

**MORPHOLOGICAL ANATOMICAL AND PHYSIOLOGICAL
STUDIES ON *GALANTHUS IKARIAE* BAKER AND
G. RIZEHENSIS STERN (AMARYLLIDACEAE)
GROWN AROUND NE TURKEY**

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Abstract

External morphological, anatomical and physiological studies on *Galanthus ikariae* Baker and *G. rizehensis* Stern (Amaryllidaceae), the bulbous perennial plants collected from Trabzon, Turkey are described.

Introduction

Galanthus L., commonly known as pigbulb or crowbulb (Baytop, 1994) also called Kardelen in Turkey is an ornamental plant in the Black Sea region. The bulbs which have the alkaloid Galantamine are used in pharmaceutical industry in the physical therapy after poliomyelitis and the treatment of some vascular diseases. Although taxonomical (Stern, 1956; Schwarz, 1963; Artyushenko, 1966; Delipawlov & Angeliw, 1970), cytotaxonomical (Sveshnikova, 1971; Popova, 1972; Kamari, 1981, 1982; Papanicolaou & Zacharof, 1983) and pharmacological (Van Damme, 1987; Van Damme, 1988) studies of *Galanthus* L., have been made, the present report describes the morphological anatomical and physiological nature of *Galanthus ikariae* and *G. rizehensis* collected from Trabzon, Turkey.

Materials and Methods

Samples of specimens viz., *Galanthus ikariae* A7, (Trabzon: Caykara; Uzungöl, Sürmene; Zeytinli, Sahin 1), and *G. rizehensis* A7, (Trabzon: Macka; Cosandere, Bagisli, Meryemana, Yomra; Yenice, Arsin; Harmanli, Sahin 2) collected during January, February, March and April between 1991-93 (Fig.1) were kept in the herbarium of the Karadeniz Technical University. For physiological studies the plants were grown in the laboratory. For anatomical studies, specimens of leaf, scape, root, ovary and seed tissue were fixed in FAA and after passing through ethanol embedded in paraffin. Sections were stained with Safranin and Fast Green (Sass, 1940; Algan, 1981) and then covered with entellan.

Surface sections were taken from outer surface and inner surface of fresh leaves by hand for examination of stomata and epidermal cells. The standard error used in the statistical evaluation of the results has been calculated (Arici, 1984). For physiological investigations, 1 g of fresh leaf pieces from each species were suspended in distilled

Table 1. Outer morphological observations of *Galanthus ikariae* and *G. rizehensis*.

Species	Measurement (mm)	Bulb	Breadth of leaf	Length of leaf	Length of sheath	Scape	Spathe	Length of outer perianth	Breadth of outer perianth	Length of inner perianth	Breadth of inner perianth	Length of inner of anther	Length of Filament
<i>Galanthus ikariae</i>	Min-Max	12-25x5-11	15.27	80.245	30-90	110-290	25-50	17-23	7-15	8-11	4-6	4-6	0.5-1
	Mean	19-8	19.1	155.7	60.4	214.5	40	20.5	13	10.3	6	6	0.5
	Standard deviation		± 0.33	± 5.16	± 1.97	± 5.27	± 0.71	± 2.09	± 1.93	± 0.95	± 1.02	± 0.48	± 0.28
<i>Galanthus rizehensis</i>	Min-Max	10-20x4-10	3-7	40.105	35-125	76-190	20-36	9-15	4-8	8-9	4-7	4-6	0.5-1
	Mean	12-6	5.1	59.9	77.1	114.7	27.9	10.7	6	9	5	5	0.8
	Standard Deviation		± 0.38	± 1.79	± 2.65	± 2.92	± 0.53	± 1.67	± 1.04	± 1.05	± 0.80	± 0.75	± 0.33

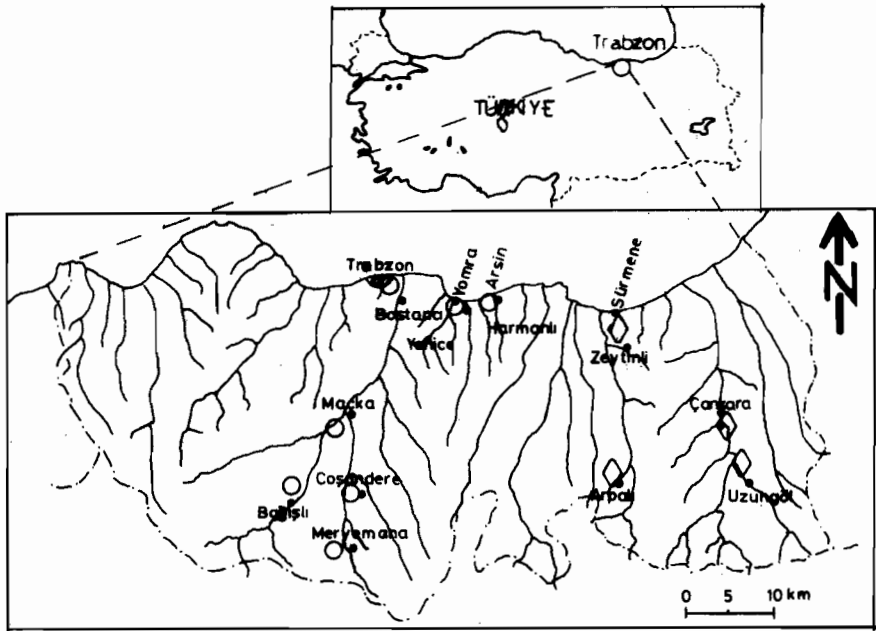


Fig.1. The distribution of the *Galanthus ikariae* and *G. rizehensis* around Trabzon (<> *G. ikariae*, O *G. rizehensis*).

water, 4 volumes of absolute acetone were added to 1 volume of leaf suspension (80% acetone). The flasks of leaf suspension were then placed on a shaker at 4°C. Forty hours later, the chlorophyll had been extracted by acetone and the leaf pieces appeared white. Five ml of solution were removed from each flask in a tube and centrifuged at nearly 30,000 g. for 5 min. Optical density of the supernatant was measured with a Shimadzu UV 1201 Spectrophotometer at wavelengths of 645 nm, 663 nm and 450 nm. One tube filled with 5 ml of 80% acetone was used as control and were subjected for calculating mg of total chlorophyll a, chlorophyll b and carotenoid in chlorophyll solution extracted from 1 g of fresh leaf (Harborne, 1984; Holden, 1965).

Results

Outer Morphology

Galanthus ikariae: Bulbs ovoid, 8-19 mm ($\pm 0.17-0.33$). Leaves lanceolate, venation parallel, bright green, flat and recurved. Apex mucronate and cuculate (Fig.2). Length of leaves 155.7 mm (± 5.16), size of leaves 19.1 mm (± 0.42). Scape 214.5 mm (± 5.2), sheath 60.4 mm (± 1.97), spathe rather regular, length 40 mm (± 0.781). Outer perianth segments 20.5 mm (± 2.09) x 13 mm (± 1.93) and convex. Inner perianth segments 10.3 mm (± 0.95) x 6 mm (± 1.02) and outer side of inner perianth with heart shaped green spot only at the apex. Filaments 0.5 mm (± 0.28), anthers 6 mm (± 0.48) (Fig.3). Ovary, subglobose 14.4 mm (± 1.29) x 14.7 mm (± 1.10) (Table 1). Seed ellipsoidal, 1.82 mm (± 0.33) and dark brown testa (Fig.4).



Fig.2. General appearance of *G. ikariae*.

G. rizehensis: Bulbs ovoid, 6-12 mm ($\pm 0.23-0.38$). Leaves linear, dark green, flat and recurved, apex obtuse (Fig.5). Length of leaves 59.9 mm (± 1.79), size of leaves 5.1 mm (± 0.11). Scape 114.7 mm (± 2.92). Sheath 71.1 mm (± 2.65). Spathe rather regular and length 27.9 mm (± 0.53). Outer perianth segments slightly convex 10.7 mm (± 1.67) x 6 mm (± 1.04). Inner perianth segments 9 mm (± 1.05) x 5 mm (± 0.80) and

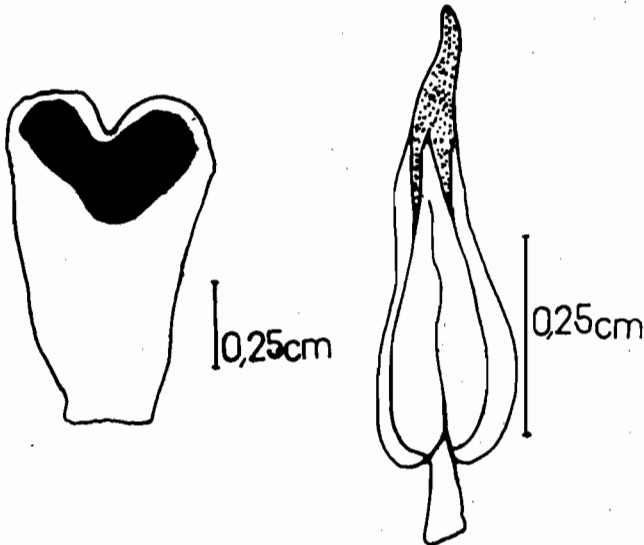


Fig.3. Inner perianth segment and stamen of *G. ikariae*.

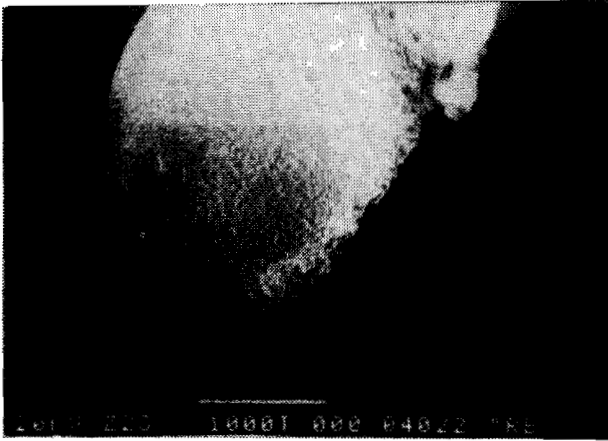


Fig.4. *G. ikariae* seed (SEM).

outer side of inner perianth with heart shaped green spot only at apex. Filaments 0.8 mm (± 0.33), anthers 5 mm (± 0.75) (Fig.6). Ovary subglobose, 5.75 mm (± 1.28) x 4.87 mm (± 1.12) (Table 1). Seed ellipsoidal, 1.7 mm (± 0.34), dark brown testa (Fig.7).

Anatomical observations

Both the *Galanthus* species showed similarity in anatomical features. In the surface section of leaf, epidermal cells are quadrangular in shape and there is stomata which is amaryllis type, between two epidermal cells (Fig.8-9). The leaves are amphistomatic. There is no subsidiary cells around guard cells in stomata. The structure of mesophyll is isobilateral. In the cross section of the leaves, there is no pronounced palisade and



Fig.5. General appearance of *G. rizehensis*.

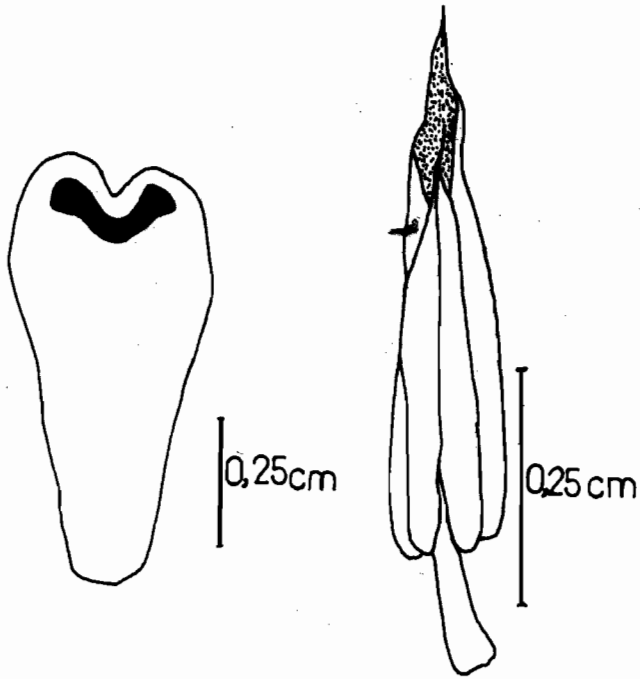


Fig.6. Inner perianth segment and stamen of *G. rizehensis*.

spongy parenchyma. The leaves have big hollows between the bundles of vessels with calcium oxalate crystals in the cells near the bundles. The bundles are surrounded by parenchymatic sheath which have not the same cells (Fig.10-11a, b; Table 2).



Fig.7. *G. rizehensis* seed (SEM). E = Eleisom



Fig. 8. Surface section from *G. ikariae* leaf (Epidermis and stomata).

**Table 2. The anatomical observations of leaves.
Standard deviations are given in parentheses (n=35)**

Species	(μ)	<i>Galanthus ikariae</i>	<i>Galanthus rizehensis</i>	
Thickness of cuticle	Min-Max	2.5-7.5	3.75-5	
	Mean	4.3	4.1	
	Std. Deviation	(± 2.21)	(± 0.60)	
Thickness of leaf	Min-max	950-1500	700-950	
	Mean	1005	795	
	St. Deviation	(± 6.12)	(± 3.01)	
Epidermis	Upper	Breadth	37.4 (± 8.88)	29 (± 3.94)
	Surface	Length	314 (± 73.5)	464 (± 106.76)
	Outer	Breadth	23 (± 4.17)	21.5 (± 3.37)
	Surface	Length	264 (± 70.86)	302 (± 85.08)
Stomata	Upper	Breadth	42.66 (± 3.16)	36.27 (± 2.05)
		Surface	Length	38 (± 3.21)
	Outer	Stoma	5.75 (± 4.27)	30.25 (± 5.90)
		(mm ²)		
	Surface	Breadth	32.5 (± 2.19)	31.33 (± 1.92)
		Length	40.95 (± 1.51)	39.30 (± 2.59)
Stoma	89.6 (± 13.86)	83.3 (± 16.2)		
(mm ²)				

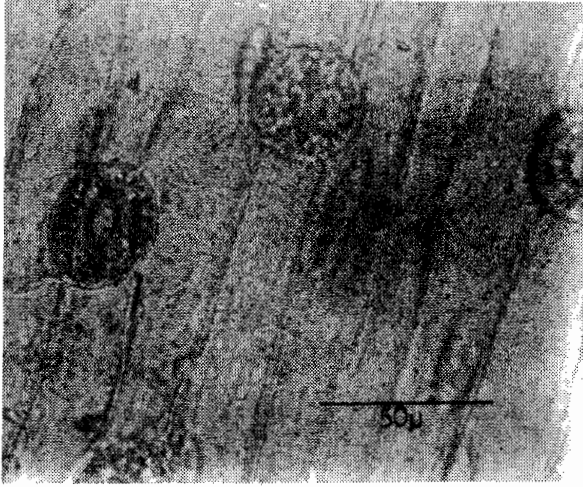


Fig.9. Surface section from *G. rizehensis* (Epidermis and stomata).

In *Galanthus* sp., the stem are scape with epidermal cells quadrangular or pentagon in shape, thickness $19 \mu (\pm 1.36)$, one layer at transverse section of scape. Parenchyma cells with chloroplast and calcium oxalate crystals. There are cavity between cells. The vascular bundles are arranged in two circles, the outer circle consisting of smaller, $57.14 \mu (\pm 13.8)$ and additional number (8-9), inner of the larger $292 \mu (\pm 74.4)$ and less number (5). Vascular bundles are collateral with large hollows in the pith (Fig.12-13).

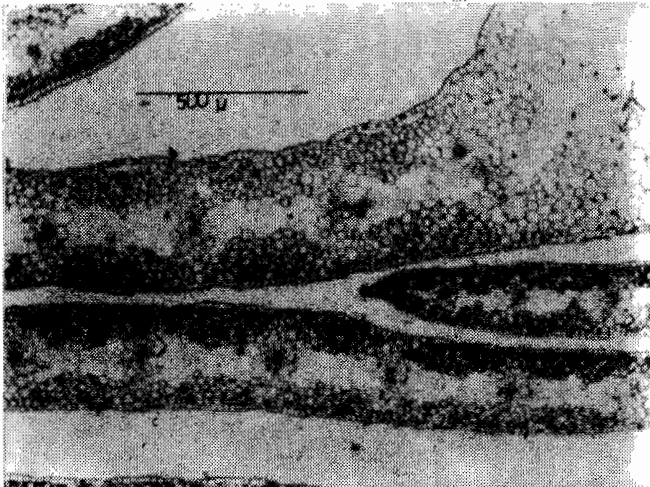


Fig.10. Cross section of *G. ikariae* leaf.



Fig.11a-b. Cross sectin of *G. rizehensis* leaf. Cr = Crystal.

Epidermal cells of root are thin walled and usually square shaped. The cortex under the epidermis consists of 6-7 layer of parenchyma cells, with intercellular spaces. The inner layer of the cortex constitutes the endodermis with smaller cells thicker than the corical cells. The thickened endodermal cell walls are homogenous. Vascular cylinder occupies the central portion of the root. Pericycle consists of one layer of thin walled parenchyma cells. Vascular bundles are tetrarch in the vascular cylinder. Phloem strands are separate concentrated on the periphery of the vascular cylinder. Xylem strands reach the center of the vascular cylinder which is then occupied by a pit. Xylem appears star-shaped in cross section. The metaxylem occupies the central portion of the pith hollow and the protoxylem occupies the periphery of the vascular cylinder. There are parenchyma cells between strands which have intercellular spaces. In cross sections the young roots appear a pith-hollow (Fig.14a) whereas in old roots these hollows are filled by trachea of metaxylem (Fig.14b).

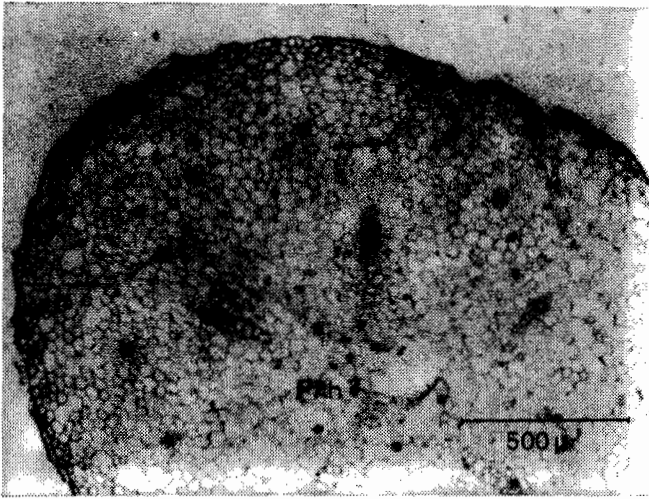


Fig.12. Cross section of *G. ikariae* scape.

The ovary is composed of 3 carpels. In transverse section taken from ovary, upper epidermis $23.5 \mu (\pm 2.83)$ in thickness and cutting by the stomata. In mesophyll, parenchyma cells are of different shapes and sizes. The vascular bundles (ovary traces) have two layers. The proportion of xylem is more than that of phloem (Fig.15-16).

Structure of the seed coats are reticulate. It has elongated cells, $10.6 \mu (\pm 2.98)$ in thickness. In surface sections of testa, cells are hexagon (Fig.17a). Endosperm under the testa covers a large area consisting of thick-walled cells with simple pits (Fig.17b). Embryo is rudimentary.

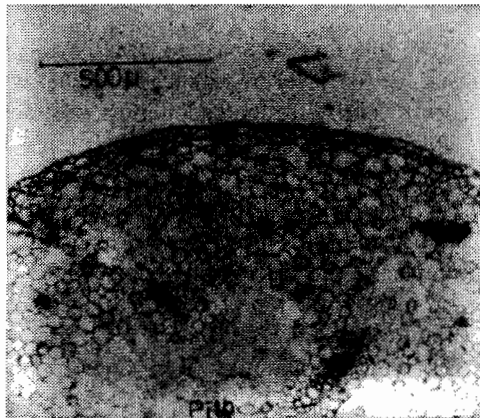


Fig.13. Cross section of *G. rizehensis* scape.

Physiological Differences

Leaves of *Galanthus* species showed differences in the total chlorophyll, chlorophyll a, chlorophyll b and carotenoid proportions (Table 3).

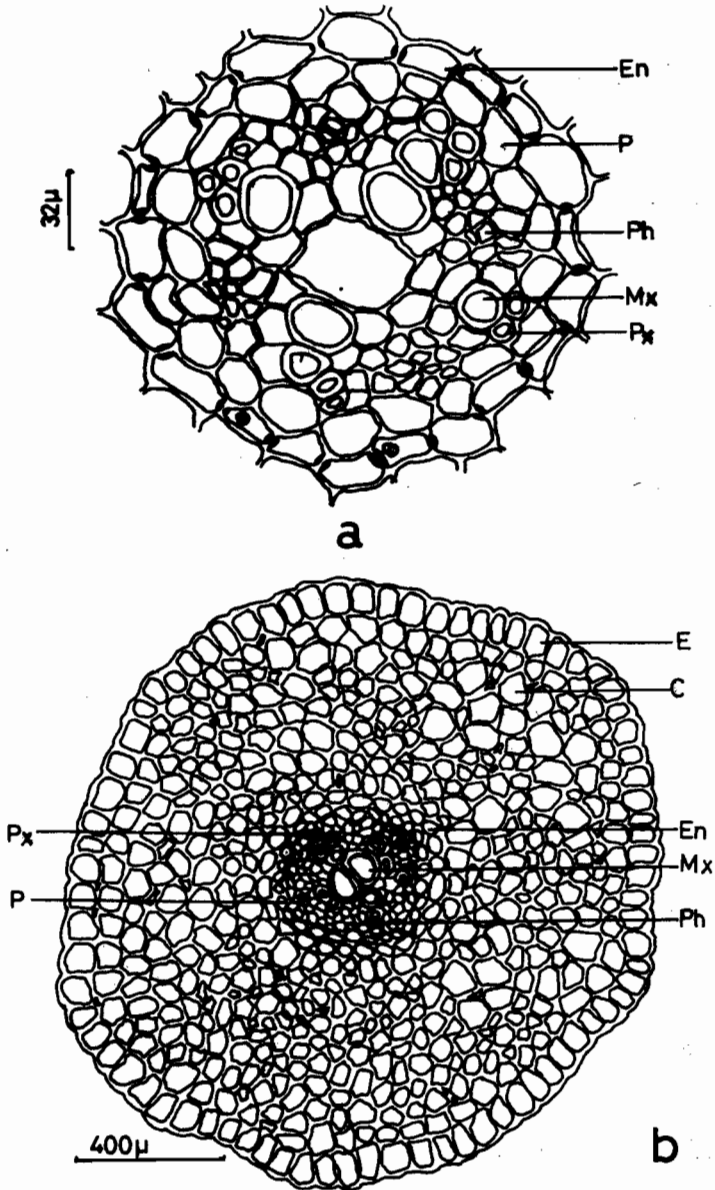


Fig.14. Cross section of *G. ikariae* root.
a= Young root, b= old root.

E= Epidermis, C= Cortex, En= Endodermis, P= Pericycle,
Px= Protoxylem, Mx= Metaxylem, Ph= Phloem

Table 3. The physiological results of *Galanthus ikariae* and *G. rizehensis* leaves (mg/g).

Species	Chlorophyll a	Chlorophyll b	Chlorophyll a/b	Total Chlorophyll	Carotenoid
<i>G. ikariae</i>	0.513	0.257	0.050	0.780	0.121
<i>G. rizehensis</i>	0.604	0.484	0.032	1.087	0.050

Discussion

The external morphological properties of the 2 *Galanthus* species showed differences when compared with the Flora of Turkey (Brickell, 1984). This may be due to different collection sites (Table 4). This is a feature of monocotyledonae leaves in which lack of certain palisade and spongy parenchyma (Fig.8-9) has also been reported (Metcalf, 1972). There has been differences in terms of the number of stomata in a unique area, the length of the epidermal cells, thickness of cuticle and the leaves thickness (Table 2). In the *Galanthus* species, the parenchymatic cells which have a thin wall, develop hollow air cavity instead of disintegration of cells (Kasaplilil, 1961).

The general features of the root anatomies of the herbaceous monocotyledon are the thickening of three sides of the endodermal cell wall and the increasing number of xylem strands (Esau, 1966; Fahn, 1967). There are conspicuous similarities between the root features of *Galanthus* species and primary structure of herbaceous plants. The most important feature of tetrarch roots are not having three sides thickness in the endodermis. The same feature has also been observed in the *Leucojum aestivum* from the same family (Kutbay, 1993). The pith usually doesn't grow at the root of the monocotyledon. The parts of the metaxylem take place inside of the central cylinder. On the other hand, the pith was filled by metaxylem vessel in older roots (Fig.14a,b).

**Table 4. A comparison of the outer morphologic results with Flora of Turkey
A: The measurements in Flora of Turkey, B: Our measurements**

Values (mm)	<i>Galanthus ikariae</i>		<i>Galanthus rizehensis</i>	
	A	B	A	B
Bulb	18-35x16-28	10-20x4-10	15-20x11-15	10-20x4-10
Leaf	50-300x5-30	80-245x15-27	90-200x3-10	40-105x3-7
Scape	110-250	110-290	10-20	76-190
Outer perianth segment	17x26x6-11	17x23-7-15	15-20x6-8	9-15x4-8
Inner perianth segment	9x5	8-11x4-6	8-9x4-5	8-9x4-7
Filament	0.5-1.5	0.5-1	0.5	0.5-1
Anther	5-6	4-6	5	4-6
Ovary	15x14-15	12-16x13-16	7-8x5-6	4-8x3-6
Spathe	-	25-50	-	26-36
Sheath	-	30-90	-	35-125
Seed	-	1.8	-	1.7

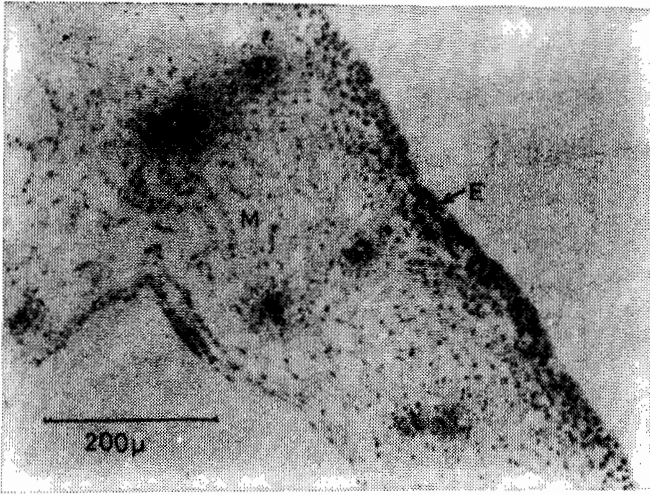


Fig.15. Cross section of *G. ikariae* ovary.
E= Epidermis, M= Mesophyl

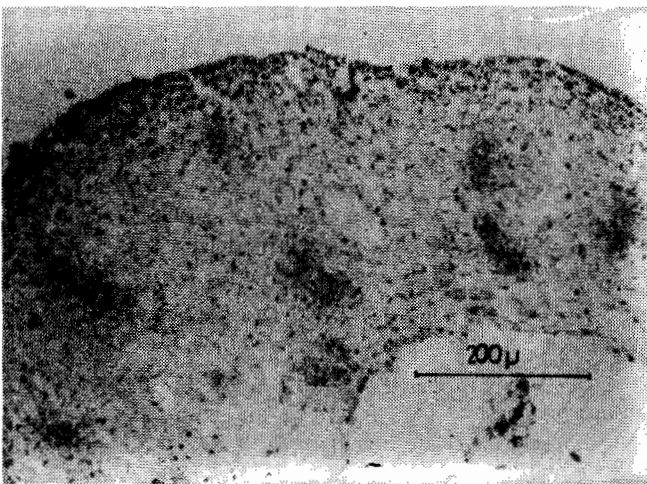


Fig.16. Cross section of *G. rizehensis* ovary.

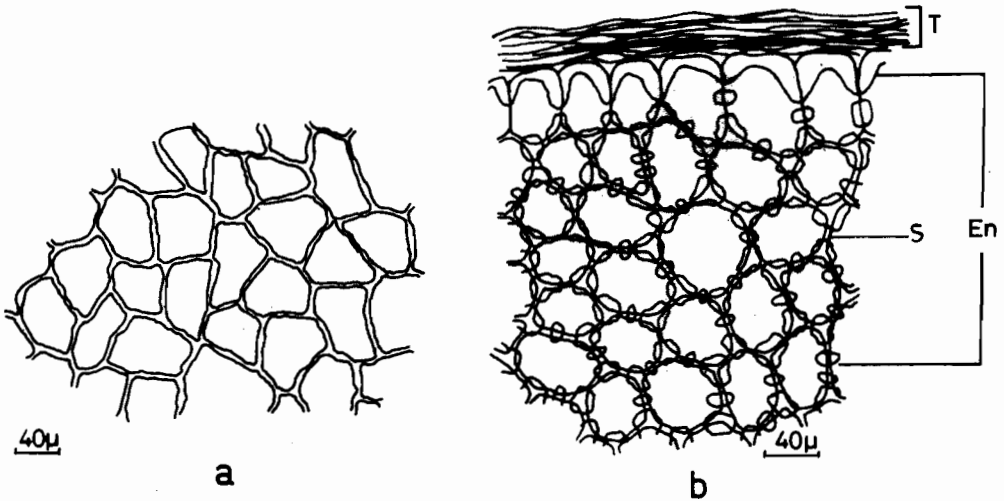


Fig.17. a- Surface, b- Transverse sections of *G. rizehensis* seed. En= Endosperm, S= Simple Pit.

There are calcium oxalate crystals in scape cortex like the one in leaf-mesophyl and intercellular spaces in cross section from ovary of the *Galanthus* species.

Eleisom which is seen in *Amaryllidaceae* has been observed in *Galanthus* species too (Fahn, 1967). The seed coat has the feature of reticular structure which is not deep (Fig.7). No differences has been observed in anatomical structure in the longitudinal section of seed samples (Fig.17a,b). Since the two *Galanthus* species showed differences in terms of photosynthetic pigments, the morphological results are thus supported by the physiological features found in this study.

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