YYÜ TAR BİL DERG
(YYU J AGR SCI)
2018, 28(1): 10-18

Geliş tarihi (Received): 17.04.2017
Kabul tarihi (Accepted): 08.02.2018
doi: 10.29133/yyutbd.306727

Araştırma Makalesi/*Research Article (Original Paper)*

# Seed Morphology, Leaf Anatomy and Karyotype Analysis of the medicinal and ornamental plant; *Vaccaria hispanica* (Miller) Rauschert

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**Abstract:** *Vaccaria hispanica* (Miller) Rauschert is a monotypic genus which is in the family Caryophyllaceae. The plant is known as ‘‘Ekinebesi’’ in Turkey. It has been recorded in pharmacopoeias and also it has been cultivated as ornamental plant in Canada. The leaf anatomy *V. hispanica* has been determined, seed and chromosome morphology have been confirmed in this study. Somatic chromosome number has been determined as 2n=30. Relative variation in chromosome length and mean centromeric asymmetry of the species has been estimated. The examined species has bifacial and amphistomatic leaf. The lower stomatal index was estimated at 27.14%, the upper stomatal index was estimated as 13.20%.

**Keywords:** Anatomy, Cow cockle, Karyotype, Seed, *Vaccaria*

## Tıbbi ve Süs Bitkisi Olan *Vaccaria hispanica’* nın Tohum Morfolojisi, Yaprak Anatomisi ve Karyotip Analizi

*Vaccaria hispanica* (Miller) Rauschert, Caryophyllaceae familyasındaki monotip bir cinstir. Türkiye’de Ekinebesi ismiyle bilinir. Farmakopelerde kayıtlıdır ve ayrıca süs bitkisi olarak Kanada’da kültürü yapılır. Bu çalışmada *V. hispanica*’nın yaprak anatomisi belirlenmiştir, tohum ve kromozom morfolojisi daha önceki çalışmalarla teyit edilmiştir. Somatik kromozom sayısı 2n = 30 olarak belirlenmiştir. Türlerin kromozom boyu ve sentromerik asimetri ortalaması hesaplanmıştır. İncelenen türün bifasyal ve amfistomatik yaprakları vardır. Yaprak alt yüzeyindeki stoma indeksi %27.14, üst yüzeydeki stoma indeksi %13.20 olarak hesaplanmıştır.

**Anahtar kelimeler:** Anatomi, Ekinebesi, Karyotip, Tohum, *Vaccaria*

## Introduction

*V. hispanica* is a monotypic genus which is in the family Caryophyllaceae. Due to the different systematic interpretations of different authors about the genus *Vaccaria*, a large number of synonyms have emerged as taxa (Greuter 1995). For example, the genus *Vaccaria* recorded as four different species in Iranian flora (Rechinger 1988), these species were interpreted as subspecies in the Mediterranean Checklist I (Greuter et al. 1984). Also four varieties of *Vaccaria pyramidata* were accepted in the Flora of Turkey and the East Aegean Islands (Cullen 1967). In the last molecular studies all taxa were considered synonymous with *V. hispanica* (APG III 2009). The plant also known as Ekinebesi (Turkish name) is an annual weed commonly found in grain fields of the Turkey (Tepe 2014).

The seeds of *V. hispanica* have been recorded in pharmacopoeias for about 2000 years in China because of galactagog effects and for menstrual disorders, also it is known as well as accelerate blood circulation in humans (Jiang et al. 2013). It has been used for eyestrain symptoms in female, shingles treatment (Zhang et al. 2012) and prostate treatment (Huo 2009). It may also be an alternative agricultural product as an ornamental plant (Mazza et al. 1992; Weiss 2002; Waiganjo et al. 2008). In our country, it has been determined that the roots of the plant were used as diaphoretic (Çakılcıoğlu and Türkoğlu 2010). Also, neurobiological activities were evaluated by Orhan et al. 2016. The chemical components of *V. hispanica*

are grouped as triterpenoid saponins, cyclic peptides, flavonoids, and others (Zhou et al. 2015; 2017). Seeds contain more than %60 starch and %15-16 protein (Price et al. 1987). Antifungal activity of seeds revealed by Wong at al 2017.

Seed and chromosome morphology have been considered useful for solving taxonomic and evolutionary problems (Bergreen 1981; Akbari and Azizian 2006; Abid and Ali 2010; Rajbhandary and Shrestha 2010; Ackin and Binzet 2011). The chromosome numbers and the karyotypes of *V. hispanica* were investigated (Clavijo and García-Panta-León 1986; Luque and Lifante 1991; Talavera 1978; Vachova 1976). Also seed morphology of *V. hispanica* was examined by scanning electron microscopy (SEM) (Kanwal et al. 2012; Mazza et al. 1992; Memon et al. 2014). Leaf anatomy is a significant characteristic in the system of many plant groups (Inamdar and Chohan 1969a; Bhatia 1984; Stace 1984; Jones 1986; Baranova 1972; Adedeji and Dloh 2004; Rudgers et al. 2004; Celka et al. 2006; Zou et al. 2008; Yasmin et al. 2009). The epidermal cells of *V. hispanica* were examined, but there is a lack leaf anatomy in detail (Ahmad et al. 2010).

*V. hispanica* is a typical summer annual species. It has blue-gray, waxy herbage and pale pink flowers. So, it has potential for used a bedding plant or an outdoor ornamental plant (Arı et al. 2014; Duddu and Shirtliffe 2014). Ornamental plant is an important sector of the horticultural industry. Ornamental plant production even exceeds vegetable and fruit production in some countries of the world. So, new crops are brought to the ornamental plants market (Brickell 2001; Linde et. al. 2007). Development of a new crop includes a combination of superior plants material, production technology and an efficient marketing strategy. If any of these three factors is not properly developed and fully implemented, the chance of success is greatly diminished (Hentig 1998). The development and introduction process for new commercial crops introduction is complex, lengthy, expensive and filled with hazards. The introduction process can be divided into five stages. The first stage is basic genetic and anatomy studies (chromosome number and incompatibilities, leaf anatomy and so on) are under taken (Wilkins and Erwin 1998).

The present study aims to determine some basic genetic and anatomy feature. In this framework; the leaf anatomy, also to confirm and to examine in detailed seed and chromosome morphology of the *V. hispanica* which is the medicinal and ornamental plant.

## Materials and Methods

*Plant material*

The study was conducted on *V.hispanica*, The specimens were collected between May 2015 and July 2015 from eastern Turkey (Van Yüzüncü Yıl University Campus area, Van) (Figure 1). The plant materials were stored at The Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE) with numbered ISTE 113103.

Figure 1. Habitat of *V. hispanica* in the eastern Turkey (ISTE 113103).

*Chromosome analysis*

Chromosome counts were obtained from somatic metaphases (Figure 2) using a standard squash technique. 10–15 metaphase plates were studied. Seeds collected from three populations were germinated on filter paper in petri dishes, and root tip meristems were collected for analysis. The samples were pretreated with alphabromonaphthalene at 4°C for 24h before fixing in Carnoy solution. The classification of chromosomes based on location of centromeres as metacentric (m), submetacentric (sm), subtelocentric (st), and telocentric (t) was done according to Levan et al. (1964). Karyograms and idiograms of the investigated species are presented in Figures 3 and 4. Morphometric data of the species is summarized in Table 1.

*Leaf anatomy*

Fresh leaves were preserved in a 70% ethyl alcohol solution. Cross-sections were obtained by sectioning the leaves from the tip to 15–20 mm, and then the cross-sections were stained with a SARTUR solution (Çelebioğlu and Baytop 1949). Several slides were made and photographed for each species with the aid of a light microscope (Olympus BH-2 and Canon A 640 digital camera). Stomatal index (SI) was calculated according to the formula of Salisbury (1927): $SI = S/(E+S) \times 100$, where S is the number of stomata and E is the number of epidermal cells per unit leaf area.

*Seed morphology*

The seeds were principally observed by a light microscope to make sure that they were of normal size and maturity. Three samples were prepared for scanning electron microscopy (SEM) analysis. The samples were stayed on stubs and covered with gold before they were studied with an FEI Quanta 450 FEG-EDS scanning electron microscope in Istanbul University.

## Results and Discussions

*Chromosome analysis*

In this study, chromosome observations for the *V.hispanica* were presented with microphotographs of diploid metaphase, karyogram and ideogram (Figures 2, 3, 4). The chromosome numbers and morphology of were reported as detailed and morphometric data of the species was given in Table 1. Somatic chromosome numbers and karyotype formula were determined as 2n=30=28 MC + 2 SMC. Relative variation in chromosome length (CVCL=14.171 ± 0.04) and mean centromeric asymmetry (MCA = 6.94 ± 0.03) of the species were estimated according to Peruzzi and Eroğlu 2013. The chromosome number of examined the *V. hispanica* supports findings from earlier scholars (Ruíz de Clavijo and García-Panta-León 1986; Luque and Lifante 1991; Talavera 1978; Vachova 1976); however, there was a difference in satellite chromosome included in the karyotype of the species (Huss 1981). In this study satellite was not observed in the karyotype of all examined metaphase cells. The basic chromosome number was observed as x=15. The smallest chromosome length was measured as 1.112 µm, while the longest length of 1.994 µm was estimated. Also total chromosome lengths were measured as 21.586 ± 0.04 µm.

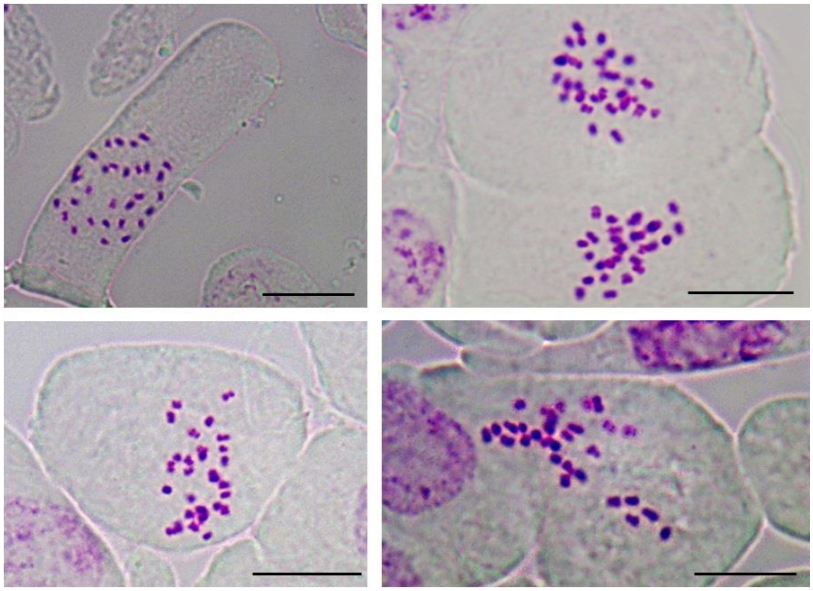

Figure 2. Microphotographs of five diploid metaphase cells (scale bar 10 µm).

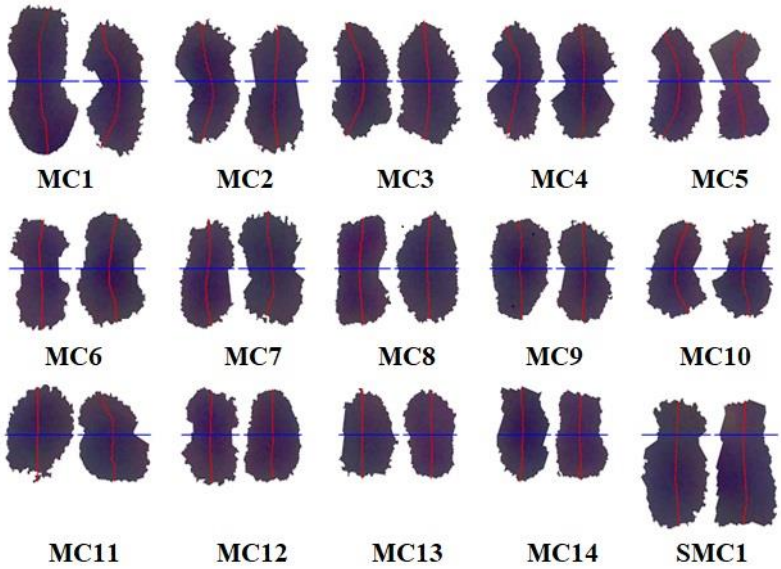

Figure 3. Diploid karyogram of the *V. hispanica*

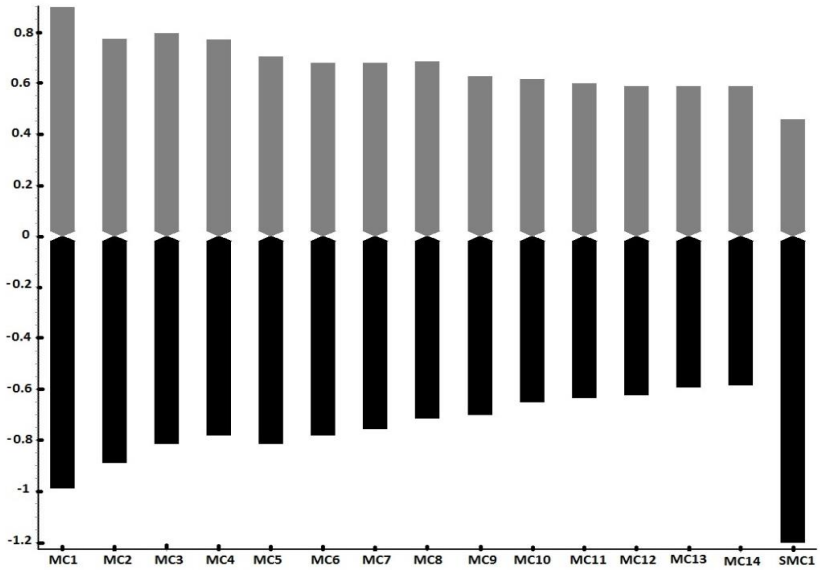

Figure 4. Haploid ideogram of the *V. hispanica*

*Leaf anatomy*

The leaf anatomy of the *V.hispanica* was assessed in terms of its overall aspect including examination of leaf and midrib in transverse section, also stomatal index, and stomata structure for the first time in this study.

*Midrib in transverse section:* Epidermis, the leaf epidermis on both the upper and lower surfaces consists of a single, uniform layer of cells, approximately 0.5–0.8 µm thick, covered with a thin cuticle, approximately 0.1–0.2 µm. Just below the epidermis, there are usually 3–5 layers (the number of layers can vary) of hexagonal-to-rounded parenchyma cells, approximately 1 mm wide. Some idioblasts, with starch grains, are also present. Well-developed sclerenchymatous cells surround the vascular bundles, forming a bundle sheath. Sieve tubes and companion cells are narrow. The phloem is distinct, and the xylem is composed of narrow vessels and tracheids (Figure 5).

*Leaf in transverse section:* The mesophyll consists of palisade and spongy parenchyma (bifacial leaf). The palisade parenchyma comprises a layer of thin-walled and compactly arranged cylindrical cells. Spongy parenchyma usually comprises isodiametric cells with intercellular space and it is located underneath the palisade parenchyma (Figure 6).

Table 1. Mean (±) standard deviation of morphometric data for the *V. hispanica*

| | Centromer positions | *Total lengths (µm) | Short arms (S) (µm) | Long arms (L) (µm) | Arm ratio |
|---|---|---|---|---|---|
| **1** | MC1 | 3.774± 0.107 | 0.896 ± 0.101 | 0.991± 0.006 | 1.11 |
| **2** | MC2 | 3.327 ± 0.01 | 0.773 ± 0.064 | 0.891± 0.054 | 1.15 |
| **3** | MC3 | 3.224± 0.025 | 0.794 ± 0.024 | 0.818± 0.001 | 1.03 |
| **4** | MC4 | 3.104± 0.014 | 0.768 ± 0.007 | 0.784± 0.007 | 1.02 |
| **5** | MC5 | 3.038± 0.012 | 0.702 ± 0.015 | 0.817± 0.004 | 1.16 |
| **6** | MC6 | 2.921± 0.007 | 0.678 ± 0.033 | 0.782± 0.04 | 1.15 |
| **7** | MC7 | 2.87 ± 0.005 | 0.678± 0.022 | 0.757± 0.027 | 1.12 |
| **8** | MC8 | 2.8 ± 0.016 | 0.684± 0.008 | 0.716± 0.024 | 1.05 |
| **9** | MC9 | 2.656± 0.003 | 0.625± 0.01 | 0.704± 0.006 | 1.13 |
| **10** | MC10 | 2.538± 0.022 | 0.615± 0.007 | 0.654± 0.015 | 1.06 |
| **11** | MC11 | 2.469± 0.007 | 0.597± 0.024 | 0.638± 0.017 | 1.07 |
| **12** | MC12 | 2.42 ± 0.006 | 0.586± 0 | 0.624± 0.006 | 1.06 |
| **13** | MC13 | 2.363± 0.006 | 0.587± 0.007 | 0.595± 0.001 | 1.01 |
| **14** | MC14 | 2.346± 0.061 | 0.587± 0.03 | 0.587± 0.03 | 1.00 |
| **15** | SMC1 | 3.322± 0.007 | 0.457± 0.005 | 1.204± 0.012 | 2.63 |

*Total Diploid Length of chromosome set

*Leaf surface section:* The epidermal cells are arranged as undulate. Our results support previous studies about the leaf surface section of the *V.hispanica* (Ahmad et al. 2010; Memon et al. 1014). Both surfaces of epidermis have stomata (amphistomatic leaf), with guard cells surrounded by 3-4 epidermal cells (anomocytic stomata). Stomata slightly sink from the epidermis. Stomatal type is often used as species-diagnostic (Metcalfe and Chalk 1979). In the family Caryophyllaceae, diacytic type stomata usually are observed (Yentür 2003), but anomocytic stomata were observed in the examined species. Stomata were measured as 6.37 × 12.2 µm. The lower stomatal index was estimated as % 27.14, upper stomatal index was estimated as % 13.20 (Figure 7.).

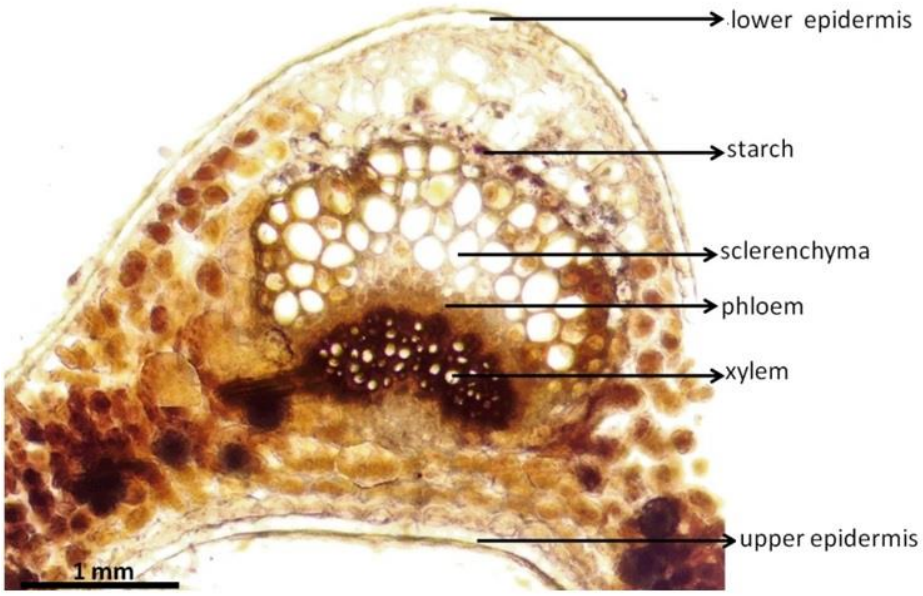


Figure 5. Midrib of the *Vaccaria hispanica* in transverse section

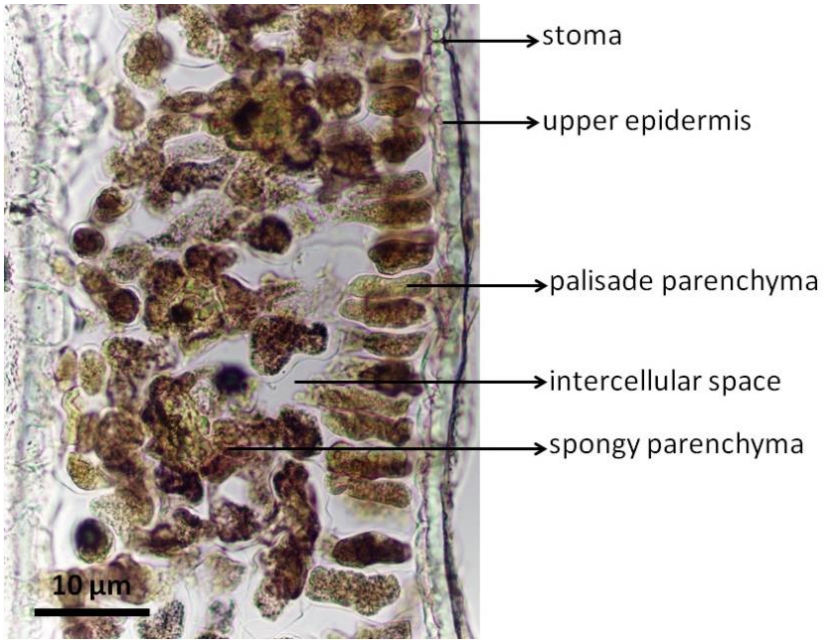

Figure 6. Leaf of the *Vaccaria hispanica* in transverse section

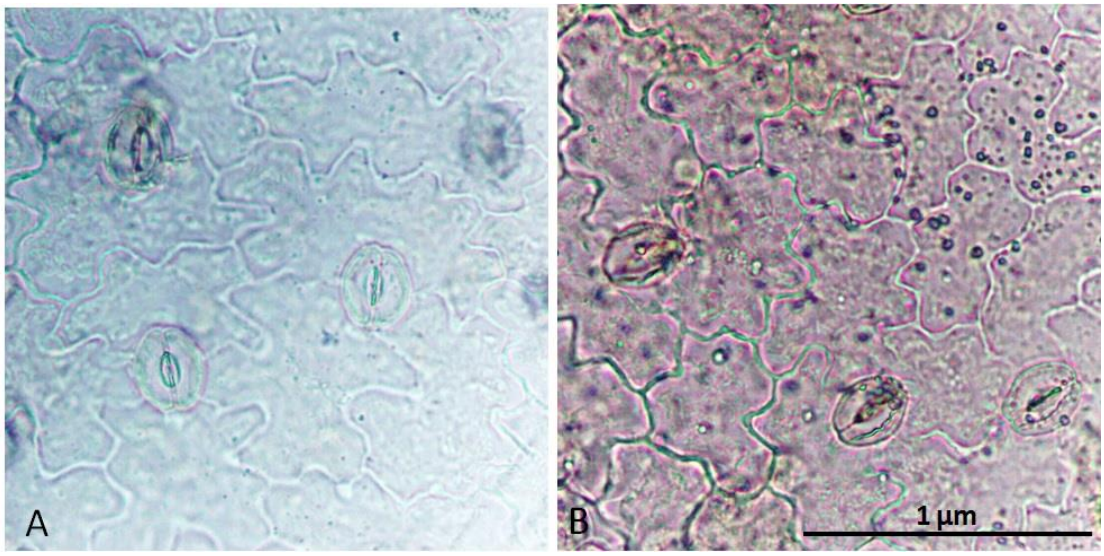

Figure 7: Leaf surface sections of *V. hispanica* A) Lower surface, B) Upper surface.

*Seed morphology*

The seed shape and surface characteristics of the *V.hispanica* were presented with scanning electron micrographs (Figure 8). Seeds 1.4-2.2 × 1.5-1.6 mm, angular and globose-subglobose, dark brown and shiny surface covered with linear-round plates, margin crenate, surface rugose, hilum distinct. The seed morphological data is significant enough to correlate the taxonomic delimitation of the family Caryophyllaceae at various levels. The genus *Vaccaria* remains distinct due to the presence of globose-subglobose seeds, similarly, the two genera *Dianthus* and *Petrorhagia* can be coupled due to the presence of ovate seeds and different seed size and plate margin (Kanwal et al. 2012). Previous studies (Memon et. al. 2014; Mazza et al. 1992) about the seed morphology of *V.hispanica* were confirmed by our results in this study.

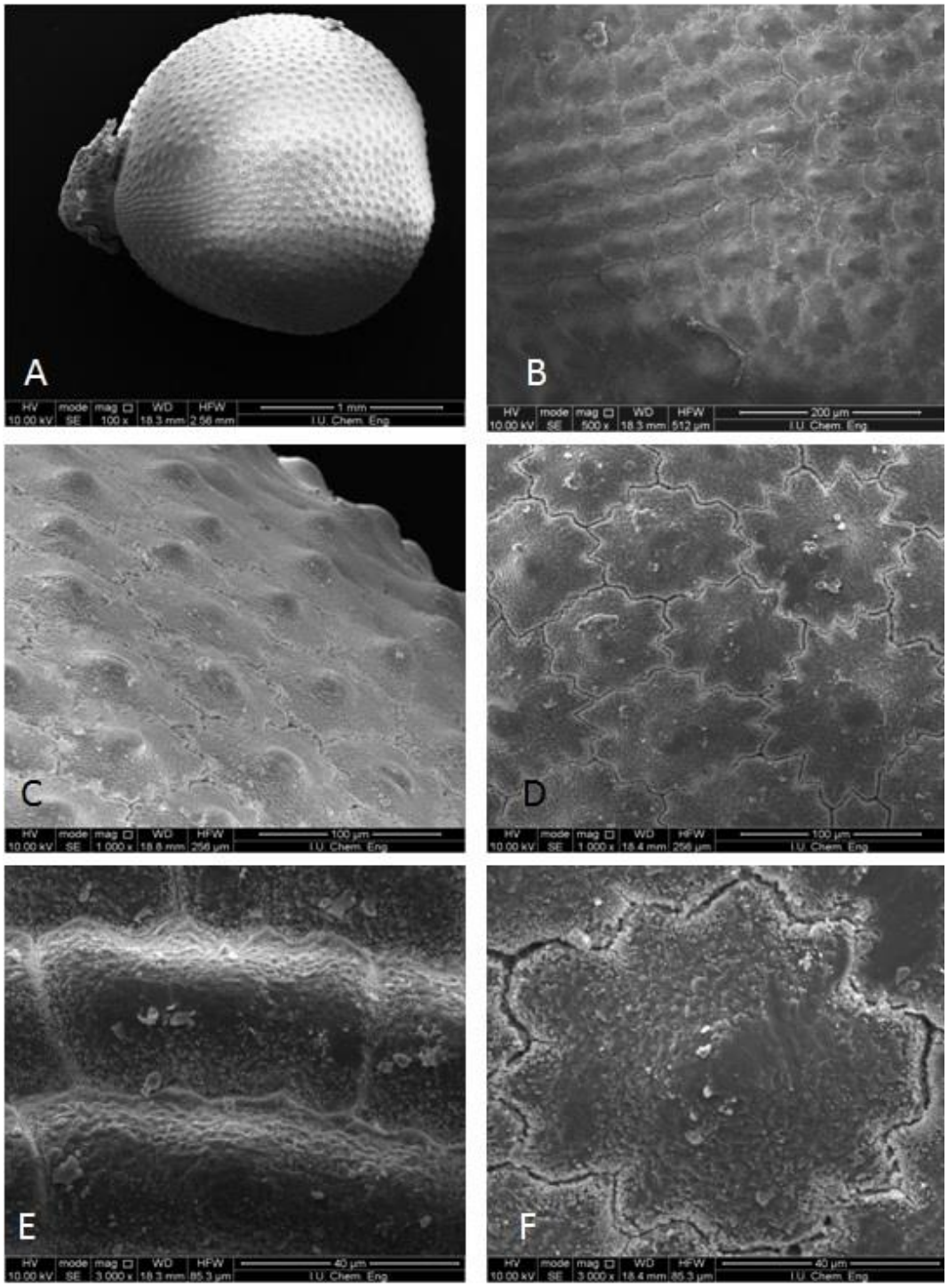


Figure 8: Scanning electron micrographs of *V. hispanica:* A) Seed general view, B) Hilum line, C) Rugose surface, D) Round plates with crenate margin, E) A plate of hilum line, F) A round plate with crenate margin.

## Conclusion

Cytogenetic studies of *V. hispanica* is limited and there are no reported from Van/Turkey. The study showed the chromosome numbers and karyological analysis of *V. hispanica*. The basic chromosome number is relevant to the systematic position of a taxon at high taxonomic levels. *V. hispanica* comprise a polyploid complex with the fifteen base chromosome numbers. Not surprisingly, the present investigations regarding the base chromosome number agree completely with previous reports. The chromosome morphology has been found to be stable. This feature makes it suitable for the methods used to develop the variety of ornamental plants. Results revealed the leaf anatomy and chromosome analysis

of the *V.hispanica* for the first time. Also, the seed morphology was confirmed in this study. Eventually, there is a need of more exhaustive studies on the ornamental and medicinal plants for comparing. Thus, such distinct characteristics have been proved as good taxonomic indicators and also a potential add in taxonomic description of these species.

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