MORPHOLOGICAL AND ANATOMICAL STUDY OF THREATENED ENDEMIC HELIOCARYA MONANDRA BGE. (BORAGINACEAE) IN IRAN

M. Yousefi

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Heliocarya monandra is a rare endemic species of *Boraginaceae* that grows in a limited area in central Iran. Since there has been no comprehensive study of this species, morphological and anatomical properties of it were investigated. For this, plant samples were collected from a locality on the Soffeh Montain in the south of Isfahan city in central Iran. Sections of leaf, stem and root were taken manually and prepared according to the current laboratory methods for light microscopic examination. The measurements were done in at least 5 replicates. Some properties of fruit and seed were described. Fruit consisted of an ovoid or orbicular one-seeded nutlet with 4-6 (-7) mm length, 2.5-4 mm width and 0.8-1 mm thickness. Seed was also 1.8-2 mm long, ovoid, with a curved beak. The leaf was isobilateral with anomocytic stomata. The root showed secondary structure. The stem had also both primary and secondary tissues. The results in morphology and anatomy were in agreement with general characteristics of the family *Boraginaceae* except for some differences. Details of fruit and seed are reported for the first time. Fruit characters are especially important to clarifying taxonomic relationships in the family. According to field observations, there is only one population of this species in Isfahan province and it seems that this population is at risk of disappearing in nature.

Mehdi Yousefi, Payam Noor University of Isfahan, Department of Biology, P. O. Box 81395-671, Ashrafi Isfahani Blvd., Isfahan, Iran. E-mail: muosofi@yahoo.com

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مطالعه ریخت‌شناسی و تشریحی گونه انحصاری و در معرض خطر *Heliocarya monandra* (Boraginaceae) در ایران

مهدی یوسفی، استادیار گروه زیست‌شناسی دانشگاه پیام نور اصفهان.

Heliocarya monandra گونه‌ای انحصاری و نادر از خانواده گاوزبانیان (*Boraginaceae*) است که در ناحیه محدودی در مرکز ایران می‌روید. با توجه به نبود مطالعات جامع، صفات ریخت شناختی و تشریحی این گونه بررسی شد. برای این منظور نمونه‌هایی از کوه صفه در جنوب شهر اصفهان در مرکز ایران جمع‌آوری شد. از برگ، ساقه و ریشه آنها برش‌های عرضی به‌صورت دستی گرفته شد و به روش‌های رایج در آزمایشگاه برای مطالعه با میکروسکوپ نوری آماده گردیدند. اندازه‌گیری‌ها حد اقل در ۵ تکرار انجام شد. برخی از ویژگی‌های میوه و دانه شرح داده شدند. میوه شامل فندقه‌ای یک دانه‌ای، تخم مرغی یا دایره‌ای به طول (۷–) ٦–٤ میلی‌متر، به عرض ٤–۲/۵ میلی‌متر و به ضخامت ۱–۰/۸ میلی‌متر بود. دانه نیز به طول ۲–۱/۸ میلی‌متر، تخم مرغی، با نوکی برگشته بود. برگ از نوع دو طرفی با روزنه‌های آنموسیتیک بود. ریشه ساختار ثانوی نشان داد، ساقه نیز دارای بافت‌های اولیه و ثانوی بود. نتایج ریخت‌شناختی و تشریحی به جز در برخی موارد با ویژگی‌های عمومی خانواده گاوزبانیان منطبق بود. جزئیات میوه و دانه در این گیاه برای اولین بار گزارش می‌شوند. صفات میوه به‌طور اخص برای روشن نمودن روابط آرایه‌شناسی این خانواده مهم است. بر طبق مشاهدات صحرائی تنها یک جمعیت از این گونه در استان اصفهان وجود دارد که این جمعیت نیز در معرض خطر ناپدید شدن از صحنه طبیعت قرار دارد.

INTRODUCTION

Heliocarya monandra Bge., an endemic species belonging to the monotypic genus of *Heliocarya* Bge. (*Boraginaceae*), is found in a restricted area between Isfahan and Yazd provinces in central Iran (Hedge & Wendelbo 1978; Ghahreman & Attar 1999). Phytogeographically, this species is an Irano-Turanian element (Riedle 1967; Khatamsaz 2002) that has no

local name and the economic importance, probable medicinal properties or other potential usages of it are not clear.

According to Riedle (1963), *H. monandra* was first collected by Bunge in April 1859 from Iran and was described as a new species by the same author in 1871, approximately 12 years later. Bossier (1879) based on type specimens, presented diagnostic description of this species and Engler (1921) based on Bossier description, explained some of morphological properties of it. Johnston (1924) renamed *H. monandra* as *Caccinia monandra* (Bge.) Johnst., but Riedle (1963) declined this renaming and therefore, the previous name was retained.

Although a variety of morphological and anatomical characteristics of the family *Boraginaceae* (Metcalfe & Chalk 1979; Fahn 1982) or of some genera and endemic species of this family (for instance: Azizian et al. 2000; Akcin & Engin 2001; Selvi & Bigazzi 2001; Dasti et al. 2003; Akcin 2004; Akcin & Engin 2005; Ozdemir & Altan 2006; Akcin & Ulu 2007; Akcin 2007; Akcin & Ulu 2008; Ovchinnikova 2009; Pakravanfard et al. 2009) have been described, anatomical studies on *H. monandra* has not been done so far and morphological studies on it were also low. In the present work, morphological and anatomical properties of this species were investigated for the first time.

H. monandra is a rare endemic species (Jalili & Jamzad 1999). Therefore, any additional information about this species can be useful to more introduction of it. Metcalf & Chalk (1983) pointed out that anatomy of the vegetative organs of flowering plants could be taxonomically useful in the identification of fragmentary material, the preliminary identification of herbarium specimens, and as an aid toward establishing the interrelationships of taxa at and above the species level.

MATERIAL AND METHODS

Plant samples were collected from a locality at 32º 35.471' N and 51º 38.722' E with an altitude of 2000-2100 m above sea level on the Soffeh Mountain in the south of Isfahan city (central Iran). The voucher specimens were identified according to Riedle (1967) and Khatamsaz (2002) and saved in Isfahan Payam Noor University Herbarium and TARI. Fresh samples were used for morphological studies. The materials for anatomical studies were also fixed in FAA (Johnsen 1940) and preserved in refrigerator at 4º C. Sections of leaf, stem and root were taken manually, cleaned with sodium hypochlorite, stained with methyl green and Carmen-vest and mounted in glycerin gelatin. An Olympus BX40 light microscope and a Nikon binocular, equipped with digital camera and camera lucida, were used to examination of slides, photography and drawing. All measurements given herein were taken from fresh and dried materials in at least 5 replicates.

RESULTS

Notes on morphology

The plant is a perennial herb, 15-30 (-35) cm tall with a thick taproot. The stem is erect, robust and hollow in maturity. Lower leaves included petiole 4-10 cm long and 1.5-3 (-3.5) cm wide. Upper and middle leaves are similar to but smaller than the lowers. Inflorescence is an elongated panicle composed of many scorpioid cymes (Fig. 1), and relatively lax in fruiting stage. Pedicel of flower 4-11 mm long and is thicker and longer after anthesis (up to 16 mm long in fruiting stage). Calyx 5-7 (-8) mm long, thicker and longer in fruit. Corolla 8-11 (-12) mm long (Fig. 2-a), longer than the calyx; lobes are 5 and unequal (zygomorphic). Stamens 5; 4 sterile and 1 fertile; fertile anther 1.8–2 mm long (Fig. 2-b). Style gynobasic, erect, 4-5 mm long; stigma globular (Fig. 2-c). Fruit consists of an ovoid or orbicular one-seeded nutlet, ventrally connected to the calyx and remains in this status even after fruit falling (Fig. 3-a); rarely tow nutlets are developed in one flower (Fig. 3-e). The nutlet is variable in size; 4-6 (-7) mm long, 2.5-4 mm wide at the widest part, and approximately 0.8-1 mm thick (Fig. 3-b), surrounded by a flattened and thickened ring composed of 12-14 (-17) stiff prickles (glochids) that are in radial arrangement; the ring is formed by fused bases of the prickles (Fig. 3-a and b). Prickles are glabrous, 1.5-2.2 mm long; each of them has a white stellar hair on the apex (Fig. 3- c). Dorsal surface of nutlet is covered with white hairs that among them one or occasionally 2 of them are stellate (Fig. 3-d). Seed is ovoid with a curved beak, 1.7-2 mm long; glabrous with black and shiny surface (Fig. 3-f). Some quantitative characteristics described above were also compared with the related literatures (Table 1).

Anatomical observations

Leaf

A single layered epidermis with a relatively thick cuticle, dense trichomes and sunken stomata covers on both surfaces of leaf (Fig. 4). The mesophyll was isobilateral, consisted of 2-3 layered palisade parenchymas on the adaxial surface, 1-2 layered palisade parenchymas on the abaxial surface and 2-3 layered spongy parenchyma were between them. The

Fig. 1. *Heliocarya monandra*, at flowering stage in natural habitat (photo by the author).

palisade cells were compact and elongated with straight or a little wavy walls, and spongy cells were polygonal or irregular and bigger in size. The veinlet was surrounded by a parenchyma layer and midrib composed of a solitary arc shaped vascular bundle supported with a few layered collenchymas (Fig. 4). Stomata were mostly anomocytic or occasionally anisocytic as saw on the abaxial surface view (Fig. 5-e). Leaf was densely covered with 3 types of trichomes; long with setaceous bodies, medium with warty walls and short non-glandular trichomes, all of them with prominent white calcareous bases (Fig. 5).

Stem

In surface view, simple non-glandular trichomes covered the outer surface of epidermis. A transverse section taken from the middle part of stem showed a single layer epidermis with thick cuticle in the outer surface, and 2-3 layered collenchymas, 3-5 layered cortex, phloem, xylem and a few layers of pith cells (Fig. 6). Cortex consisted 3-5 layers of compressed parenchyma cells and its innermost layer was distinguished as endodermis. Secondary growth was seen in the stem; the vascular tissues were of primary and secondary phloem and xylem. Stem was hollow in maturity due to removing of pith cells. Therefore, 2-3 layers of pith cells were seen in the innermost part of the stem (Fig. 6).

Root

The root, as saw in a transverse section (Fig. 7), consisted a multilayered peridermis, 5-8 layered

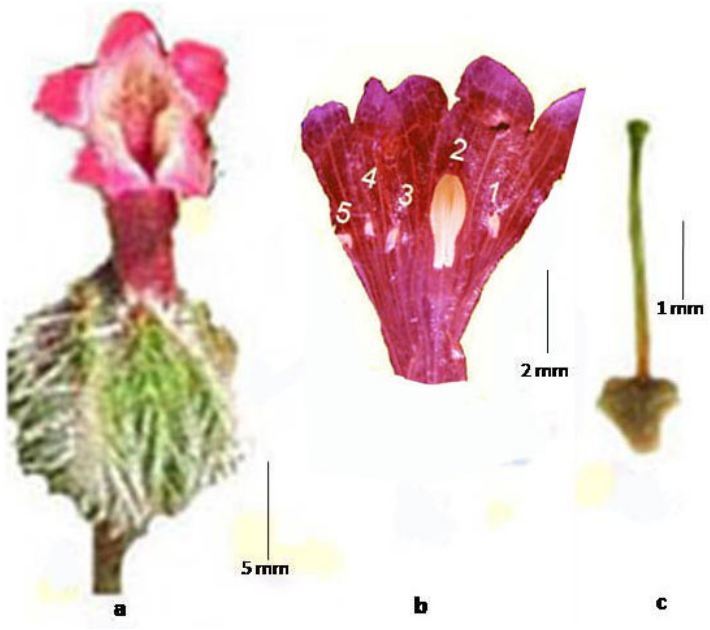

Fig. 2. Details of flower in *Heliocarya monandra*: a- calyx and corolla; corolla with 3 bigger and 2 smaller lobes (zygomorphic), b- stamens; 1, 3, 4 and 5 are sterile and 2 is fertile, c- ovary, style and stigma.

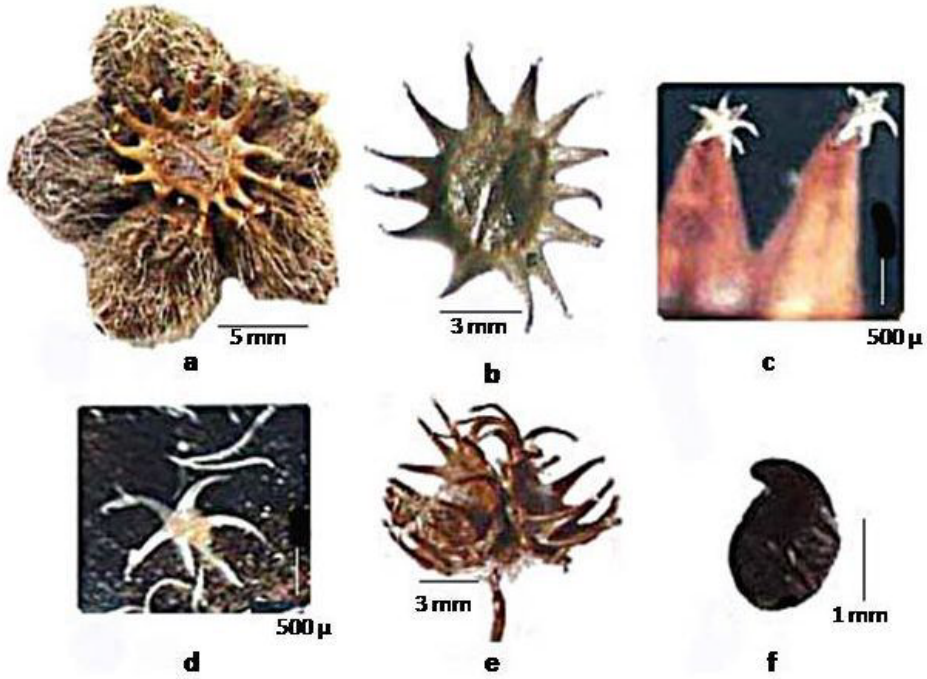

Fig. 3. Fruit and seed in *Heliocarya monandra*, a- nutlet attached to the calyx, b- nutlet with marginal appendages (prickles), c- 2 prickles with stellate hairs on the apex, d- one stellate hair on the dorsal surface of fruit, e- two nutlets developed in one flower, f- seed.

Table 1: Comparison of some quantitative morphological properties of *Heliocarya monandra* with 3 related literatures.

Characteristics	Engler 1921	Riedle 1963	Khatamsaz 2002	This work
Plant height (cm)	15-20	15-20	15-30	15-30 (-35)
Leaf length (cm)	6-7	6-7	6-9	4-10
Leaf width (cm)	1.5-2	1.5-2	1.5-3	1.5-3 (-3.5)
Pedicel in flower (mm)	-	-	5-10	4-11
Pedicel in fruit (mm)	-	-	up to 20	up to 16
Calyx length (mm)	7	± 7	5-7	5-7 (-8)
Corolla length (mm)	10	± 10	8-12	8-11 (-12)
Nutlet length (mm)	7	± 7	-	4-6 (-7)
Nutlet width (mm)	-	-	-	2.5-4
Nutlet thickness (mm)	-	-	-	0.8-1
Nutlet prickle length (mm)	-	-	-	1.5-2.2
Number of nutlet prickles	12-16	-	-	12-14 (17)
Fertile anther length (mm)	-	-	-	1.8-2
Style length (mm)	-	-	-	4-5
Seed length	-	-	-	1.7-2

primary cortex, distinct bundles of sclerenchyma fibers, phloem, xylem and primary pith rays. The outline of root seems irregular, corresponding to longitudinal furrows on the outer surface of the root (Fig. 7-a). Peridermal cells are compressed and seen in crushed conditions. Primary cortex was not a continuous tissue due to disruption by the fiber bundles surrounding the phloem patches (Fig. 7-b and c). The xylem was also made up of 10-13 branches; that were in radial arrangement and alternate with the same number of primary pith rays (Fig. 7- b, c and d). The pith ray cells are elongated in radial direction.

DISCUSSION

H. monandra is an endemic species that grows in small populations on low mountains in central Iran (Hedge & Wendelbo 1978). Due to limited distribution, unknown economic usages and far from being available, this species is poorly known. The only available information about this plant was a few morphological descriptions based on type specimens (Boissier 1879; Engler 1921). Therefore, the results of the present investigation revealed some morphological characteristics including shape and size of seed and details of fruit, and all anatomical properties of this rare species for the first time. Fruit characteristics are especially important to clarify taxonomic relationships of the tribes and genera in *Boraginaceae* (Ovchinnikova 2009).

In general, results in morphology were in agreement with the descriptions in the Flora Iranica (Riedle 1967) and Flora of Iran (Khatamsaz 2002), except for a few differences (Table 1). The measurements given in the present work for nutlet, fertile anther, style and seed have not been done in the works of Engler (1921), Riedle (1963) and Khatamsaz (2002). The other measurements for plant height, leaf length and width, calyx, corolla and nutlet length (Table 1), are exactly similar in Engler (1921) and Riedle (1963), while they are different in Khatamsaz (2002) and in the present work. The height of plant has been reported as 15-20 cm in Engler (1921) and Riedle (1963), and as 15-30 cm in Khatamsaz (2002). But in the studied area, the plants were up to 35 cm tall, especially in fruiting stage.

The anatomical properties resulted in this study were also in agreement with general anatomical features of the family *Boraginaceae* that have been described by Metcalfe & Chalk (1979) and Fanh (1982). The anatomical characteristics of the leaf, including dominance of anomocytic stomata and isobilateral type of it, were in agreement with some studies in *Boraginaceae* (for instance: Selvi & Bigazzi 2001, in tribe *Boraginaceae*; Akcin & Ulu 2007, in the endemic *Anchsa leptophylla*). According to Metcalfe & Chalk (1979) there are both anomocytic and anisocytic stomata in the *Boraginaceae* family but anomocytic type is dominant. Dasti et al. (2003) reported the same results in 31 species belonging to different genera and tribes of family *Boraginaceae* in Baluchistan. The cells of palisade parenchyma were highly compact (Fig. 4). The compactness of the palisade parenchyma depends directly upon light intensity (Metcalfe & Chalk, 1979; Fahn, 1982). This characteristic is compatible with the ecological conditions of studied region; this plant grows on the south slope of a mountain where it is exposed to intense sunlight.

The anatomical findings of stem were consistent with a lot of related studies in the other taxa of

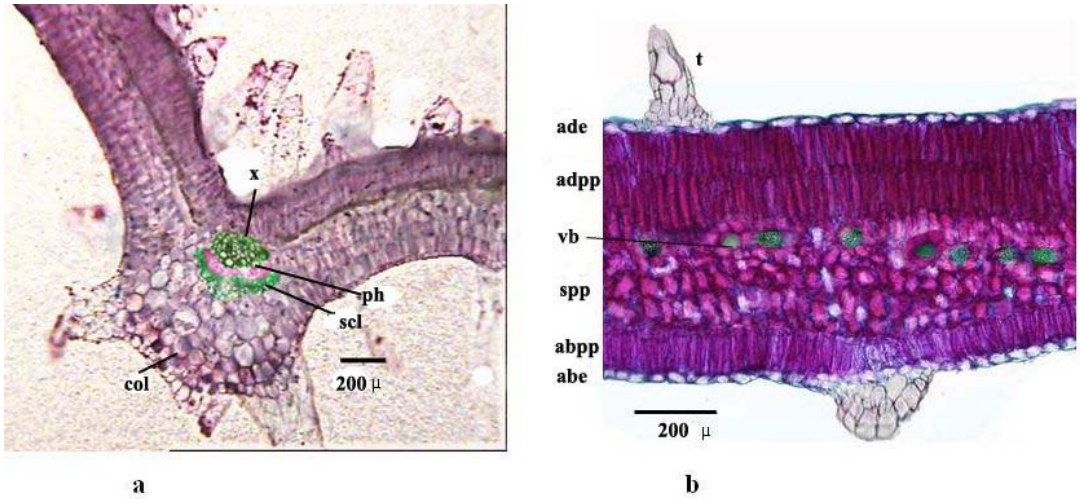

Fig. 4. Anatomy of leaf in *Heliocarya monandra*, a- transverse section taken from the midrib, b- tissues: ade) adaxial epidermis, abe) abaxial epidermis, adpp) adaxial palisade parenchyma, abpp) abaxial palisade parenchyma, spp) spongy parenchyma, x) xylem, ph) phloem, scl) sclerenchyma, col) collenchyma, t) trichome, vs) vascular bundle.

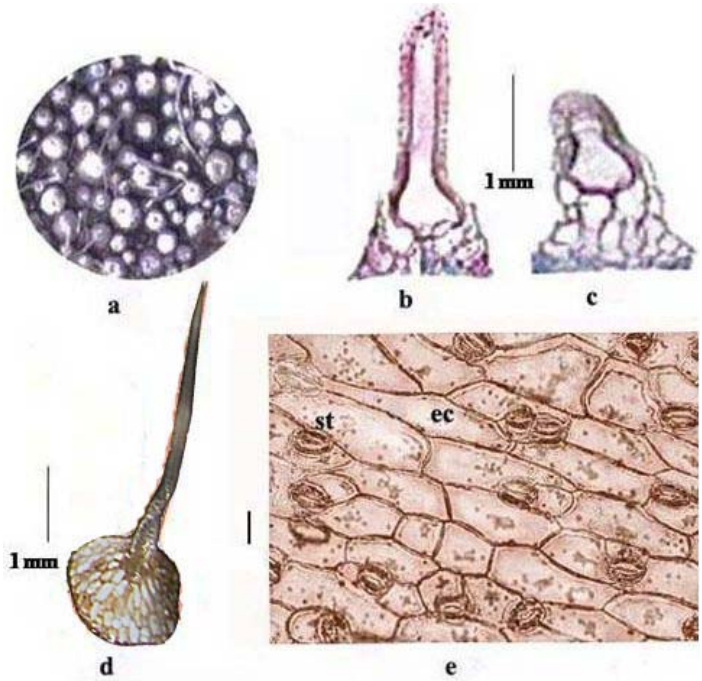

Fig. 5. Trichomes and stomata; a- surface of leaf covered densely by coarse trichomes, b- medium simple trichome with warty wall, c- short simple non-glandular trichome, d- long simple trichome with setaceous body and prominent white calcareous base, e- stomata: st) stoma; ec) epidermal cell.

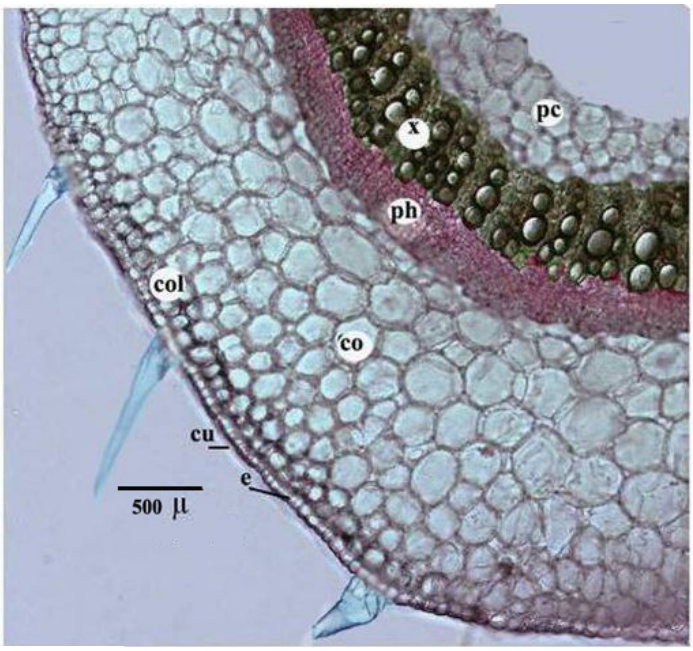

Fig. 6. Transverse section of stem in *Heliocarya monandra*; cu) cuticle, e) epidermis, col) collenchyma, co) cortex, phl) phloem, x) xylem, pc) pith cell..

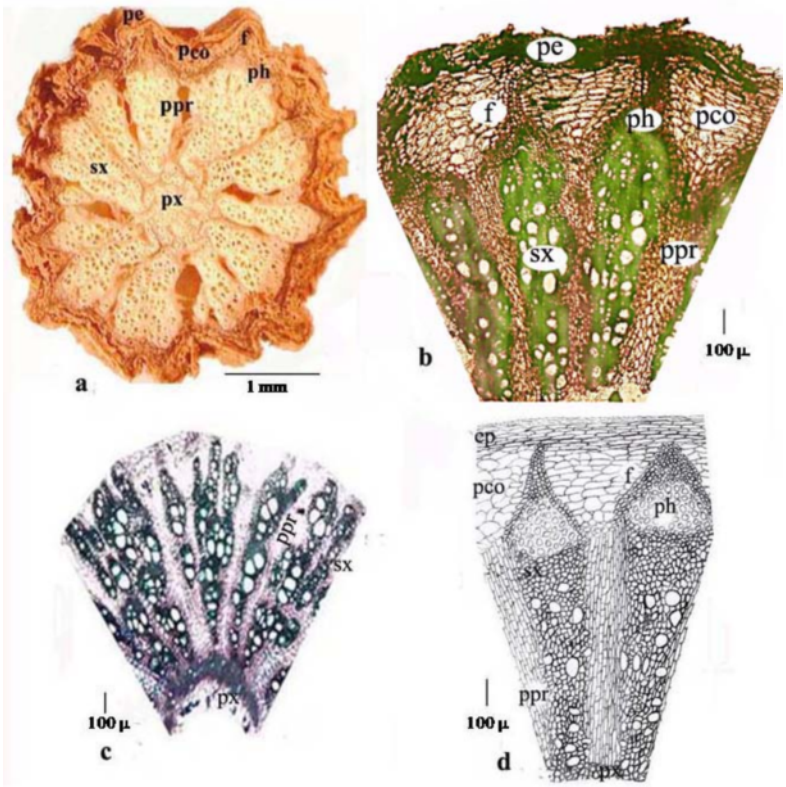

Fig. 7. Transverse section of root in *Heliocarya monandra*; a- general view under binocular showing the branches of xylem and pith rays, b and c- tissues, d- primary and secondary xylem: pe) peridermis, pco) primary cortex, f) sclerenchyma fibers, ph) phloem, px) primary xylem, sx) secondary xylem, ppr) primary pith ray.

Boraginaceae (for example, Akcin 2004, in the endemic *Alkanna haussknechtii* in Turkey; Akcin & Engin 2005, in the endemic *Onosma bracteosum*; Ozdemir & Altan 2006 in some endemic *Alkanna* species; Akcin & Ulu 2007 in *Anchusa leptophylla*; Akcin & Ulu 2008, in some *Anchusa* species). According to plant life form terminology (Archibold 1995), this plant is initially a Hemicryptophyte. Field observations also confirmed the fact; the stem is dried at the end of growing season and is produced in the next year. The stem was also hollow at maturity. Published documents in relation to this property in other species of the *Boraginaceae* were not found.

The root showed secondary structures similar to the other perennial species of *Boraginaceae* (Metcalfe & Chalk 1979). Primary pith rays in the root (Fig. 7) have been reported in some species of the family (Akcin & Ulu 2007 in *Anchusa leptophylla*), but the number of them, and consequently the number of xylem branches were apparently more than the other species of *Boraginaceae* that have so far been studied.

The genus *Heliocarya* Bge. has been considered to be related to the genus *Caccinia* Savi. Davis (1978) has also pointed out that the genus *Caccinia* in Turkey is closely related to Iranian endemic genus of *Heliocarya*. The closest species of *Caccinia* to *H. monandra* is *C. actinobole* Bge., an idea sustained by Jahnston (1924) who renamed *H. monandra* as *Caccinia monandra*. But as Riedle (1963) had already expressed, these two taxa have basic differences; having one fertile stamen by *H. monandra* while 5 by *C. actinobole* is the most important one. Present research findings, especially in related to fruit and seed characteristics, support the separation of the two genera.

The status of extinction risk of *H. monandra* has been in doubt. Due to insufficient information from its distribution, direct or indirect assessment of risk of extinction in this rare species has been almost impossible. Therefore, Jalili & Jamzad (1999) placed it in the category of Data Deficient (DD) in Red Data Book of Iran. Based on observations made by the author, it seems that at least the Isfahan population of this species is at risk of declining and the probability of extinction is very high due to a sharp reduction in rainfall in recent years, extensive constructions in habitat in order to urban green space development, and the exposure route Tourism. Therefore, more investigation to introducing this plant as an Endangered or Vulnerable (IUCN 2009) is strongly recommended.

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