

Study on the Morphology of Genus *Arabidopsis* in Iran

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ABSTRACT

In the present research, we have investigated morphological traits of different organs of *Arabidopsis* species in Iran. Trichomes on the aerial parts of three species were studied under anatomical microscopy. The species exist in Iran include: *Arabidopsis thaliana* (Heynh), *Arabidopsis pumila* (steph) and *Arabidopsis wallichii* (Hook & thomas). The form of trichomes, basal and stemleaves, fruit length, the attitude of fruit, the existence of trichomes on the fruit, and the color of petal are morphological traits which separate the species. Anatomical traits of the leaf and stem of studied species were analyzed using an optical microscope. In the stem, some traits such as the shape of stem in transverse incision, the thickness of skin, the existence of supporting tissue around phloem, the number of xylem and phloem and the wavy form of stele were distinct in the three species. In the leaf, some traits such as the shape of leaf, the number of palisade parenchyma layers, the uniformity of mesophyll tissue, and the density of trichomes were different in the three species. No significant difference has been observed in the study of reproductive meristem, except in *A. wallichii*. The only difference was related to the size of reproductive meristem of two species and the number of flowers produced from the meristems.

Keywords: Morphological Traits; Anatomical Traits; *A. wallichii*; *A. pumila*; *A. thaliana*; Meristems

1. Introduction

The genus *Arabidopsis* belongs to *Cruciferae* family. The members of this family are distributed in all continents of the world. However, north temperate regions, mediterranean and the Iran—Turan regions show the most population [1]. As currently delimited, the Brassicaceae comprise 49 tribes, 321 genera, and 3660 species. Of these, 20 genera and 34 species remain to be assigned to tribes. These figures differ substantially from those estimated five years ago, in which 25 tribes, 338 genera, and 3709 species were recognized. Of those 338 genera, 37 are treated herein as synonyms, and 21 genera (10 re-established and 11 new) have since been added [2].

The species previously introduced in Iran was: *A. wallichii*, *A. pumila* and *A. thaliana* [3]. There are no supportive data regarding its geographic origin. *A. thaliana* evolved in SW Asia [4]. *A. thaliana* species is mostly distributed in northern cities of Iran. *A. wallichii* is north east of Iran and *A. pumila* is observed in most provinces of Iran [5]. The name of *Arabidopsis* was first recom-

manded by Decandoll as a section of genus *Sisymbrium* Linneaus [6]. Since the first half of the twentieth century, when Redei introduced *A. thaliana* as a proper herbal model in scientific researches, taxonomists decided to remove *Arabidopsis* from the rank of section and promoted it to the rank of genus [7]. The characteristics such as the smallness of plant, short life cycle, abundant production, self-fertilization, low chromosome number, small genome size, and etc, make *A. thaliana* suitable for experimental tests [4]. The genus *Arabidopsis* was first introduced by *A. thaliana* species. Indiscriminate expansion of the genus, merely due to the similarities in linear fruits and branched trichomes, brings the number of species of genus to 59 species [8]. Unscientific development of the genus provoked the researchers to start extensive studies on the field of molecular, genetic and phylogenetic. Research results led to the removal of 50 species from this genus [9]. At present, *Arabidopsis* genus includes 9 species and 8 subspecies. 9 species are all native to Europe and 2 species are seen in the north and north-east Asia. Nitrogen and sulphur-rich compounds such as

glucosinolates and indole alkaloid are observed in *Cruciferae* family. These materials have a defensive role against microorganisms and herbivore insects. Researches revealed that indole alkaloids such as camalexin, indole glucosinolates oxin are synthesized in this genus. Researchers believe that the ability to synthesize and accumulate cytotoxic alkaloids depends on more than one active enzyme complex [10]. The goal of this research is collecting more information regarding those morphological and morphogenesis traits that result in segregation and the separation of species and are important in taxonomy science.

2. Material and Methods

A. Thaliana and *A. Pumila* were collected in the anthesis stage from the natural habitat (Table 1). Followed by confirming their identification in Evin herbarium, some of them were maintained as herbaria. Some fragments of stem, leaf and reproductive meristims were fixed in FAA and glutaraldehyde 5% solution and some fragments of stem and leaf of each three species were maintained in 8% formalin solution. Samples stored in 8% formalin were cut with razor blade and the samples were stained by green methyl and red carmen. Samples stored in FAA and 5% glutaraldehyde were molded after washing with water, dehydration by increasing degree of alcohol, clearing with toluene and paraffin infiltration into the tissue [11]. The molds were cut into 10 micrometer sections using a Leica 2045 microtome. The sections were glued onto lamella by using albumin glue. After deparaffinization, were stained by allendorfer, hematoxylin, methyl green pyronine, and periodic acid schiff (PAS) solutions. Samples were dehydrated and then became permanent on the lamella by antilugue. In order to observe the trichomes and to compare them, SZ51 Anatomical microscope was applied.

At the end, all samples were carefully observed by means of optical microscope and photos were taken by Canon camera, SD1000.

The collected samples in the nature were powdered under dry shadow and by mill, except *A. wallichii*. About 20 gram powder was obtained from each species. The powders were extracted by methanol separately for 24 hours. The filtered extract was partially purified by vacuum distillation (Bush 461 model). 5 grams of each extract was mixed with 2N HCl and NaCl and then infiltrated. The obtained extracts were examined into experimental tubes by Myers' reagent. To confirm the existence of alkaloid, Ammoniac was added to each acidic extracts till the pH reaches to 9. Then 10 cc chloroform was added to each basic solution and the mixture was poured into a separating funnel. After gently shaking the mixture, the chloroform phase was separated from the mixture. Chloroform adding was repeated three times

Table 1. Collective list and placement of various study types in anatomical survey.

Name of species	The specification of collect samples placement
1) <i>A. thaliana</i>	Mazandaran state: Tonekabon, from the sea level to 1000 meters high. Khorasan state: Chenaran, Dare abshar, top of Akhlamad, high of 1600 - 1800 meters.
2) <i>A. pumila</i>	Khorasan state: Chenaran, Golmakan, high of 1000 - 1500 meters. Khorasan state: Neyshabour, Khoro mountain, 1700 meters, Amini Rad, Iran 44847-2. Khorasan state: Neyshabour, Khoro mountain, Reshinger, Esfandiari, Iran 14152.
3) <i>A. wallichii</i>	Khorasan state: Torbat-e-heydarie, Zave flat, Jouharchi.

and chloroform solutions were collected in a container. After distillation of the solutions and adding 2N HCl, sample was examined by Myers' reagent

3. Results

Three species studied were similar in many morphological and anatomic traits (Figures 1, 2). The most important morphological traits which cause the species be differentiated from each other is shown in Table 2. The most important morphological traits from the stem cross-sections of studied species are as follows (Figures 3, 4).

In *A. thaliana*: relatively round stem in the cross section, monolayer and stomatous epidermis, vascular bundle with almost 9 - 11 bundles, wavy stele, and simple, 2 or 3 branched trichomes.

In *A. pumila*: polyhedral and angular stem in the cross section, monolayer and stomatous epidermis, vascular bundle with 11 - 15 bundles, wavy form stele, 2 - 4 branched trichomes, and outgrowth the base

In *A. wallichii*: relatively round stem in the cross section, monolayer and stomatous epidermis, very thick cuticle, vascular bundle with 11 - 13 bundles, stele with a little wave, tall dendroid trichomes, and the existence of a supporting tissue on top of the phloem (Figure 5).

The most important anatomical traits of leaf in studied species are as follows (Figure 6).

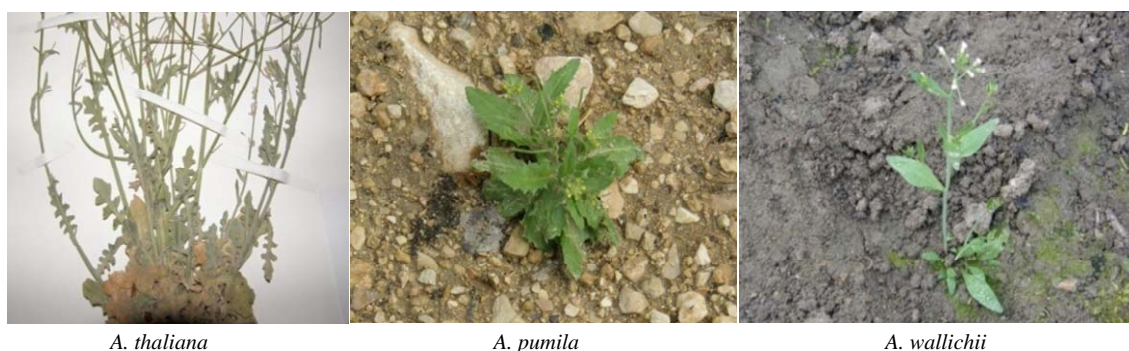
In *A. thaliana*: A monolayer upper and lower epidermis, variable mesophyll tissue, palisade parenchyma on the top, sponge parenchyma under the leaf, variable mesophyll in the main vein, a little outgrowth of distal surface of leaf at the main vein.

In *A. pumila*: A monolayer upper and lower epidermis, variable mesophyll tissue, uniform mesophyll on the main vein, a relatively much outgrowth of distal surface of leaf on the main vein.

In *A. wallichii*: A monolayer upper and lower epidermis, more than one layer in the palisade tissue of paren-

Table 2. Compared of same various type of morphology traits in case study.

Morphology traits of organs			<i>A. thaliana</i>	<i>A. pumila</i>	<i>A. wallichii</i>
Stem	Trichom		Bottom of stem has trichom Top of stem is trichomeless	From the bottom to the top of stem has trichom	
Leaf	Basic leaves	Form	complete	Toothed	Pectinate
			Low	Many	A lot
	Stem Leave	Form	Oblong-lanceolate	Sagittate-auriculate	Rare auriculate
		Petiole	Short petiole	Epetiolate	Epetiolate
Fruit	Length		5 - 17 mm	15 - 60 mm	40 - 70 mm
	Shape		ascending	Rararearcuatus	Arcuatus
	Trichome		eptiolate	Full of petiole	Puberulus
	Fruit tail		eptiolate	Pubescens	Pubescens
Flower	Color of petal		white	Yellow	Violet

**Figure 1. Samples of herbarium.****Figure 2. The species collected in Iran.**

chyma, uniform mesophyll tissue at the main vein, a relatively small protrusion on the dorsal surface of leaf at the midvein.

The results of the histological structure and the reproductive meristem of species:

A. thaliana and *A. pumila*: In terms of meristem structure, there was no significant difference between the two reproductive meristems of these two species. We can see the floral meristem of *A. thaliana* in **Figure 7**.

The size of meristem and the number of produced

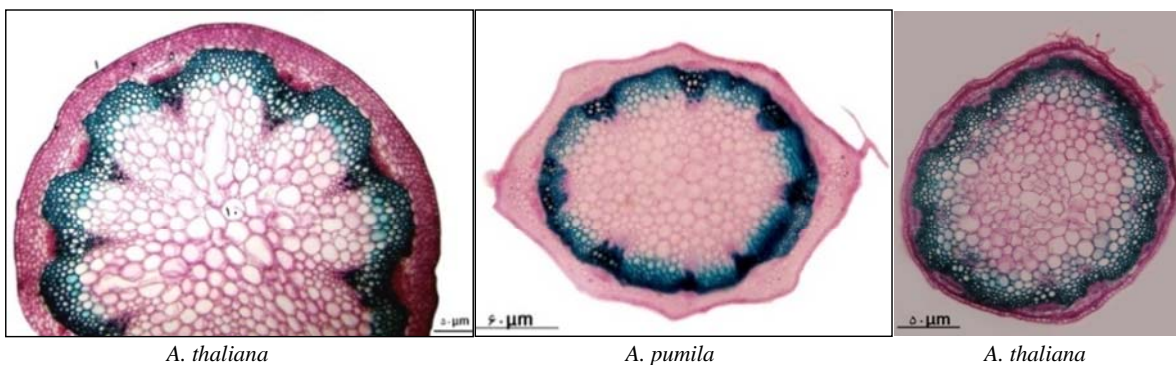


Figure 3. The cross section of stem in three study species.

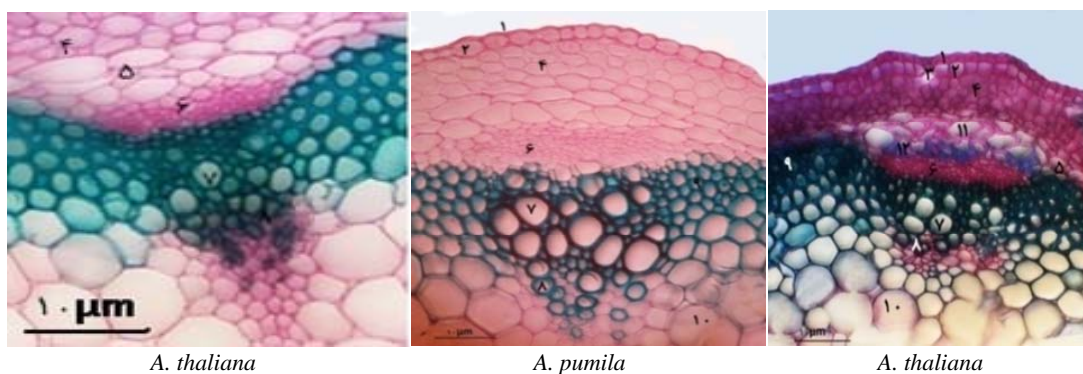


Figure 4. Part of stem in three study species: 1—cuticule; 2—epiderme; 3—stomata; 4—cortexparenchyma; 5—endoderm; 6—phloem tissue; 7—metaxylem; 8—protoxylem; 9—fiber; 10—collenchyma; 11—sclerenchyma.

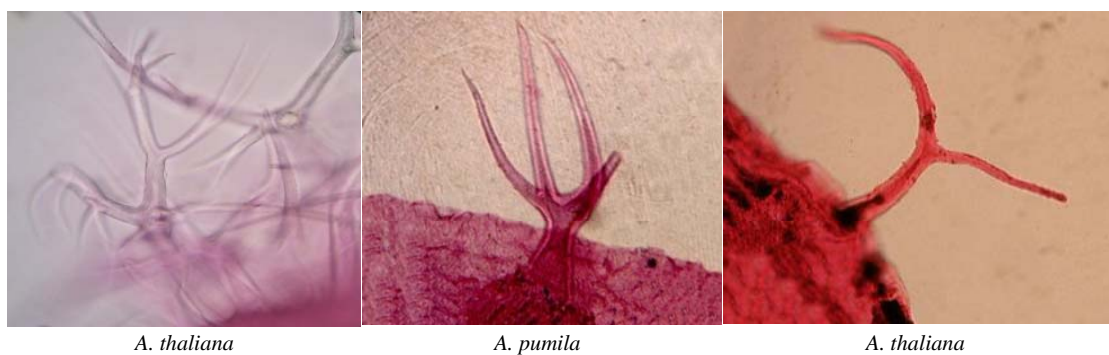


Figure 5. The trichome in three study species.

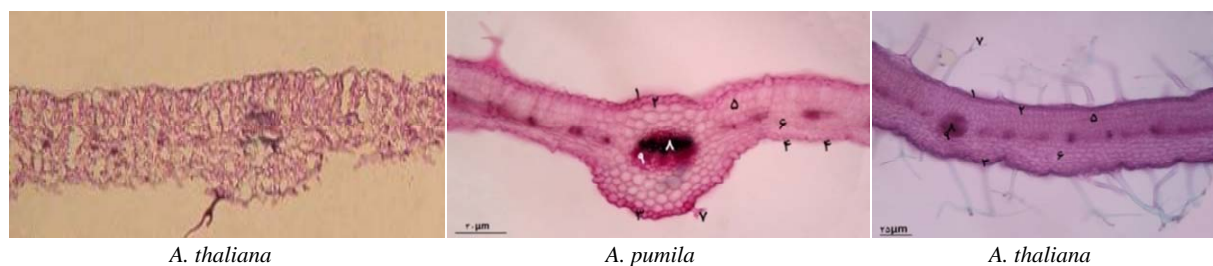
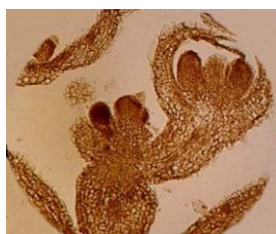


Figure 6. The cross section of leaf in three study species: 1—cuticle; 2—adaxial epiderme; 3—abaxialepiderme; 4—stomata; 5—palisadparenchyma; 6—spongyparenchyma; 7—trichume; 8—xylemtissue; 9—phloem tissue.

flowers in *A. pumila* was more than *A. thaliana*.

The results of alkaloid test, in the two stages of experi-

ment, were positive for *A. thaliana* and were negative for *A. pumila*. The experiment was repeated three times and



A. thaliana

Figure 7. The radial section of floral meristem.

the results of experiment were similar in all three cases.

4. Discussion

With regard to the fact that *A. thaliana* is a unique species of the genus *Arabidopsis* and most of its morphological, anatomical, cellological, and genetic traits are known in the world. Therefore, all traits studied on *A. pumila* and *A. wallichii* were compared with *A. thaliana*. According to the extensive studies performed on the plants of genus *Arabidopsis* by herbal researchers, plants are included in the genus *Arabidopsis* which are in the context of following attributes:

Having a simple trichome, not having a lug in stem and leaf, flowers in white to light purple, trichomeless fruit, fruit with perpendicular and glabrous tail, aflat or round fruit in the section.

In addition to the above morphological traits, in the present study most other traits are shown such as:

The presence of palisade tissue in main vein, the wavy form of stele, and the positive alkaloid test are of traits which separate *A. thaliana* from two other species. This difference in alkaloid may be related to time and stage of growth of plant. Therefore this test was not the suitable case for us to separate this species.

First time, *A. parvula* has been reported by Akhany from Mighan desert region of Arak, Iran in 1988 [12]. It is different from *A. thaliana* due to lack of petals, no trichome in the whole plant, leaves without petioles and stems with a difference of calcareous or salty environment. These species outside the genus *Arabidopsis* was transferred to the genus *Thellungiella*.

With regard to the research results of researchers such as Price (1994), Al Shehbaz (1994) and O'kane (1997), [9,13,14], *A. pumila* was removed from the genus *Arabidopsis* due to the morphology, anatomy and genetic differences and was moved to the genus *Olimarabidopsis*.

A. wallichii species was removed from the genus *Arabidopsis* and was moved to the genus *Crucihimalaya*. According to the extensive scientific researches and the present study, only one species of the genus *Arabidopsis* remains in Iran, which is the unique species of *Arabidopsis thaliana*.

Therefore previous researchs introduced three species allegedly belonging to *Arabidopsis* in Iran but they be-

long to three different genera of three unrelated tribes. *Arabidopsis thaliana* belongs to the tribe Camelinae; *A. wallichii* *Crucihimalaya* of the tribe Crucihimalayae, and *A. pumila* *Olimarabidopsis* of the tribe Alyssopsideae.

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