

## Morphological and anatomical investigation on the genus *Vaccaria* Wolf. (Caryophyllaceae) In addition to its geographical distribution in Iraq

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**Abstract:** The morphological, anatomical, micro-morphological features and geographical distribution of *Vaccaria* Wolf. have been studied on plant samples collected from different northern and middle Iraqi districts where the plants usually grow, In general, the morphological results were similar with the description of the genus in Iraqi flora but with more details, the micro-morphological features of each seeds and pollen grain have been studied by SEM and gave a good distinguished characters. The anatomical studies of the roots, stems and leaves were carried out to obtain precise characteristics which are consider as original information about the genus in Iraq.

**Keywords:** Anatomy, Geographical distribution, Iraq, Morphology, Micro-morphology, *Vaccaria*.

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### I. Introduction

The genus *Vaccaria* Wolf. belong to Caryophyllaceae family which is consist of annual or perennial herbs, rarely shrubs or trees, distributed worldwide especially in the Northern Hemisphere [1].

Genus *Vaccaria* represented by 4 species in Europe, Mediterranean, Temperate Asia as mentioned by [2], others such as [3] treat them as subspecies, and a majority as [4] have them as varieties, while in Iraq the genus represented by one species which is *Vaccaria hispanica* (Miller.) Rauschert. [5].

The seeds of this plant have a wide range of chemical compounds, including saponins, triterpene, alkaloids, cyclic peptide, phenolic acid, flavonoids and steroids, and its well known in traditional Chinese medicine for activate blood circulation, relieve carbuncle and promote milk secretion, so its consider as one of an important medicinal herbs.[6] Some anatomical and Palynological information about the family and the genus was mentioned previously in some references [7; 8; 9; 10; 11) and to provide more accurate morphological and anatomical information, and to determine the distribution of *Vaccaria* plants in Iraqi districts, this study was conducted.

### II. Materials And Methods

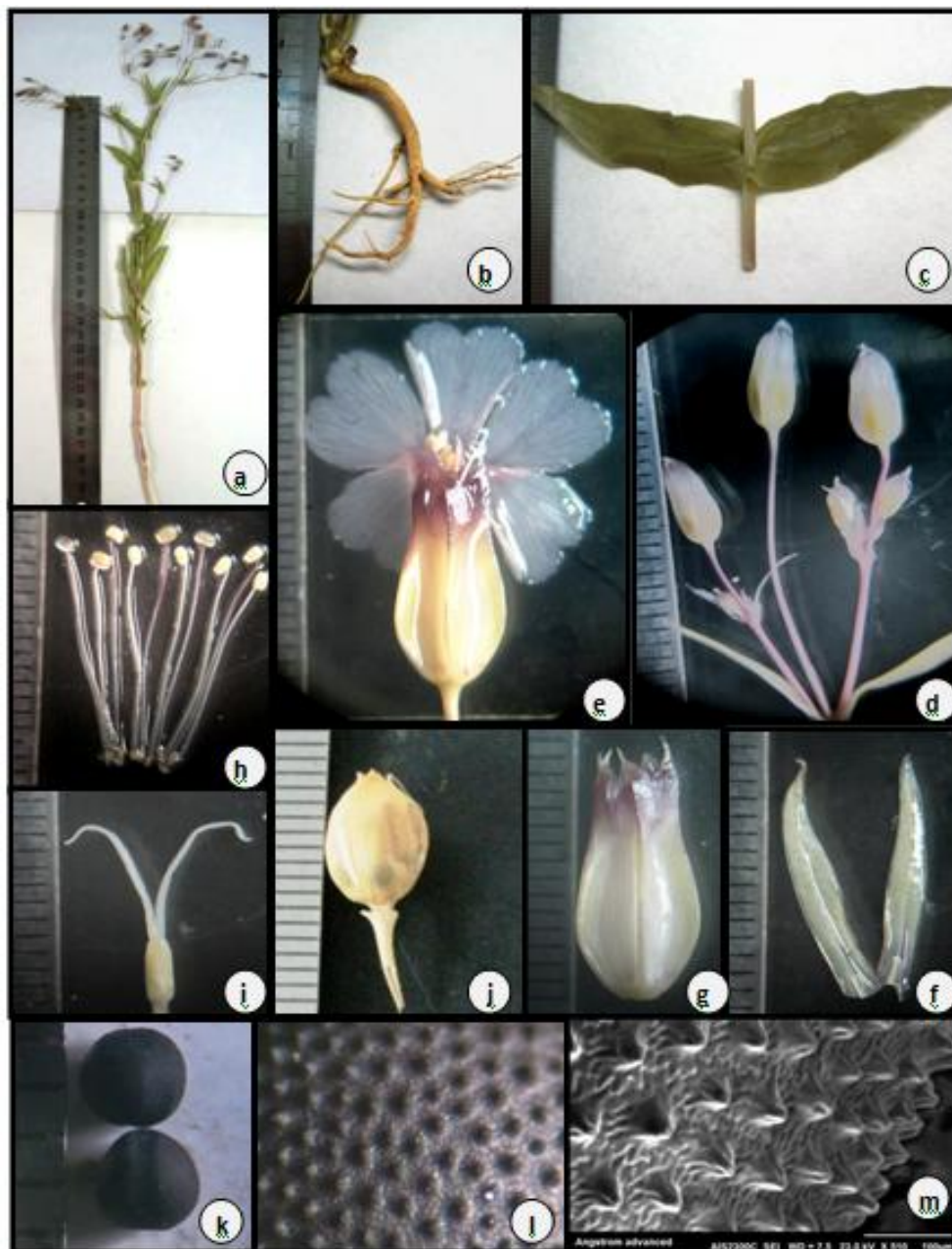
Plant samples were collected from different northern Iraqi district in 2016 and saved either by FAA solution (Formalin, ethanol, acetic acid solution) or by drying them according to standard herbarium techniques and stored in Iraqi herbarium, the morphological study was conducted by taking measurements and observing the different characteristics of all plant parts from both fresh collected samples and dried herbarium samples from Iraqi herbarium, Anatomical cross sections of vegetative parts were prepared by either free hand sectioning or by standard method of paraffin wax [12], Epidermis of both stem and leaf were peeled from fresh specimen and stained with safranin 0.5%, then the slides were studied by Leica compound microscope and photographed by digital cannon camera, geographical distribution information collected from the labels of drying herbarium samples, The seed, and pollen grains of the plant were examined by LEO 1450VP Scanning Electron Microscopy (SEM).

### III. Results

#### 3.1. Morphological study "figure 1"

Annual herb with yellowish tap root, plant glabrous divaricately branched from above; stem cylindrical and erect, 90-520 mm; Basal and Cauline leaves sessile and opposite, usually connate at base, lanceolate to broadly lanceolate with acute apices, entire margins and semi-cordate bases, (5-25x35-90)mm, Inflorescence compound dichasium with many- loosed flowered. Pedicels (22-25) mm, erect-spreading, with pair of narrowly lanceolate bracts usually connate at base (1.5-2x6-13) mm. Calyx cylindrical and winged, composed of 5 lanceolate leaves with acuminate green-purplish teeth, (9-14) mm. Petals consist of narrow-long claws and cordate limbs (16-20) mm, pink in color. Androecium consist of ten stamens, five of them have short light

yellowish filaments (13-14) mm and the other five with yellow-purplish long filaments (14-15) mm, anthers oblong yellowish dehiscent by longitudinal slits, Gynoecium with cylindrical white ovary, and two styles, Capsule ovoid and yellowish, opened with 4 teeth, surrounded by calyx (7-13x15-20) mm. Seeds spherical with central groove, spinulate, each spinule surrounding by undulated circle, and black in color, 10-12 in the capsule, (1.5-2) mm. Flowering 4-6.



**Figure 1:** morphological characters of *Vaccaria hispanica*, a: whole plant, b: root, c: leaf, d: inflorescence, e: flower, f: bracts, g: calyx, h: stamens, i: gynoecium, j: capsule, k: seeds, l, m: seed surface configuration

### 3.2. Palynological study "Figure 2"

Pollen grains Spheroidal, (26-31)  $\mu\text{m}$  diameter, poly, panto-porate, 8-10 pored. Pores operculate and sunken (3-6)  $\mu\text{m}$  diameter with spinulate membrane. The sculpture of pollen exine was granulatae with microechinatae.

### 3.3. Anatomical study "Figure 3"

#### 3.3.1. Root "figure 3-a"

The cross section was taken from the middle part of the root and it was covered externally with a thin periderm (3.2-8)  $\mu\text{m}$ . The cortex consist of parenchyma with druses crystals. Phloem is arranged in triangular shapes consist of 6-8 layered of irregular cells (39-52)  $\mu\text{m}$ . The cambium is thin. The xylem extends all around the primary xylem and filled the root centre.

### 3.3.2. Stem "Figure 3-b,c"

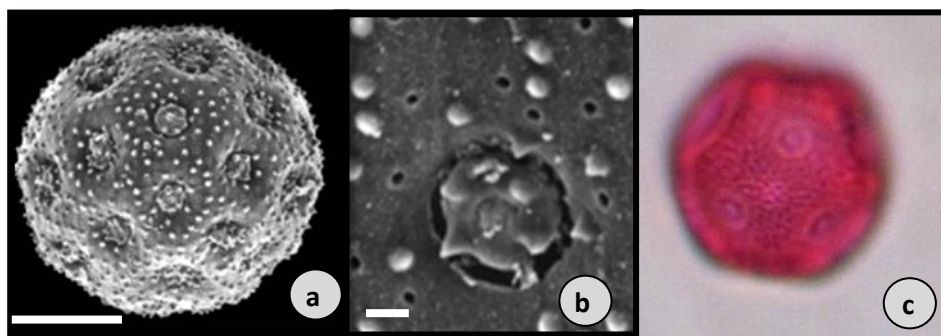
The cross section was taken from the middle part of the stem and showed one layer of epidermis composed of rectangular cells (15-18) $\mu$ m. The upper surface is glabrous and covered with a relatively thick cuticle (2.5-7.8)  $\mu$ m. The cortex is 4 layered and consists of Chlorenchyma (46-52)  $\mu$ m. The single-layered endodermis consists of ovoid cells. The pericycle is wide and consists of 8-9 layered of sclerenchyma cells (299-338) $\mu$ m. The phloem is 2-3 layered and consists of irregular cells arranged in continuous ring (20.8-26) $\mu$ m. The cambium is thin and consist of 2-3 compacted rows (5-7.8) $\mu$ m. The xylem cells arranged in continuous ring (78-143) $\mu$ m. The center of section represented by pith which is consists of large semispherical or polygonal parenchymatic cells with druses crystals.

### 3.3.3. Leaf "Figure 3-d,e"

Leaf section was glabrous covered by single layer of each upper and lower epidermises which consist of rectangular cells, The mesophyll was bifacial composed of one row of elongated palisade layer and 4-5 rows of irregularly lobed Spongy cells with druses crystals. The midrib was semi orbicular in shape and covered by uniseriate epidermis, the cortex consist of parenchyma tissue only, the vascular tissue represented by one arced-shape vascular bundle surrounded by one layer of bundle sheath.

### 3.3.4. Cuticular study "Figure 4"

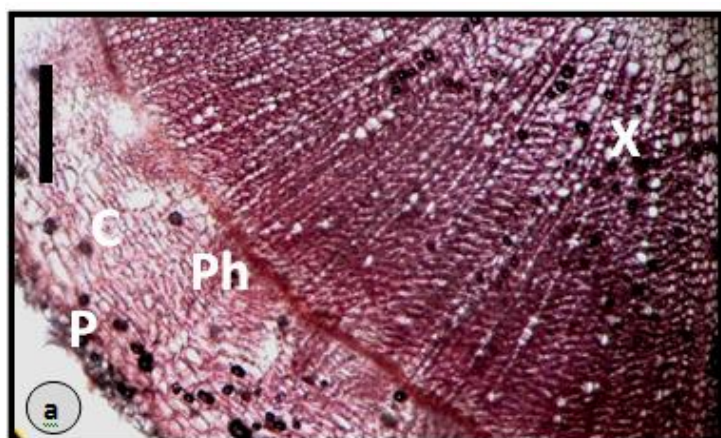
Both upper and lower leaf epidermises as well as stem epidermis were glabrous and covered with a thin cuticle. Ordinary epidermal cells of leaf were polygonal but varied in their Anticlinal walls shapes, so they were curved in upper epidermis and undulate in lower one, stem epidermis cells were rectangular with oblique end wall. The Stomatal types were caryophyllaceous (Diacytic type) and ranunculaceous (Anomocytic type), they occurred on the surfaces of both sides of leaf so its amphistomatic, being more abundant on the lower surface. They are located on the same level as the epidermal cells.

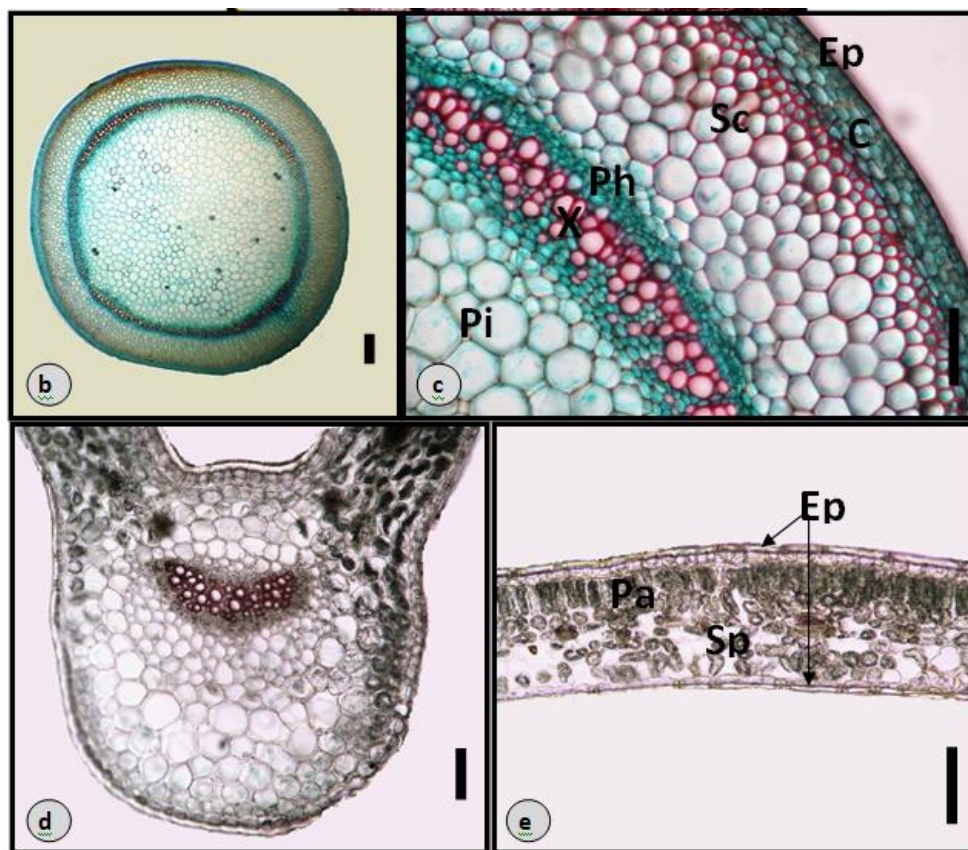


**Figure 2:** Pollen grain of *Vaccaria hispanica*, a: pollen under (SEM) bar= 10  $\mu$ m., b: pollen pore bar= 1  $\mu$ m., c: pollen in light microscope under 40 X.

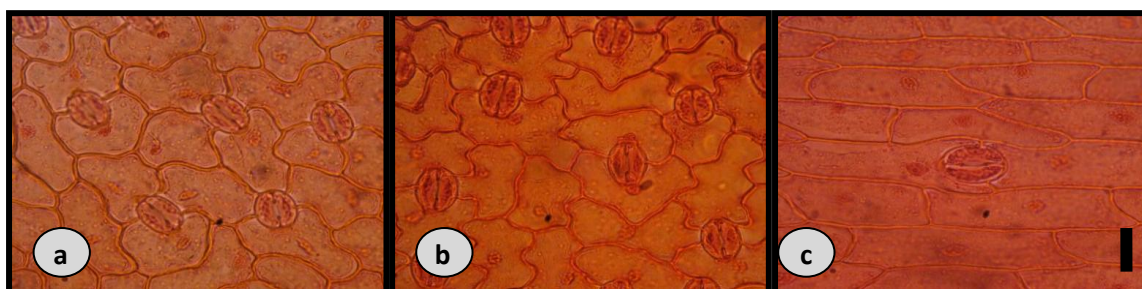
### 3.4. Habitat and geographical distribution "Figure 5"

Plant widely distributed in northern districts of Iraq and less in districts of middle region (Upper plains and foothills region, Desert plateau region, Lower Mesopotamian regions) as showed in Table (1). The habitat of the genus represented mainly by shady places under trees in forest and in mountain slopes as well as in cultivated field of cereals, grown in different kinds of soils.





**Figure 3:** Anatomical characters, a: root cross section, b, c: stem cross section, d: leaf midrib longitudinal section, e: leaf lamina section. Bar= 100µm.  
(abbreviation, C= cortex, Ep= Epidermis, P= Periderm, Pa= palisade layer, Ph= Phloem, Pi= Pith, Sc= Sclerenchyma, Sp= Spongy layers, X= Xylem)



**Figure 4:** cuticular characters, a: upper leaf epidermis, b: Lower leaf epidermis, c: Stem epidermis, bar= 20 µm.

**Table 1:** geographical distribution of *Vaccaria* in different regions of Iraqi districts.

Districts	Regions	Altitude and Habitat
Amadiya District (MAM)	Dohuk, Zakho to Sharanish, between Zakho to Turkish border, Gali Mazurka, Qaradagh mountain, Baidara	590-1700 m. Loamy soil road side, clay hill side, rocky mountain slope.
Rowanduz District (MRO)	Pushtashan, Rayat, Qarano village, Habat sultan, Haj omran, Koi sanjak, Salah aldine	440-1700 m. Caly soile in low depression, in valley, Cultivated field, river edge, stony clay hill side, Mountain slope, clay soil between Oak tree.
Sulaimaniya District (MSU)	Penjwin, Sulimanya, Zizan, Kamarspa, Tawela, Darbandikhan, Chemechemal, Rania, Halabja	300-1350 m. Road sides, cultivated clay hill side, Quercus forest, rocky mountain
Jabal Sinjar District (MJS)	Jabal sijar	Sandy clay soil, mountain slope, clay hill side
Upper Jazira District (FUJ)	Road from Mosul to Sinjar	400 m. Cultivated land
Nineveh District (FNI)	Mosul, Hamam alalil	300m. Loamy soil, road side cultivated land.

Arbil (FAR)	District	Erbil, Jabal Qara	350-410 Clay hill side, foot hill, Corn field.
Kirkuk (FKI)	District	Karkuk	250 m. Clay gravelly soil
Persian foothills District (FPF)		Saadiya, Bagsaya	110-190 m. Sandy gravelly soil, gravelly hill side
Ghurfa-adhaim District (DGA)		Sad al- Adhaim, between Samara and Tekrit	Gravelly soil in Barely field
Western Desert District (DWD)		Ramadi, Ana, west of K3, Rutba	550 m. Sandy gravelly soil, Cotton field, clay soil in depression, road side.
Central alluvial plain District (LCA)		Zafaraniya, Abu- Ghraib, near Khan Bani saad, Sudur	40 m. Road side, cultivated field, clay soil in Barely field, Cotton field

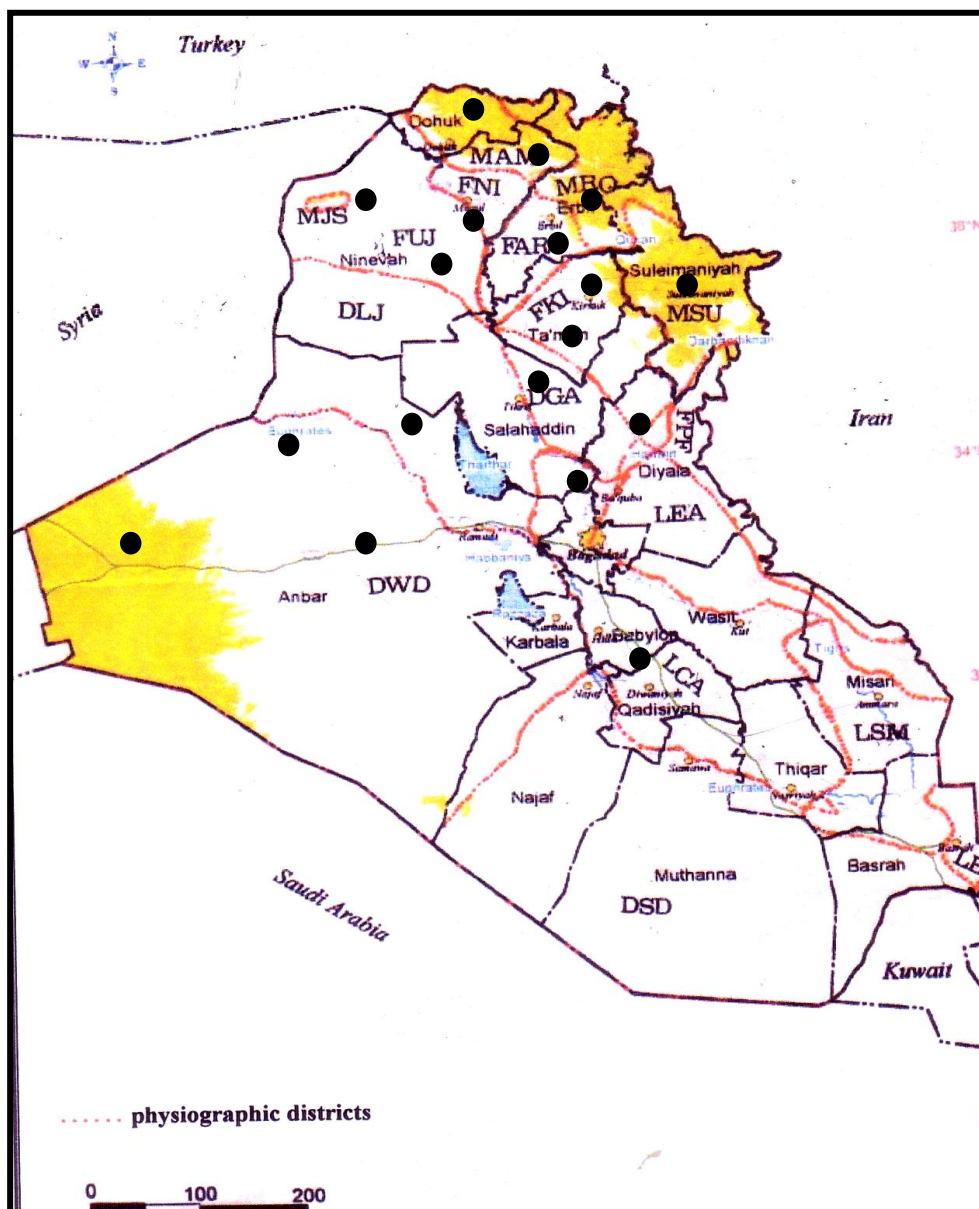


Figure 5: distribution of *Vaccaria hispanica* in Iraqi districts.

#### IV. Discussion

The morphological, Anatomical and micro morphological characters of *Vaccaria* have been studied to consider as segregated characters. The morphological studies results were generally in line with the description of genus in Flora of Iraq [5] but with more details, and the features with a diagnostic value of the genus were glabrous epidermises, cylindrical calyx with wings, and spherical seeds with central grooves.

The Palynological study showed the poly- panto porate pollen grain which is the common type of the family Caryophyllaceae as mentioned by [7] while the pollen of genus *Vaccaria* can be recognized by the sunkin pores with spinulated membrane.

The anatomical results of this study considered as the first detailed description of *Vaccaria*. The study of the root cross section showed that the root is covered externally by a thin periderm as a result of secondary growth, the secondary phloem arranged in triangular shapes as mentioned by [13]

The description of the stem cross section, showed that the epidermis is uniseriate and glabrous which is a good character to diagnostic the genus, cortex is narrow ended at endodermis which is often well characterized. The pericycle is characterized by a sclerenchymatous ring which is one of the characteristic feature of the family and its width variation considered as a good distinguished character between different genera and species of the Caryophyllaceae family as mentioned by [14], The xylem and phloem form a continuous ring. These results are consistent with the description given by[13].

Anatomical studies on the leaf longitudinal section showed that the mesophyll consists only of one row of palisade parenchyma and 4-5 rows of spongy parenchyma., These anatomical features observed on the leaves are in line with those of [13]. In addition, calcium oxalate is present in the form of large, druses crystals found in root cortex, stem pith and leaf mesophyll.

Furthermore, Epidermal study showed that the stomata are mainly caryophyllaceous type and are present on both surfaces of the leaf as well as stem, ordinary epidermal cells showed that Anticlinal walls were curved in upper surface of leaf and undulate in lower one and this agree with the opinion of increasing the waviness or undulating in lower surfaces of leaves as mentioned by [15].

The study of geographical distribution showed the possibility of plant propagation in the different northern and middle districts of Iraq and in various environments indicating that it has a high fitness to withstand various environmental conditions, excluding the southern districts with high temperature degrees.

## V. Conclusion

From the results it can be concluded the importance of each morphological and anatomical feature in identification of genus plants and segregation from other family genera, in addition to determine the common feature between *Vaccaria* and other family plants, farther more we cam used these data in the updating process of the Iraqi flora information.

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