

Currently, *A. stipitatum* complex comprises about 10–15 taxa in Iran and neighboring countries classified into several sections and subsections (Fritsch and Abbasi, 2013). Iran is also the main center of diversification ofthis complex, with approximately 80% of its species being endemic (Wendelbo, 1971; Fritsch and Abbasi, 2013). Within *A. stipitatum* complex, some species are supposed to be related and known under the vernacular name 'Bon–Sorkh' in Iran including *A. kazerouni*, *A. jesdianum*, *A. remediorum* and *A. bakhtiaricum* (*A.* sect. *Procerallium* subsect. *Costatae*) and 'Mooseer' including *A. altissimum* and *A. stipitatum* (*A.* sect. *Procerallium* subsect. *Elatae*); thus, the taxonomic circumscription of these taxa in this complex is still not clearly defined (Khorasani et al., 2018a).

Previously published studies have revealed that size and shape of seeds are variable characters among *Allium* species (Bednorz et al., 2011; Veiskarami et al., 2018), and even among various populations of certain species, while seed color is a stable character state within species of *Allium* sect. *Allium* (Veiskarami et al., 2018). These morphological features of seeds (color and size of seeds) were taxonomically considered as unimportant traits in this genus (Neshati and Fritsch, 2009), except for few taxa of a certain section (Hanelt, 1992; Gregory, 1996; Veiskarami et al., 2018). In contrast, previous ultrastructural studies have shown that the characteristic features of testa cells could provide practical tools for taxon delimitation at different taxonomic levels in *Allium* (Kruse, 1984, 1986, 1988, 1994; Fritsch et al., 2006; Bednorz et al., 2011; Celep et al., 2012; Choi et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018). According to these reports, members of subg. *Melanocrommyum* differ in the details of testa sculpturing, i.e. cellular arrangement, amplitudes and wavelengths of anticlinal wall, and size, shape, density and position of verrucae and granules onpericlinal wallsurface (Fritsch et al., 2006; Celep et al., 2012; Lin and Tan, 2017). Nevertheless, some investigated species of this subgenus mostly shared the general patterns of testa micromorphology, such as epidermal cellshape (from circular, elliptic to ovate), types and patternsof periclinal walls (with convexsurface carrying several verrucae), as well as undulation type of anticlinal walls (from S-, U- to Omega-like) (Fritsch et al., 2006; Neshati and Fritsch, 2009; Lin and Tan, 2017).

Notably, the most recent reports on different sections and subgenera of *Allium* showed that general patterns of seed coat sculpturing can be constant within a species and relatedmembers within certainsections (Lin and Tan, 2017; Veiskarami et al., 2018), but characters associated with shape and ornamentations of testa cells, occurrence of straight and raised anticlinal walls, and strip-like sculptures, presence of flat periclinal wall, presence of dense granules, and the features of verrucae on the periclinal surface were considered as characteristic features for delimitation of *Allium* taxa from subgeneric to species rank (Fritsch et al., 2006; Bednorz et al., 2011; Celep et al., 2012; Choi et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018). Additionally, high variation in undulation and sculpturing of seed coat may reflect different evolutionary trends. Accordingly, loose arrangement of testa cells compared to tight ones, straight anticlinal walls compared to undulatedones, granulate periclinal compared to verrucate walls onesare shown to represent ancestral states (Lin and Tan, 2017). The results of the latter study corroborate the phylogenetic classifications by Friesen et al. (2006) and Li et al. (2010), but contradict the results of Celep et al. (2012) that indicated the above mentioned characters as ineffective to verify the evolutionary patterns.

According to previous ultrastructural studies, characters of seed coat are informative and can provide efficient tools for resolution of taxonomic problems in *Allium*. Hitherto, no comprative investigation of seed morphology and testa ultrastructure has been performed in *A. stipitatum* complex, although testa sculpturing has been reported for few species of *A.* sect. *Megaloprason* (Fritsch et al., 2006; Neshati and Fritsch, 2009). The current investigation aimed at: (i) describing the seed outline and testa ultrastructure in the members of *A. stipitatum* complex in detail, (ii) assessing the systematic importance of obtained characters within the complex to distinguish inter- and intraspecific taxa, subsections and sections, (iii) inferring and evaluating the potential phylogenetic implication of seed testa features in *A. stipitatum* complex.

2. Materials and methods

2.1. Taxon sampling

During field trips to Zagros mountain ranges and parts of Khorasan province in 2014–2015, we collected representatives of *Allium* focusing on members of *A. stipitatum* complex. For micromorphological studies of

seeds in the complex, 25 populations representing 13 species and five sections of *Allium* (including *Procerallium, Pseudoprason*, *Compactoprason*, *Decipientia* and *Megaloprason*) were investigated using stereomicroscope and scanning electron microscope (SEM). Mostly two populations were selected for each species of the complex, except for *A. pseudohollandicum*, *A. chelotum*, *A. koelzii* (not a member of the complex) each represented by only one population. *Allium stipitatum* as a widely ditributed species was represented by four populations.

Most ripe capsules bearing black mature seeds were prepared from living plants in the field while other specimens were taken from herbarium sheets deposited in FUMH, IRAN and TARI. Collection data of sampled species along with their voucher numbers and taxonomic positions are performed in Table 1.

2.2. Morphological and micromorphological studies

Morphological features of seeds (including outline, colour and size) were examined under a stereomicroscope (Leica/Wild M3Z, Leica Microsystems AG, Heerbrugg, Switzerland) coupled with Canon digital camera (Canon Inc., Tokyo, Japan). For SEM, dry and mature seeds were attached onto aluminum stubs using Leit-Tabs, and covered with gold in a ion-sputter (model DST1). Finally, prepared materials were observed under SEM (Hitachi SU3500, Hitachi Ltd., Tokyo, Japan) at 5 kV acceleration voltage, in the Central Laboratory of the Shahid Beheshti University, Tehran, Iran.

The terminology of seed ultrastructure follow Barthlott, 1981; Fritsch et al., 2006; Choi et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018. For each accession, 5–10 (–20) seeds in average were measured. Finally, nine qualitative characters, including seed shape (Ss), wrinkled degree of seed surface (Ds), shape of epidermal cells (Sc), alignment of epidermal cells (Ac), type of anticlinal walls (Ta), type of periclinal walls (Tp), verrucatype (Vt), density of verrucae (Dv) and density of granules (Dg) were scored and also six quantitative traits, i.e. seed length (Sl), seed width (Sw), epidermal cells length (Tl), epidermal cells width (Tw), wavelengths (Wa) and amplitudes (Am) were evaluated by a stereomicroscope and ImageJ 1.4 software.

2.3. Numerical analysis

To assess the statistical significance of qualitative characters, nonparametric test of Kruskal–Wallis (or chi-square), and ANOVA, analysis of variance for quantitative traits implemented in SPSS ver. 16.0 were performed. Factor analysis of selected characters with respect to results of above statistical tests was also used to determine the most effective characters in the identifiation and delimitation of the studied *Allium* taxa. Finally, the principal component analysis (PCA) was carried out to infer the ordination of taxa of *A. stipitatum* complex, using the NTSYS-pc ver. 2.1 software (Rohlf, 2005).

3. Result

The qualitative characteristics of the examined seeds and testa cells are summarized in Table 2, and the quantitative data are presented in Table 3. The photographs of the investigated samples illustrating the general appearance of seedsand the detailed features of testa cells are also arranged in Figures 1–4.

3.1. Qquantitative macro- and micromorphological traits

The seeds of all examined taxa were black and ranged from 2.0 (in *A. giganteum* of *A.* subsect. *Erectopetala*, sect. *Compactoprason*) to 4.8 mm (in *A. altissimum* of*A.* subsect. *Elatae*, sect. *Procerallium*) in length and 2.0 (in most species, even in *A. giganteum*) to 3.5 mm (in *A. stipitatum* of *A.* subsect. *Elatae*, sect. *Procerallium*) in width. The smallest seeds were found in *A.* sect. *Compactoprason* (*A. giganteum*) and the largest ones insect. *Procerallium* (*A. altissimum* and *A. stipitatum*). Length of epidermal cells ranged from 25.1 µm for *A. kazerouni* (*A.* subsect. *Costatae* of sect. *Procerallium*) to 97.3 µm in *A. stipitatum*, and their width ranged from 14.16 (*A. kazerouni*) to 69.4 µm (*A. stipitatum*). Also, the longest wavelengths and amplitudes were observed in *A. stipitatum* (19.5 and 15.8 µm, respectively), and the lowest values for these characters were observed in *A. chelotum.*

3.2. Qualitative characters of seed and testa

The shape of seeds varied among different populations of most species, with outlines ranging from ovate, rounded to ovate-roundish, wrinkled on surface. Also, few species showed long ovoid or nearly oblongoid (*A. altissimum*, Figure 1E) and broadly ovoid seeds with smooth surface (*A. chelotum*, Figure 1W). The shapes of testa cells were variable among the investigated species, ranging from elliptic to circular. Also, polygonal cells were observed in *A. stipitatum* (Figure 2D), *A. altissimum* (Figures 2E and 2F), *A. orientoiranicum* (Figure 2G), *A. kazerouni* (Figures 2J and 2K) and *A.chelotum* (Figure 3K), as an unusual feature compared to other species. The cellular arrangement was loose (or depressed) with intercellular spaces, except in *A. chelotum.*

The periclinal cell walls of many taxa investigated were convex with different type, size and density of sculptures (granules or verrucae), but flat walls with more or less granulose ornamentation and one to few largecentral verrucae occurred in *A. chelotum* (Figures 3K and 4E). In most species, the granulate verrucae with raised surfacewere prominent on the periclinal walls (Figure 4D), except in *A. kazerouni*, which showed fairly smooth or flat to granulate verrucae (Figure 4C). Other types of verrucae were found in some species: verruculose in *A. altissimum* and *A. stipitatum*, (Figure 4B; see also Choi et al., 2012), and sulcate in *A. chelotum* (Figure 4E). Most studied taxa showed dense distribution of verrucae (except *A. kazerouni* and *A. jesdianum*) and granules (except *A. stipitatum*, *A.*

Table 1. Voucher information of *Allium* spp. in present study. The systematic position of species follows Fritsch and Abbasi (2013) (pop: population).

Species	Section/subsection	Collection data
A. stipitatum Regel	*Procerallium/ Elatae*	Pop1: Kordestan, Dehgolan, Gazgazareh village (35°11'N, 47°10'E, 1790 m, 10.5.2015, Khorasani 6002; GUH). -Pop2: Zanjan to Bijar, Gholi-Kandy village (36°46'N, 48°14'E, 2238 m, 26.5.2015, Mahmodi 6003; GUH). -Pop3: Khorasan, Central Kopedagh, Tandureh National Park (37°23'N, 58°52'E, 950 m, 25.6.2014, Saeidi et al. 6028; GUH). -Pop4: Chaharm. Bakhtiyari, NE of Chelgerd, Kuhrang (32°34'N, 50°12'E, 2397 m, 6.5.2015, Khorasani 6127; GUH).
A. altissimum Regel	*Procerallium/ Elatae*	Pop1: Khorasan, Shirvan toward Esfarayen, Glian village (37°12'N, 57°32'E, 1000 m, 25.4.2014, Khorasani 6015; GUH). -Pop2: Khorasan, Binalood mount, Kang village (36°19'N, 59°13'E, 1800 m, 22.5.1990, Zangooie and Neshati 18639; TARI).
A. orientoiranicum Neshati, Zarre & R.M.Fritsch	*Procerallium/ Costatae*	Pop1: Khorasan, SW Torbate jam, Bezd mount (35°11'N, 60°22'E, 1500 m, 6.5.2007, Faghinia and Zangooie 38758; FUMH).-Pop2: Khorasan, Kuh-e Bezgh, 50 km NE Kashmar (35°37'N, 58°35'E, 1900–2500 m, 13.6.1981, Assadi and Mozaffarian 35733; TARI).
A. pseudohollandicum R.M.Fritsch	*Procerallium/ Costatae*	Pop1: W Azarbaijan, Ghasemloo valley, Khan village, 40 km to Oshnaviyeh (37°18'N, 45°06'E, 1529 m, 16.5.2015, Khorasani 6004; GUH).
A. remediorum R.M.Fritsch	*Procerallium/ Costatae*	Pop1: Kordestan, Dehgolan, Gazgazareh village (35°11'N, 47°11'E, 2224 m, 10.5.2015, Khorasani 6006; GUH). -Pop2: Kordestan, Divandareh to Sanandaj, road of Kooleh to Dozakh darreh, Kapak village (35°43'N, 47°55'E, 2132 m, 11.5.2015, Khorasani 6007; GUH).
A. kazerouni Parsa	*Procerallium/ Costatae*	Pop1: Fars, Marvdasht, Kamfiruz, Tange Bostabak (30°19'N, 52°11'E, 2000 m, 25.5.2009, Zarre 6126; GUH). -Pop2: Kohgil. Buyerahmad, Yasuj 51 km Fahlian road (30°23'N, 51°30'E, 1660 m, 28.4.1972, Foroughi 3542; TARI).
A. jesdianum Boiss. & Buhse	*Procerallium/ Costatae*	Pop1: Yazd, Deh Balla, Shirkuh (31°32'N, 54°13'E, 3200 m, ?, Zarre 6009; GUH). -Pop2: Chaharm. Bakhtiyari, Sabz Kuh, Chahartagh (31°45'N, 51°53'E, 2597 m, 7.5.2015, Khorasani 6017; GUH).
A. bakhtiaricum Regel	*Procerallium/ Costatae*	Pop1: Mrkazi, Arak to Malayer, Chepeghli (34°03'N, 48°33'E, 2300 m, 24.5.1984, Termeh, Karavar and Tehrani 287; IRAN). -Pop2: Chaharm. Bakhtiyari, 5 km S Farsan, Deh Cheshmeh Pirghar (32°13'N, 50°33'E, 2000 m, 6.5.2015, Khorasani 6010; GUH).
A. sarawschanicum Regel	*Megaloprason/ Keratoprason*	Pop1: Golestan, Khosh-yeilagh, 60 km SE of Azad-Shahr (36°51'N, 55°21'E, 1700 m, 22.5.1995, Faghinia, Rafeies and Zangooie 25453; FUMH). -Pop2: Khorasan, Dargaz, Tandureh National Park, Tivan toward Urteh-Bulagh, 2 km to Tivan (37°28'N, 58°34'E, 2215–2280 m, 19.5.2004, Memariani and Zangooie 35521; FUMH).
A. hooshidaryae Mashayekhi, Zarre & R.M.Fritsch	*Pseudoprason*	Pop1: Kordestan, Divandareh to Sanandaj, road of Kooleh to Dozakh-darreh, Kapak village, Sabzposh mount (35°44'N, 46°55'E, 2182 m, 13.5.2015, Khorasani 6012; GUH). -Pop2: Kordestan, Doozakh-darrehvillage (35°46'N, 46°53'E, 2136 m, 13.5.2015, Khorasani 6013; GUH).
A. koelzii (Wendelbo) K.Perss. & Wendelbo	*Pseudoprason*	Pop1: Kordestan, Narran village, 38 km SE Sanadaj, Sanandaj to Kamyaran (35°02'N, 47°00'E, 2200 m, 15.6.1987, Assadi 60408; TARI).
A. giganteum Regel	*Compactoprason/ Erectopetala*	Pop1: Khorasan, Central Kopedagh, Dargaz-Chelmir (37°32'N, 58°37'E, 1360 m, 25.6.2014, Saeidi et al. 6010; GUH). -Pop2: Khorasan, SE Dargaz, Zangranlo waterfall (37°10'N, 59°10'E, 2350 m, 20.5.2014, Amiri 6014; GUH).
*A.chelotum*Wendelbo	*Decipientia*	Pop1: Golestan forest (37°21'N, 56°00'E, 1700–2000 m, 6.5.1973, Iranshahr 41834; IRAN).

Table 2. List of examined qualitative characters and their states in studied *Allium* spp."Pop" refers to corresponding population in Table 1.

Species/populations	Ss	Ds	Sc	Ac	Ta	Tp	Vt	Dv	Dg
A. stipitatum/Pop1	0	1	2	0	2, 3 (U to Omega-like)	0	0	1	0
A. stipitatum/Pop2	0	1	1, 2	0	2, 3	0	0	1	0
A. stipitatum/Pop3	2	1	0, 2	0	2, 3	0	0	1	0
A. stipitatum/Pop4	2	1	0,3 (irregular)	0	1	0	0, 1	1	0
A. altissimum/Pop1	0 (long ovate)	1	3 (irregular)	0	1	0	0, 1	1	0
A. altissimum/Pop2	2	1	0,3 (irregular)	0	1 (Straight to S-like)	0	0	1	0
A. orientoiranicum/Pop1	0	1	3	0	1(Straight to S-like)	0	0	1	1
A. orientoiranicum/Pop2	0	1	2	0	1	0	0	1	1
A. jesdianum/Pop1	0	1	2	0	1	0	0	0	1
A. jesdianum/Pop2	0	1	2	0	1	0	0	0, 1	1
A. kazerouni/Pop1	2	1	3	0	1 (Straight to S-like)	0	0 (flat)	0	1
A. kazerouni/Pop2	0	1	0, 3 (irregular)	0	1	0	0	0	1
A. remediorum/Pop1	0	1	2	0	1	0	0	1	1
A. remediorum/Pop2	0	1	2	0	1	0	0	1	1
A. bakhtiaricum/Pop1	0	1	2	0	1	0	0	1	1
A. bakhtiaricum/Pop2	0	1	2	0	1	0	0	1	1
A. sarawschanicum/Pop1	0	1	2	0	2, 3 (U to Omega-like)	0	0	1	1
A. sarawschanicum/Pop2	0	1	2	0	1, 2 (S to U-like)	0	0	1	1
A. pseudohollandicum/Pop1	2	1	2	0	1	0	0	1	1
A. hooshidaryae/Pop1	2	1	2	0	1	0	0	1	1
A. hooshidaryae/Pop2	0	1	2	0	1	0	0	1	1
A. giganteum/Pop1	1, 2	1	0	0	3 (S to omega)	0	0	1	1
A. giganteum/Pop2	2	1	2	0	3 (S to omega)	0	0	1	1
A. chelotum/Pop1	0 (broad ovate)	0	3 (regular)	1	0	1	0, 2	1	0
A. koelzii/Pop1	0	1	0	0	2, 3 (U to Omega)	0	0	1	1

Seed shape (Ss): ovate (broad or long, 0), round (1), ovate-roundish (2); wrinkled degree of seed surface (Ds): smooth (0), wrinkled (1); shape of epidermal cell (Sc): elliptic (0), circular (1), elliptic-circular (2), polygonal (regular or irregular, 3); alignment of epidermal cell (Ac): loose (0), tight (1); type of anticlinal wall (Ta.): straight (0), S-like (= straight to s-like, 1), U-like (= S to U-like, 2), Omega-like (U, S to Omega-like, 3); type of periclinal wall (Tp): convex (0), flat (1); verruca type (Vt): granulate verruca (raised or flat,0), verruculose verruca (1), sulcate verruca (2); density of verruca (per area, Dv): a few (0), numerous (1); density of granule (per area, Dg): a few (0), numerous (dense or loose, 1).

altissimum and *A. chelotum*) on their periclinal surface. We also observed the dense granulose ornamentation in *A. orientoiranicum* (Figure 4F).

Most variation observed was related to the undulation type of the anticlinal walls showing transitions from S-, U-like to Omega-like with different wavelengths and amplitudes (see Figures 4A–4F, 2, and 3A–3L), although we also found deeply straight walls without any undulation (in *A. chelotum*, Figure 3K). Transitions from U- to Omega-like undulations were observed within each of *A. stipitatum*, *A. sarawschanicum* and *A. koelzii* (with different amplitudes and wavelengths, see Figures 2A–2C, 3D and 3L), and from S- to Omega-like in *A. giganteum* (Figures 3I and 3J). In other taxa the S-like (or nearly S-like) undulation was dominated.

3.3. Numerical analysis of qualitative and quantitative data

The statistical results of the chi-square and ANOVA analyses displayed a significance level of $P \leq 0.01$ for qualitative and quantitative characters (Tables 4 and 5,

Table 3. List of measured quantitative characters in studied *Allium* spp. (Sl: seed length, Sw: seed width, Tl: epidermal cells length, Tw: epidermal cells width, Wa: wavelength, Am: amplitude, M: mean value, SD: standard deviation) (pop: population).

Species/populations	Sl (mm)	Sw (mm)	Tl (µm)	Tw (µm)	Wa (µm)	Am (µm)
	Min (M±SD) Max	Min (M±SD) Max	Min (M±SD) Max	Min (M±SD) Max	Min (M±SD) Max	Min (M±SD) Max
A. stipitatum/Pop1	4.0 (4.1 ± 0.1)4.3	2.3 (2.5 ± 0.1) 2.7	39.7 (56.7 ± 13.3) 71.3	32.3 (41.4 ± 9.5) 56.9	4.2 (8.0 ± 3.2) 12.0	5.9 (8.3 ± 1.6) 10.2
A. stipitatum/Pop2	3.9 (3.9 ± 0.05) 4.0	3.0 (3.2 ± 0.2) 3.5	46.4 (58.1 ± 7.1) 63.9	36.8 (48.9 ± 12.2) 69.4	8.8 (11.0 ± 2.9) 15.8	6.8 (8.5 ± 1.5) 11.0
A. stipitatum/Pop3	4.0 (4.2 ± 0.3) 4.5	3.2 (3.3 ± 0.1) 3.5	66.8 (78.4 ± 11.6) 97.3	36.0 (45.2 ± 8.0) 54.2	5.8 (8.3 ± 2.3) 11.1	4.3 (8.1 ± 2.6) 10.6
A. stipitatum/Pop4	3.0 (3.1 ± 0.1) 3.2	2.0 (2.6 ± 0.3) 3.2	32.9 (51.8 ± 17.3) 80.2	33.1 (38.3 ± 3.3) 42.4	1.9 (3.4 ± 1.5) 5.9	14.1 (16.2 ± 2.0) 19.5
A. altissimum/Pop1	4.0 (4.4 ± 0.4) 4.8	2.2 (2.5 ± 0.2) 2.9	45.0 (60.9 ± 12.8) 76.7	32.7 (38.4 ± 5.8) 47.6	4.1 (4.5 ± 0.4) 5.2	3.0 (4.5 ± 1.2) 5.8
A. altissimum/Pop2	2.8 (2.9 ± 0.1) 3.0	2.2 (2.4 ± 0.1) 2.6	41.3 (47.8 ± 4.8) 54.5	25.3 (32.9 ± 4.9) 39.0	0.8 (0.9 ± 0.08) 1.0	0.9 (1.2 ± 0.2) 1.5
A. orientoiranicum/Pop1	3.0 (3.8 ± 0.4) 3.0	2.5 (2.7 ± 0.1) 2.8	35.8 (41.9 ± 3.5) 44.5	21.1 (24.4 ± 0/2) 26.1	3.7 (4.1 ± 0.3) 4.5	3.6 (4.8 ± 1.2) 7.0
A. orientoiranicum/Pop2	3.0 (3.4 ± 0.4) 3.9	2.5 (2.6 ± 0.1) 2.7	35.5 (49.0 ± 10.9) 65.2	26.1 (34.2 ± 5.9) 42.3	2.5 (4.0 ± 1.2) 5.5	3.1 (4.5 ± 1.0) 5.6
A. jesdianum/Pop1	2.5 (2.7 ± 0.2) 3.0	2.1 (2.2 ± 0.07) 2.3	32.7 (40.3 ± 5.3) 47.1	20.8 (26.0 ± 4.9) 31.2	2.4 (4.3 ± 2.0) 7.8	3.4 (5.7 ± 2.3) 8.6
A. jesdianum/Pop2	2.5 (2.7 ± 0.2) 3.0	2.0 (2.4 ± 0.3) 2.8	42.5 (51.3 ± 6.1) 59.1	26.7 (33.1 ± 5.9) 39.8	3.3 (3.9 ± 0.5) 4.6	4.4 (7.4 ± 2.1) 10.1
A. kazerouni/Pop1	2.8 (2.9 ± 0.1) 3.1	2.1(2.2 ± 0.1) 2.4	25.1 (34.9 ± 8.4) 44.8	14.6 (19.1 ± 3.1) 23.6	3.1 (3.8 ± 0.5) 4.3	1.7 (2.3 ± 0.5) 3.1
A. kazerouni/Pop2	2.8 (2.9 ± 0.08) 3.0	2.5 (2.5 ± 0.08) 2.7	35.0 (39.1 ± 4.5) 45.2	21.8 (31.3 ± 6.9) 38.6	2.0 (3.7 ± 1.0) 4.8	2.8 (3.8 ± 0.7) 4.9
A. remediorum/Pop1	2.5 (2.8 ± 0.2) 3.0	2.1 (2.2 ± 0.1) 2.4	48.7 (55.0 ± 5.8) 62.2	24.8 (39.7 ± 13.0) 56.1	2.4 (4.7 ± 2.2) 7.8	6.5 (9.0 ± 1.2) 7.3
A. remediorum/Pop2	3.5 (3.6 ± 0.1) 3.8	2.4 (2.6 ± 0.2) 3.0	46.5 (52.8 ± 6.0) 62.6	37.6 (38.0 ± 0.3) 38.5	3.6 (5.5 ± 1.3) 7.2	5.8 (9.1 ± 2.3) 12.1
A. bakhtiaricum/Pop1	2.7 (3.0 ± 0.2) 3.4	2.2 (2.3 ± 0.1) 2.5	50.6 (57.9 ± 8.6) 72.8	33.4 (39.8 ± 4.5) 45.2	3.5 (4.4 ± 0.9) 5.9	6.7 (8.9 ± 1.8) 11.1
A. bakhtiaricum/Pop2	2.5 (2.6 ± 0.1) 2.8	2.2(2.3 ± 0.1) 2.4	40.7 (47.7 ± 4.9) 53.2	32.6 (36.2 ± 3.2) 41.1	2.5 (3.6 ± 0.8) 4.9	6.1 (7.7 ± 2.1) 11.4
A. sarawschanicum/Pop1	2.5 (2.7 ± 0.2) 3.0	2.1 (2.3 ± 0.1) 2.5	38.8 (50.1 ± 9.9) 63.9	28.9 (35.4 ± 4.5) 41.5	3.9 (5.1 ± 1.0) 6.4	5.1 (5.9 ± 0.7) 7.2
A. sarawschanicum/Pop2	2.7 (2.8 ± 0.08) 2.9	2.1 (2.3 ± 0.1) 2.5	40.2 (55.4 ± 9.1) 65.2	34.8 (44.0 ± 7.3) 54.5	3.2 (4.8 ± 1.4) 6.7	0.6 (0.7 ± 0.8) 8.0
A. pseudohollandicum/Pop1	2.8 (2.8 ± 0.08) 3.0	2.3 (2.4 ± 0.1) 2.6	51.8 (70.0 ± 11.3) 81.6	38.3 (47.3 ± 7.4) 55.6	3.0 (4.2 ± 0.8) 5.5	6.7 (9.0 ± 1.6) 10.8
A. hooshidaryae/Pop1	2.7 (3.0 ± 0.2) 3.5	2.2 (2.4 ± 0.1) 2.7	44.5 (51.8 ± 7.1) 59.3	39.1 (45.2 ± 4.3) 50.2	3.2 (3.9 ± 0.4) 4.5	4.1 (6.2 ± 1.6) 8.5
A. hooshidaryae/Pop2	2.4 (2.4 ± 0.05) 2.5	2.4 (2.4 ± 0.05) 2.5	32.4 (43.7 ± 7.7) 51.0	29.0 (33.6 ± 4.6) 39.4	1.6 (2.5 ± 1.0) 4.2	1.7 (2.7 ± 0.6) 3.6
A. giganteum/Pop1	2.0 (2.0 ± 0.05) 2.1	2.0 (2.0 ± 0.05) 2.1	32.9 (39.6 ± 5.9) 49.0	20.3 (22.5 ± 2.1) 25.0	1.6 (2.1 ± 0.5) 3.0	1.9 (2.4 ± 0.4) 3.0
A. giganteum/Pop2	2.0 (2.2 ± 0.2) 2.4	2.0 (2.1 ± 0.1) 2.2	37.8 (45.4 ± 7.7) 56.0	16.5 (21.2 ± 3.4) 25.3	2.7 (3.1 ± 0.4) 4.0	2.4 (5.2 ± 1.8) 7.2
A. chelotum/Pop1	3.0 (3.1 ± 0.1) 3.3	2.8 (2.9 ± 0.08) 3.0	40.1 (52.0 ± 8.2) 62.1	36.2 (43.0 ± 4.9) 50.0	0.0 (0.0 ± 0.0) 0.0	0.0 (0.0 ± 0.0) 0.0
A. koelzii/Pop1	3.0 (3.2 ± 0.2) 3.5	2.2 (2.4 ± 0.1) 2.5	43.6 (48.7 ± 6.5) 57.5	19.5 (26.3 ± 4.8) 32.3	4.2 (5.5 ± 1.4) 8.0	6.0 (5.7 ± 1.1) 9.0

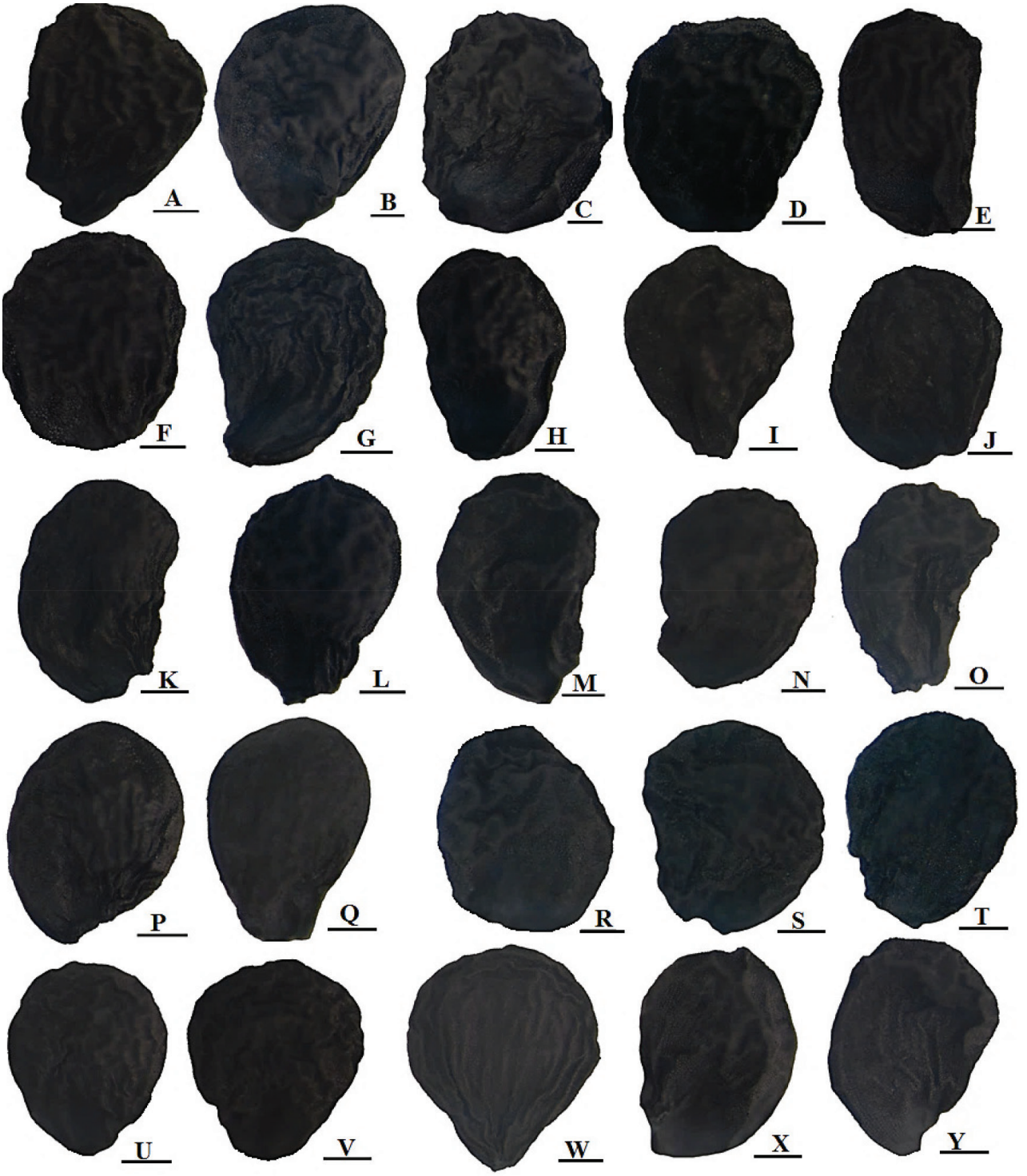

Figure 1. Stereomicroscope photographs of the general appearance of seeds in *Allium* spp. (A, B, C and D) correlate to Pop1, 2, 3 and 4 of *A. stipitatum*, respectively, (E) Pop1 of *A. altissimum*,(F) Pop2 of *A. altissimum*, (G) *A. orientoiranicum*, (H) Pop1 of *A. jesdianum*, (I) Pop2 of *A. jesdianum*, (J) Pop1 of *A. kazerouni*, (K) Pop2 of *A. kazerouni*, (L) Pop1 of *A. remediorum*, (M) Pop2 of *A. remediorum*, (N) Pop1 of *A. bakhtiaricum*, (O) Pop2 of *A. bakhtiaricum*, (P) Pop1 of *A. sarawschanicum*, (Q) Pop2 of *A. sarawschanicum*, (R) *A. pseudohollandicum*, (S) Pop1 of *A. hooshidaryae*, (T) Pop2 of *A. hooshidaryae*, (U) Pop1 of *A. giganteum*, (V) Pop2 of *A. giganteum*, (W) *A. chelotum*, and (X and Y) *A. koelzii*. Scale bars: (A)–(Y) = 0.5 mm. "Pop" refers to corresponding population in Table 1.

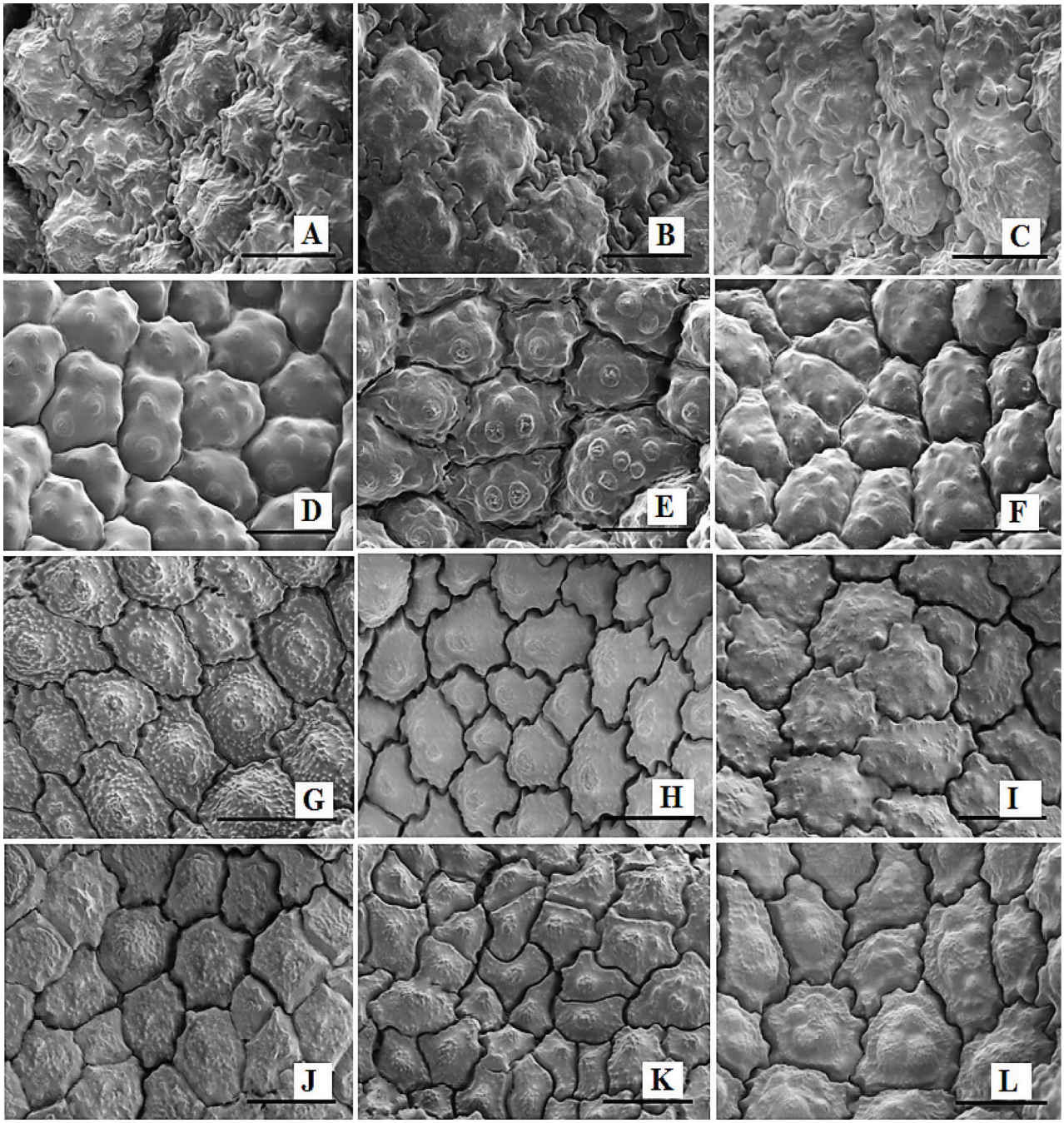

Figure 2. Scanning electron micrographs of testa cells in *Allium* spp. (A, B, C and D) correlate to Pop1, 2, 3 and 4 of *A. stipitatum*, respectively, (E) Pop1 of *A. altissimum*,(F) Pop2 of *A. altissimum*,(G) Pop1 of *A. orientoiranicum*, (H) Pop1 of *A. jesdianum*, (I) Pop2 of *A. jesdianum*, (J) Pop1 of *A. kazerouni*, (K) Pop2 of *A. kazerouni*, and (L) Pop1 of *A. remediorum*. Scale bars: (A)–(L) = 50 µm. "Pop" refers to corresponding population in Table 1.

respectively). The two main components of the factor analysis accounted for 50.94% of the total variance (Table 6). The most important traits contributed toprincipal components (PC) are listed in Table 7. PC1 (28.78% of the total variation) was significantly associated with type of periclinal wall and verruca, as well as testa cell alignment, and PC2 (22.16% of the variations) was correlated with the size of seed and testa cells, as well as wavelength and amplitude of undulations. Finally, the ordination results of PCA analysis on the

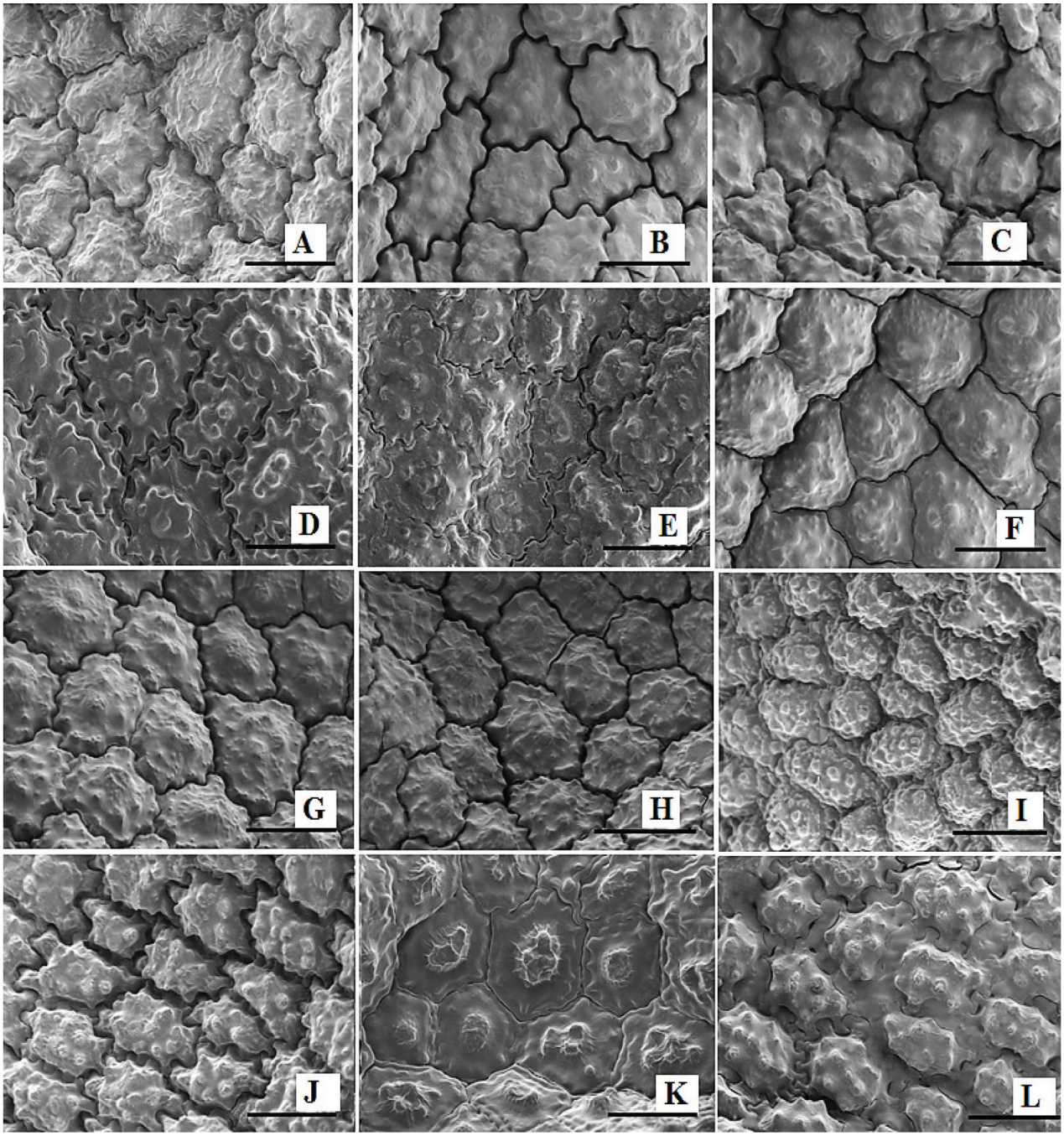

Figure 3. Scanning electron micrographs of testa cells in *Allium* spp. (A) Pop2 of *A. remediorum*, (B) Pop1 of *A. bakhtiaricum*, (C) Pop2 of *A. bakhtiaricum*, (D) Pop1 of *A. sarawschanicum*, (E) Pop2 of *A. sarawschanicum*, (F) Pop1 of *A. pseudohollandicum*, (G) Pop1 of *A. hooshidaryae*, (H) Pop2 of *A. hooshidaryae*, (I) Pop1 of *A. giganteum*, (J) Pop2 of *A. giganteum*, (K) Pop1 of *A. chelotum*, and (L) Pop1 of *A. koelzii*. Scale bars: (A)–(L) = 50 µm."Pop" refers to corresponding populations in Table 1.

basis of mentioned principal components with a high correlation coefficient (r = 0.93) underlined the close relevance among taxa of studied complex, and also the delimitation of sections and subsections within it (Figure 5).

4. Discussion

Most of the previous ultrastructural studies of seed coat revealed that shape and arrangement of testa cells, shape and sculpturing of periclinal walls (type, size, density and arrangement of verrucae and granules), as well

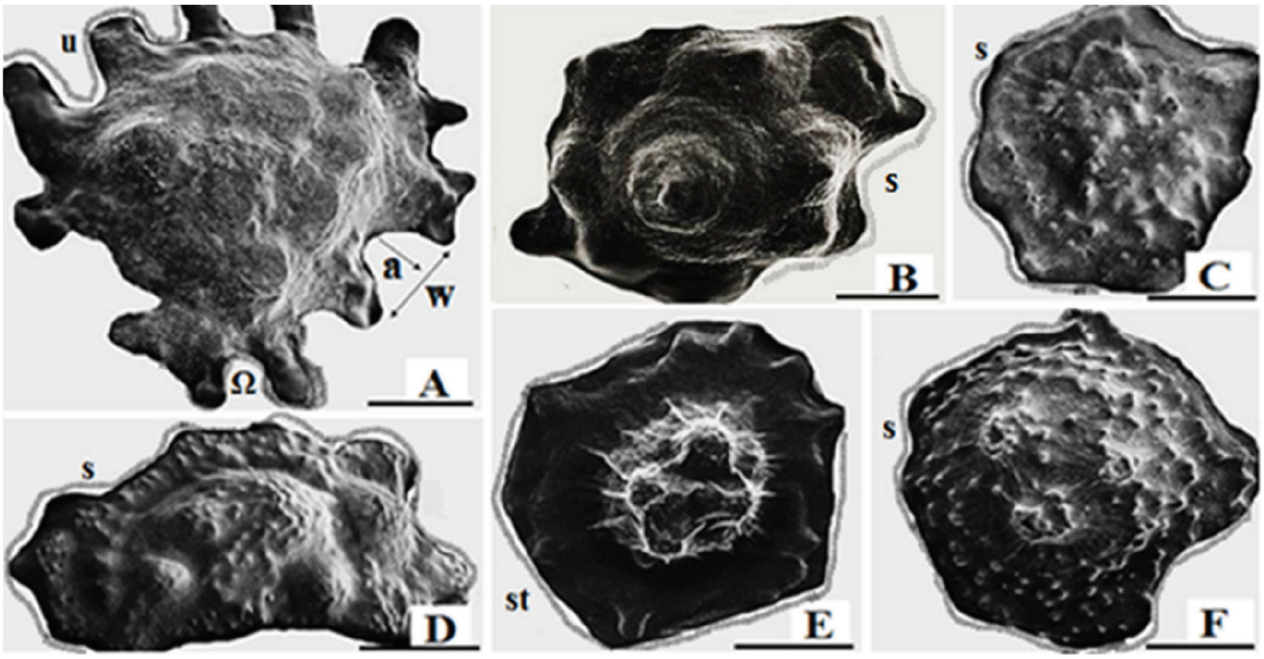

Figure 4. Micrographs of testa cells showing main ornamentation types and position of undulations. (A) anticlinal walls with U- to Omega-like undulations in A. stipitatum, (B) verruculose verrucae in *A. altissimum*, (C) flat verrucae in *A. kazerouni*, (D) granulate verrucae in *A. bakhtiaricum*, (E) sulcate verrucae in *A. chelotum*, and (F) dense granules in *A. orientoiranicum*. (a: amplitude, w: wavelength, st: straight, and S-, U-, Ω-, types of undulations). Scale bars: (A)–(F) = 20 µm.

Table 4. Kruskal–Wallis analysis results of nine qualitative traits in *Allium* spp. Abbreviations follow Table 2.

	Ss	Ds	Sc	Ac	Ta	Tp	Vt	Dv	Dg
Chi-square	8.084	10.580	9.256	10.580	12.010	10.580	19.900	6.624	2.420
Df	2	1	3	1	3	1	2	1	1
P	.000	.000	.000	.000	.000	.000	.000	.000	.000

as undulation details of anticlinal walls are useful and characteristic in species circumscription and at different taxonomic levels in *Allium*, more specifically at sectional level (Fritsch et al., 2006; Bednorz et al., 2011; Celep et al., 2012; Choi et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018).

In the present study, we evaluated all selected characters of seed and testa cells statistically. Our findings showed that the characteristic featuresrelated to two principal components (PC > 0.6, Table 7), including types of periclinal walls and verrucae, and testa cell alignment, as well as measured quantitative traits differed among the studied sections, subsections and species; hence, these characters can provide useful diagnostic evidences in the determination and delimitation of the members of *Allium*, confirming previous researches (Celep et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018). However, other characters such as type of anticlinal wall, shape of testa cells and seed, as well as density of verrucae and granules were selected as less important showing low eigenvalue (PC < 0.6, see also Table 7). Although presence of dense granules as a unique feature was seen only in *A. orientoiranicum*, which is in agreement with previous micromorphological reports suggesting a high taxonomic value for this trait (Celep et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018). Notably, type of anticlinal walls, as well as shape of testa cellsand seeds varied among different populations of one species, and even among some members of the same subsection; so they could not be considered as significant traits in taxonomy of *Allium*. This finding is in accordance with previous reports (Fritsch et al., 2006; Neshati and Fritsch, 2009; Veiskarami et al., 2018)

Below the taxonomic importance of characteristic seed features is discussedin more detailson the basis of the new classification of *Allium*. subg. *Melanocrommyum* (Fritsch and Abbasi, 2013) and in comparison with the most recent

Table 5. ANOVA analysis of six quantitative characters in *Allium* spp. Abbreviations follow Tables 2 and 3.

Characters	Groups	Sum of squares	Df	Mean square	F	P
Sl	Between groups	41.916	24	1.747	36.144	.000
	Within groups	4.832	100	0.048		
Sw	Between groups	11.760	24	0.490	14.741	.000
	Within groups	3.324	100	0.033		
Tl	Between groups	11127.567	24	463.649	5.966	.000
	Within groups	7772.132	100	77.721		
Tw	Between groups	8522.788	24	355.116	9.126	.000
	Within groups	3891.444	100	38.914		
Wa	Between groups	603.540	24	25.148	12.294	.000
	Within groups	204.556	100	2.046		
Am	Between groups	1381.156	24	57.548	21.399	.000
	Within groups	268.924	100	2.689		

Table 6. Factor analysis of macro- and micromorphological data based on principal components.

Main component	Initial eigenvalues		
	Eigenvalue	Variance %	Cumulative %
1	4.317	28.781	28.781
2	3.325	22.168	50.949
3	1.502	10.014	60.963

Table 7. Partial tabulation of principal component analysis (PCA) of correlation matrix for examined characters. Values indicated by asterisks are effective in discrimination of studied taxa. Abbreviations follow Tables 2 and 3.

	Main component		
Characters	1	2	3
Sl	0.227	0.738*	0.151
Sw	0.422	0.633*	0.037
Tl	0.185	0.664*	0.055
Tw	0.324	0.602*	0.242
Wa	0.302	0.721*	0.018
Am	–0.287	0.652*	0.119
Ss	–0.112	0.083	–0.501
Ds	–0.930	0.219	0.171
Sc	0.315	0.016	0.792
Ac	0.930*	–0.219	–0.171
Ta	–0.448	0.295	–0.559
Tp	0.930*	–0.219	–0.171
Vt	0.757*	0.004	0.043
Dv	0.162	0.430	–0.252
Dg	–0.519	–0.574	0.231

data of *A. stipitaum* complex (Khorasani et al., 2018a, 2018b).

4.1. Section *Procerallium*

According to the obtained results, taxa of this section mostly showed same sculpturing pattern of testa cells, such as type of periclinal walls (convex with verrucate and granulate surface) as well as undulation type of anticlinal walls (more or less S-like, except *A. stipitatum*). These findings are congruent with micromorphological reports of seed in other sections of *Allium* (Celep et al., 2012; Lin and Tan, 2017; Veiskarami et al., 2018), and even some species of this section (Fritsch et al., 2006; Neshati and Fritsch, 2009) addressing the testa sculpturing patterns. Also, in our study, several species differed remarkably from other members of the section in having unique shape of testa cell (irregular polygonal), combined withnearly straight undulation to S-like and Omega-like (in *A. stipitatum*, *A. orientoiranicum*, *A. altissimum* and *A. kazerouni*), verruculose verrucae (in *A. altissimum* and *A. stipitatum*) and flat granulate verrucae (in *A. kazerouni*), as well as dense granulose ornamentation of periclinal walls (in *A. orientoiranicum*). Generally, the qualitative traits were variable among members of *A.* sect. *Procerallium*; thus, such features could not separate members of this section from other sections, according to reported studies (Bednorz et al., 2011; Veiskarami et al., 2018) showing

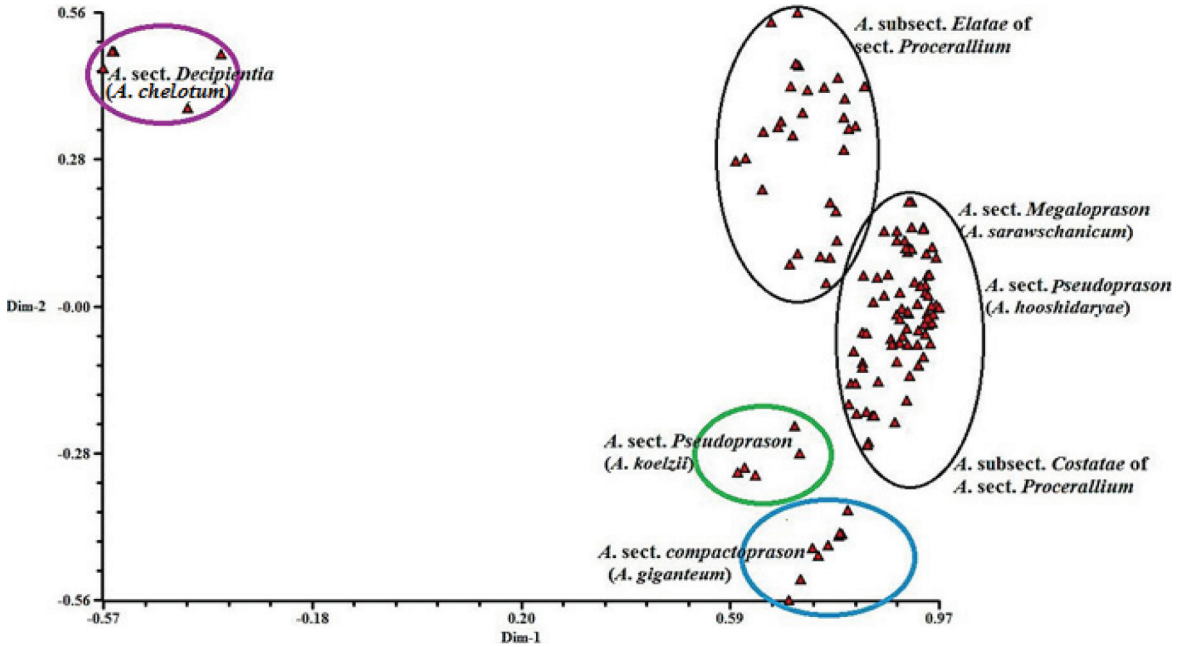

Figure 5. PCA plot based on selected characters for members of *A. stipitatum* complex and its closest taxa.

wide variation of some above mentioned traits, including the shape of seed and testa cells as well as undulation type of anticlinal walls. Besides, taxa of sect. *Procerallium* showed somewhat larger value for most of quantative data than representatives of other sections (see Tables 2, 3, and 7). These characters contributed to higher eigenvalues on the two main components in factor analysis, which is consistent with previous investigations (Neshati and Fritsch, 2009; Celep et al., 2012; Choi et al., 2012; Lin and Tan, 2017).

This section includes about eight species of *A. stipitatum* complex classified in two distinct subsections: *Elatae* (including *A. altissimum* and *A. stipitatum*), and *Costatae* (including *A. orientoiranicum*, *A. pseudohollandicum*, *A. remediorum*, *A. jesdianum*,*A. bakhtiaricum* and *A. kazerouni*) according to phylogenetic and taxonomical data (Fritsch and Abbasi, 2013). The current results highlight the separation of *A.* subsect. *Elatae* from *A.* subsect. *Costatae* by having longer wavelengths (0.8–15.8 µm to 1.6–7.8 µm, respectively), higher amplitudes (0.9–19.5 µm to 1.7–12.8 µm, respectively) of anticlinal walls, as well as larger testa cells (32.9–97.3 × 25.3–69.4 µm to 25.1–81.6 × 14.6–56.1 µm, respectively), and larger seeds (2.8–4.8 × 2.0–3.5 mm to 2.5–3.9 × 2.0–3.0, respectively), as well as verruculose verrucae (see Tables 2and 3 and Figure 5). Although taxa of subsect. *Costatae* revealed higher density of granules on their periclinal walls than subsect. *Elatae*, this feature was not turned out as a discriminator in the taxonomic delimitation of the two mentioned subsections (see also PC1 and PC2 in Table 7).

In general, seed characteristis, in line with anatomical results of scape (Khorasani et al., 2018a) and quantitative traits of bulbs and tunics (Khorasani et al., 2018b) support classifying this section into two subsections. However, we were not able to find any informative traits to infer a close relationship between *A. orientoiranicum* and members of subsect. *Elatae*, except the seed size, contradicting the above anatomical results.

4.2. Section *Megaloprason*

In the former taxonomical classifications (Wendelbo, 1971; Khassanov and Fritsch, 1994; Fritsch, 1996), *A. sarawschanicum* along with other taxa of *A. stipitatum* complex were classified in sect. *Megaloprason* with regard to the presence of overlapping leaves, dense-globose inflorescence, star-like flowers at the base of the free, and after anthesis reflexing and often spirally enrolled, and stipitate ovary, while this species was newly segregated from closely related taxa in the complex using morphological observations and ITS sequence data (Fritsch and Abbasi, 2013).

According to our results (Table 2), three types of undulations (S-, U- and Omega-like) occurredin *A. sarawschanicum* are similar to *A. stipitatum* (of sect. *Procerallium*), which is in agreement with the published reports of Fritsch et al. (2006). Also, this species is remarkably correlated with members of *A.* sect. *Procerallium*, more specifically with taxa of subsect. *Costatae*, showing high density of granules, as well as low wavelength of anticlinal walls (3.2–6.7 µm), same length of epidermal cells (38.8–65.2 µm) and almost same seed

length (2.5–3.0 mm), although the systematic importance of density of granules as well as undulation types were not supported by factor analysis (Table 7). Our conclusions are in accordance with earlier ultrastructural reports of seed coat (Fritsch et al., 2006; Neshati and Fritsch, 2009) as well as the results of ISSR markers (Samiei et al., 2015), confirming *A. sarawschanicum* as close relative of other taxa of *A. stipitatum* complex. In addition, previous reports proved considerable similarity of *A. sarawschanicum* to *A.* sect. *Procerallium* subsect. *Costatae*, based on morphological and anatomical traits of bulb and tunic, such as size of bulb and tunic cell, shape of tunic cell, texture of tunic and presence of calcium oxalate crystals (Khorasani et al., 2018b), as well as scape anatomical results (Khorasani et al., 2018a). Therefore, our data are in line with available literature, placing *A. sarawschanicum* close to members of *A. stipitatum* complex.

4.3. Section *Pseudoprason*

Both taxa assigned to this section, i.e. *A. hooshidaryae* and *A. koelzii* were examined here. Our results showed that *A. hooshidaryae*, like *A. sarawschanicum* was considerably correlated to sect. *Procerallium* by having similar seed coat patterns (referring to Table 2), and also related to taxa of subsect. *Costatae* on the basis of the most effective traits, i.e. low undulation of anticlinal walls (1.6–4.5 µm in wavelength and 1.7–8.5 µm in amplitude), granulate verrucae as well as length of epidermal cells (32.4–59.3 µm) and seed (2.4–3.5 mm). Accordingly, the most recent reports displayed close relationship of *A. hooshidaryae* with taxa of *A.* subsect. *Costatae* using anatomical elements of bulb tunic, i.e. type of tunic cells (Khorasani et al., 2018b) as well as anatomical details of scape, i.e. diameterof cross-sections and pith parenchyma, and number of vascular bundles (Khorasani et al., 2018a).

Furthermore, *A. koelzii* differed from them, especially in having U- to Omega-like undulation with different wavelengths and amplitudes (4.2–8.0 µm and 6.0–9.0 µm, respectively), consistent with former investigations of seed coat in this species (Fritsch et al., 2006). Also, separation of the two above taxa is underlined mainly by morphological differences, such as color of tepal as well as filament and anther, size and shape of tepal and filament, and type of bulb and tunic (Mashayekhi et al., 2005; Fritsch and Abbasi, 2013), while the new grouping of *Allium* placed them in same section, *Pseudoprason* (Fritsch and Abbasi, 2013). Therefore, most previous works in accordance with present results underline segregation of *A. hooshidaryae* from *A. koelzii* and its placement within *A. stipitatum* complex.

4.4. Section *Compactoprason*

Allium giganteum, as the only representative of this monotypic section (Fritsch and Abbasi, 2013), is distinguished from members of *A. stipitatum* complex by a unique type of undulated anticlinal walls from S- to Omega-like with very low wavelengths (1.6–4.0 µm) and different amplitudes (1.9–7.2µm), which is in an agreement with previous results (Fritsch et al., 2006). Also, this species differs from other taxa of the complex by having the smallest seeds (2.0–2.4 mm in length and 2.0–2.2 mm in width) which are ovate to rounded in outline. Although intraspecific variation associated with size and shape of seeds were revealed by previous researchers (Neshati and Fritsch, 2009; Bednorz et al., 2011; Veiskarami et al., 2018), these morphological characters of seed can be useful in discriminating certain taxa within a section, as for example in *A. paniculatum*, *A. rhodopeum* and *A. stamineum* of *A.* subg. *Allium* sect. *Codonoprasum*, (Veiskarami et al., 2018), and also in characterizing *A. stipitatum* and *A. altissimum* of *A.* subg. *Melanocrommyum* sect. *Procerallium*, (Neshati and Fritsch, 2009). This is in line with our morphometric results (see also Tables 3 and 7). In addition, *A. giganteum* differs clearly from *A. chelotum*of sect. *Decipientia* by having unique anticlinal walls (S- to Omega-like), convex periclinal walls, granulate verrucae, loose arrangement of testa cells, wrinkled seeds, as well as smaller seed and epidermal cells, and longer undulations (see Tables 2, 3, and 7 and Figure 5).

According to the earlier taxonomical studies (Wendelbo, 1971), *A. giganteum* was placedin *A.* sect. *Megaloprason*, but later researches transferred it to *A.* sect. *Compactoprason* subsect. *Erectopetala* based on size and shape of tepals and filaments, type of tunic texture and presence of septal nectaries at ovary base (Fritsch and Abbasi, 2013). Furthermore, the anatomical investigations of bulb tunic and scape (Khorasani et al., 2018b) recently confirmed the delimitation and separation of *A. giganteum* from members of the complex based on type of tunic cells and presence of crystals, number and distribution of vascular bundles and thickness of cross-section as well as sclerenchymatous ring. In accordance with the present study, the characteristic features of seed and testa are congruent with previously published results (Khassanov and Fritsch, 1994; Fritsch, 1996; Fritsch and Abbasi, 2013; Khorasani et al., 2018a, 2018b), placing this taxonin a distinct section, *Compactoprason.*

4.5. Section *Decipientia*

This monotypic section comprising only one species, *A. chelotum* was rather exceptional addressing the unique features of seed morphology and testa sculptures, including the broad polygonal cells of testa with tight arrangement, flat periclinal testa cell walls with sulcate verrucae and more or less granulose ornamentation, and deeply straight anticlinal walls without distinct undulation (see Tables 2–3), which accounted for high PC1 and PC2 contribution in separating this section from other investigated sections (Table 7, Figure 5). These specific types of testa sculptures

were proved as important diagnostic traits in recognization of some sections of *A*. subg. *Melanocrommyum*, i.e. *Verticillata* and *Aroidea* (Fritsch et al., 2006). However, our findings contradict the previous ultrastructural studies of seed (Fritsch et al., 2006) reporting S- to Omega-like anticlinal walls with low wavelength for *A*. *chelotum*.

Nevertheless, the recent treatment of the genus *Allium* (Fritsch and Abbasi, 2013) which contradicts the earlier taxonomical results of Wendelbo (1971), who classified this species in an independent section from members of our complex by having different size and shape of tepals as well as filaments, and ovary, which is in an agreement with our micromorphological data.

5. Conclusion

In the present study, we focused on the most influential macro- and micromorphological traits of seeds in determining the interspecific relationships within *A*. *stipitatum* complex, including shape and sculpturing of periclinal walls, undulation details of anticlinal walls and verruca type, based on performed analyses. These characters varied among sections, subsections and some species, but their general patterns such as type of periclinal walls bearing several verrucate and granulate sculptures, combined with undulation type of anticlinal walls can be steady among various populations of one species, and even among most taxa of a certain section. Our results provide reliable data not only supporting the delimitation of various subdivisions (including sections, subsections and species) in the studied complex, but also for providing further evidence in agreement with some former taxonomical treatments as well as anatomical studies to modify the taxonomic affiliation of the examinedtaxa in this complex.

One shortcoming of the current paper is the low population numbers for some taxa, which could limit the application of provided results. Some species, such as *A*. *peudohollandicum* which were presented by only one population in our study, are rare species sometimes difficult to find or identification. However, the earlier studies on testa microsculpturing in other groups of the genus *Allium* have documented the constancy of most characters assessed here. We believe that future studies including further populations would show the stability of majority of traits addressed, although some features such as seed size, shape and color may show some interpopulational variation that would be indicative of the impact of ecological conditions rather than phylogenetic relationships.

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