Greener Journal of Biological Sciences

ISSN: 2276-7762 ICV 2012: 5.99

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Research Article

Comparative Anatomical and Palynological Studies on *Rumex* L. species (Polygonaceae) in NE Iran

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ABSTRACT

In present study, anatomical and palynological characters of *Rumex* species in NE Iran were examined. For anatomical study, manual cross sections of fresh and dried stems and leaves were prepared and stained by differential staining. In palynological study, the pollen were extracted from anther, acetolysed and observed by SEM. The results of anatomical research showed, annular collenchymous tissue below the epidermis layer, different shape of vascular bundle, sclerenchymous strands around the vascular bundles in stem, dorsio-ventral mesophyll and continuous and discontinuous vascular bundles in leaves. The palynological findings indicated, panporate, tricolporate, tetracolporate, granulate, microechinate and punctate pollen in studied species.

Keywords: Anatomy, Iran, pollen, Polygonaceae, *Rumex*.

INTRODUCTION

The genus *Rumex* L. belongs to the family Polygonaceae and the order Caryophyllales which has about 200 species distributed in temperate regions, European countries, America, Asia and Australia continents (Heywood, 1978; Qaiser, 2001; Sanchez and Kron, 2008; Chase and Reveal, 2009). This genus comprises around 28 and 8 species in Iran and Khorassan Razavi Province (NE Iran) respectively (Rechinger, 1968). *Rumex* has antioxidant, anti-microbial and viral, anti-cancer activities and decreases blood sugar and cholesterol level (Jildirim et al., 2001; Cos et al., 2002; Ferreres et al., 2006; Sedaghat et al., 2010; Harshaw et al., 2010). Despite many reports about medicinal characteristics of this genus, internal structure and pollen studies have been done on a few *Rumex* species e.g. The anatomy of *Rumex* with special reference to the morphology of the internal bundles and the origin of the internal phloem in Polygonaceae (Joschi, 1936), Comparative anatomy of the leaves of *Rumex* in Jiangxi (Li et al., 2008), anatomical study of some medicinal plants of family Polygonaceae (Hameed et al., 2010), Stem anatomy of medicinally important *Rumex hastatus* D. Don (Polygonaceae) (Sahney and Vibhasa, 2012) and Taxonomic implications of pollen morphology of seven species of *Rumex* L. from Pakistan (Yasmin et al., 2010). Whereas, comparative anatomical and palynological studies have not been done on Iranian *Rumex* species, this report focused on the above research to recognize variation in internal structure and pollen micromorphological features among the five *Rumex* species in NE Iran.

MATERIALS AND METHODS

For anatomical study of vegetative organs, the base of stem and mature leaves from five species i.e. *R. crispus* L., *R. dentatus* L., *R. tuberosus* L., *R. chalapensis* Mill. and *R. conglomeratus* Murr. were fixed in FAA solution, then manually cross sections were made and stained by green methyl and carmine then observed by different magnification of light microscopy Labomed model CMZ4. The localities of studied specimens were presented in Table.1. Some anatomical characters such as the number of collenchymous layer below the epidermis tissue, the position of secretory ducts in cortex, the shape of phloem bundles, the number and type of sclerenchymous bundles above the phloem were analyzed.

Table 1: The locality of studied species of *Rumex*

Species	Locality
<i>R. crispus</i>	TorbateHeydarieh, Roud Majan, 1688 m, Soleimani, 9152 (IAUM); Quchan, Fatamehkhanom mount, 2200 m, Faghihnia and Zangouei, 29399 (FUMH); Fariman, Chehar ta Gav, 1700- 2000 m, 11313 (FUMH); Trogh, Torogh dam, 1280 m, Soleimani, 9153 (IAUM); Chenara, Ferizi, northern slope of dahaneh, 1890 m, Emadzadeh and Memariani, 36380 (FUMH).
<i>R. tuberosus</i>	Chenaran, Dahane Jaji, 1900 m, Soleimani, 9165 (IAUM); East of Quchan, Emarat mount, 1800 m, 27459 (FUMH); Kalate Nader, Khanzo, Ortekan village, 1550 m, Faghihnia and Zangouei, 24144 (FUMH); Dargaz, Tandoureh, 1750 m, Hojat and Zangouei, 39377 (FUMH).
<i>R. dentatus</i>	Torbat Heydarieh, Roud Majan, 1680 m, Soleimani, 9157 (IAUM)
<i>R. chalapensis</i>	Neyshabour, Kharv, 1460 m, Soleimani, 9150 (IAUM); Bajestan, Tangolsenjedak, 1400 m, Hojat and Zangouei, 28414 (FUMH); Kashmar, Ghaleh Jough, 1300 m, Hojat and Zangouei, 28870 (FUMH); Ferizi, 1650 m, Soleimani, 9148 (IAUM); Birjand, between Taghiabad and Roud Kaj, 1800 m, Faghihnia and Zangouei 30146.
<i>R. conglomeratus</i>	Noghondar to Kang village, 1330 m, Soleimani, 9155 (IAUM); Ferizi, 1700 m, Aiatolahi and Joharchi, 13551 (FUMH); Golmakan, Kalate Asheghan, 1500 m, Rashed and Aiatolahi, 13390 (FUMH)

For palynological study, the pollen grains of *R. crispus*, *R. conglomeratus* and *R. tuberosus* were extracted from the anther and dehydrated by Glacial Acetic Acid, then acetolised, and studied by SEM and LM (Erdtman, 1960; Moore et al., 1991). Pollen characters of *R. dentatus* and *R. chalapensis* was adapted from Yasmin's et al. (2010) report. Some characteristics like: P (polar axis length), E (equatorial axis length) and the P/E ratio were measured at magnification 5000, 20000. The pollen terminology in general followed Punt et al. (2007).

RESULTS

Anatomical results of stem, revealed uni-seriate epidermis layer, collenchymous tissue, parenchymous tissue including secretory duct, sclerenchymous bundles which surround vascular bundles and vascular bundles with different shapes. The shape of sclerenchymous strands and phloem bundles, the number of collenchymous and sclerenchymous tissues varied among the studied species (Table 2, Figures. 1A- E). All the studied species had parenchymous pith except *R. dentatus* had hollow pith in stem. Also, internal vascular bundles were observed in *R. crispus*. Druse crystals were perceived in the cortex and pith of *R. dentatus* stem. In the leaf cross section of studied species, dorsi-ventral mesophyll was noticed. The palisadic parenchyma in *R. tuberosus* and *R. crispus* was bi-seriate while in the rest of species was uni-seriate. Also the ratio of palisadic to spongy parenchyma was variable among the species (Table 2, Figure 1F).

Table 2: The stem and leaf anatomical characters in studied species of *Rumex*

Species	The number of collenchymous layer	The number of sclerenchymous layer	The shape of sclerenchymous bundles above the phloem	The shape of vascular bundle	The number of Palisadic parenchyma layer	The ratio of Palisadic to spongy parenchyma
<i>R. crispus</i>	2- 3	6- 8	Circular cap shaped	Oblong elliptical	2	1.06
<i>R. tuberosus</i>	4- 5	4- 5	Narrow crescent shaped	Triangular	2	1.32
<i>R. dentatus</i>	4- 6	2- 3	Narrow crescent shaped	Obovate with different size	1	1.16
<i>R. chalapensis</i>	2- 3	2- 4	Crescent shaped to rectangular	Obovate-oblong elliptical	1	0.76
<i>R. conglomeratus</i>	2- 3	3- 6	Broad crescent shaped	Triangular-obovate	1	0.86

Palynological results showed tricolporate, tetracolporate, panporate and spherical- triangular pollen in polar view. The ornamentation was observed porate- punctuate to granulate (Figures 1G -L). The P/E ratio and quantitative features were presented in Table 3.

Table 3: Pollen characters in studied species in *Rumex*

Species	The shape of pollen in polar view	The ornamentation of exine	P	E	P/E
<i>R. crispus</i>	Panporate	microechinate	18.27	16.22	1.12
<i>R. tuberosus</i>	Tricolporate	Perforate-echinate	16.78	15.02	1.11
<i>R. dentatus</i>	tetracolporate	Granulate	28.4	27.6	1.02
<i>R. chalapensis</i>	tetracolporate	Porate-punctuate	27	25.4	1.06
<i>R. conglomeratus</i>	Tricolporate	microechinate	23.89	22.43	1.06

DISCUSSION

In anatomical analysis of stem, the minimum and maximum number of collenchymous layer below the epidermis tissue were noticed in *R. crispus* (2- 3 layered) and *R. dentatus* (4- 5 layered). Also the minimum and maximum number of sclerenchymous strands around the vascular bundles were observed in *R. dentatus* (2- 3 layered) and *R. crispus* (6- 8 layered) respectively. Oblong elliptical bundles was just perceived in *R. crispus* while, in the rest of studied species triangular-obovate bundles were observed. Just *R. tuberosus* had sclereid above the phloem and no secretory ducts. Just *R. dentatus* had hollow pith and *R. crispus* had internal vascular bundle. Joshi pointed that most of the species of *Rumex* showed presence of internal bundles. Also, absence of internal bundles has been reported in *R. dentatus* by Joshi (1936). Joshi believes "the perennial species of *Rumex* which are without internal bundles are the oldest forms of the genus. From these have arisen the perennial forms with internal bundles and the annual forms without internal bundles". Thus all studied species in this research except *R. dentatus* represented the oldest forms of *Rumex*. Sahney and Vibhasa (2012) in anatomical studies on *Rumex hastatus*, mentioned "*Rumex hastatus* is similar to *R. dentatus* but differs from it due to presence of chlorenchyma below the furrows, tannin filled cells below the chlorenchyma and large druses in particular position in the cortex and absence of hollow pith. In *R. hastatus*, internal vascular bundles were formed below the collenchymous between the first formed bundles, though in *R. crispus* internal vascular bundles forms exactly below the older ones. In leaf anatomy of seven species of *Rumex* L. of Jiangxi, the shape of epidermal cells, stomata apparatus, glands arrangement, the shape and the number of vascular bundle of midrib were assessed by Li et al. (2008). They explained "comparative anatomy study of leaves might provide some helps to the study of phylogeny and classification of *Rumex* L." The palynological results of present research, showed tricolporate, tetracolporate, panporate type while the shape of pollen in polar view was the same in three species i.e. spherical- triangular. Also, the exine ornamentation was observed microechinate, porate- punctuate to granulate. Yasmin et al. (2010) reported, "tricolporate and tetracolporate, radially symmetrical and isopolar pollen in *Rumex* species. Moreover, tectum was granular and perforate-punctate in *R. dentatus* and *R. chalapensis* respectively". In Yasmin's et al. research, three distinct pollen types were introduced on the basis of exine ornamentation i.e. Chalapensis type (Perforate-punctate tectum), Dentatus type (granulate tectum) and Acetosa type (coarsely reticulate tectum). According to the above types, *R. crispus* and *R. conglomeratus* fall into Dentatus type and *R. tuberosus* fall into Chalapensis type. Based on Yasmin's et al. study, "there is quite considerable variation in pollen morphology especially; exine and its sculpturing under SEM is of real systematic value and make the pollen grains decidedly distinct structures which can be employed for the delimitation of closely related species" (Yasmin et al., 2010). But in present research, differences in anatomical and pollen structures are not significant features to identify studied species.

Fig. 1.

Fig. 1. Cross section of stem: A) *R. crispus*, B) *R. tuberosus*, C) *R. dentatus*, D) *R. chalapensis*, E) *R. conglomeratus*, F) Cross section of leaf of *R. crispus*; The pollen shape in polar view ×5000 and exine ornamentation ×20000: G,H) *R. crispus*, I, J) *R. tuberosus*, K, L) *R. conglomeratus*.

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