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Research Article

Leaf Epidermal Micromorphology of *Portulaca* L. Species Found in Vadodara, Gujarat, India

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Micromorphology of three species of *Portulaca* was carried out with the help of light microscopy to determine variations within the species which would aid in correct identification of the plants. Epidermal cells are polygonal with sinuous anticlinal walls in all the three species. Length of epidermal cells of *P. grandiflora* Hook. is higher than *P. oleracea* Linn. and *P. quadrifida* Linn. The leaves of *P. quadrifida* are epistomatic while the remaining species are amphistomatic with paracytic stomata in all the three species. Mean stomatal index and stomatal frequency are more in *P. quadrifida* while the mean size of stomata (both length and width) is larger in *P. grandiflora* for both adaxial and abaxial surfaces. Based on the diagnostic features, an artificial indented key is prepared.

1. Introduction

The members of the family Portulacaceae are cosmopolitan generally herbs and shrubs with 15–30 genera and 500 species [1]. According to Geesink [2], *Portulaca* Linn is divided into two subgenera: subgenera *Portulacella* (F. Muell.) Legrand with glabrous nodes and flowers in dichasia and subgenera *Portulaca* with nodal scales or hairs and terminal flowers. The presently studied species belong to subgenera *Portulaca*. All the three species are recorded from the west of North America, South America, and Africa with some representatives in Europe and Asia [3]. The plants are native to the tropics of the western hemisphere [4].

The species selected for the present study are *P. grandiflora, P. oleracea,* and *P. quadrifida* which are suggested in medicine. *P. grandiflora* is a succulent, prostrate, ascending, pretty herb with subblooming flowers [5], used for the relief of sore throat, skin rashes, and detoxification [6]. *P. quadrifida* is small succulent with elliptic to ovate leaves. Flowers are yellow, solitary, terminal with conical capsule and minutely tubercle seeds [5]. It is used for various curative purposes such as asthma, cough, urinary discharge, inflammation, ulcers, abdominal complaints, erysipelas, and hemorrhoids [7, 8]. *P. oleracea* is a succulent, erect herb with oblong ovate or spathulate leaves and cuneate or sessile base. Flowers are yellow with terminal or axillary clusters. Capsule is ovoid with black muriculate seeds. It is antibacterial, -virus, -antherosis, -caducity and enhances immunity. It is also useful in headache, stomachache, painful urination, dysentery, enteritis, astitis, lack of milk flow in nursing mothers, and in postpartum bleeding, inflammation, skin sores, and ulcers. Fresh herb is used as poultice or juice [9, 10] and in unani formulation "Qur Tabasheer" useful as antihyperglycemic and antihyperlipidemic drug [11].

Micromorphological studies are already known to be useful in elucidating the characters in the vegetative phase. Within the members of Caryophyllales, various aspects of micromorphology have been studied, for example, seed coat micromorphology of Aizoaceae, Gisekiaceae [12], Molluginaceae [13], Caryophyllaceae [14], and epicuticular waxes of Amaranthaceae members such as *Arthraerua* [15], *Gomphrena* [16], and Centrosperms [17]. Seed, pollen grains, and leaf micromorphology of *Saponaria* (Caryophyllaceae) [18] and members of Amaranthaceae such as *Gomphrena* [19] and *Achyranthes* [20] have been studied in detail. Heywood [21] reports that genera's of Portulacaceae are ill-defined and they can very well be distinguished from one another by microscopic features, which are of prime assistance in demarcation at the species level. Kadiri [22] suggests that there is insufficient information on the microscopic characters of

Table 1: Characteristics of stomata of *Portulaca* spp. (Mean ± S.E) (* μm, ** per mm^2, *** %).

Stomatal characters		*P. grandiflora*	*P. oleracea*	*P. quadrifida*
Type	Adaxial	Paracytic	Paracytic	Paracytic
	Abaxial	Paracytic	Paracytic	—
Length*	Adaxial	4.01 ± 0.12	3.88 ± 0.20	2.52 ± 0.18
	Abaxial	4.76 ± 0.22	4.26 ± 0.11	—
Width*	Adaxial	3.45 ± 0.18	2.95 ± 0.15	2.10 ± 0.23
	Abaxial	4.06 ± 0.32	3.80 ± 0.25	—
Frequency**	Adaxial	13.34 ± 0.62	13.25 ± 0.90	54.60 ± 1.06
	Abaxial	20.95 ± 0.86	14.00 ± 0.38	—
Index***	Adaxial	22.07 ± 0.45	18.35 ± 0.60	35.55 ± 0.86
	Abaxial	23.34 ± 0.41	18.03 ± 0.44	—

the members of the family Portulacaceae which can aid in their easy recognition and confirmation. His study reports that the species of *Portulaca* from Nigeria are amphistomatic but in India *P. quadrifida* is found to be epistomatic. This led us to enquire whether geographical regions are causing substantial variations within the species. It would also be helpful in understanding the taxonomical and phylogenetic relationship within the genera. Micromorphology study of these species will give an insight in identifying different species with characters like epidermal cells and stomata to prepare a good dataset to reduce the adulteration in the medicinal formulations.

2. Materials and Methods

All the three species were collected from the Faculty of Science campus, The M. S. University of Baroda, Vadodara, and were deposited in the departmental herbaria with voucher specimen number being *P. grandiflora* = 274, *P. oleracea* = 275, and *P. quadrifida* = 276. For *P. grandiflora* and *P. oleracea,* epidermal peels from the leaves were obtained by peel method, washed in water, stained in safranin, and mounted with 50% glycerine. *P. quadrifida* has minute leaves and so it was macerated with Jaffrey's solution. Fifteen observations each covering the entire leaves were done for all the three species and each slide was observed for 15 microscopic fields for both the surfaces. The epidermal characters of the cell, stomata, and trichome were studied. Quantitative features such as length of the epidermal cell, size of stomata, stomatal index, and stomatal frequency have been determined as follows:

$$\mathrm{SI} = \left(\frac{S \times 100}{E + S}\right), \tag{1}$$

where SI is the stomatal index percent, S is the number of stomata/field of study, and E is the number of epidermal cells/field of study.

$$\text{Stomatal Frequency} = \text{Number of stomata per unit leaf area.} \tag{2}$$

All observations and photomicrographs were obtained with Leica research microscope.

3. Results

For the present work, descriptive terminologies described by Metcalfe and Chalk [23] have been followed. The leaves of *P. grandiflora* and *P. oleracea* are amphistomatic while those of *P. quadrifida* are epistomatic. The stomatal type was paracytic on both the adaxial and abaxial surfaces of *P. grandiflora* and *P. oleracea,* and in *P. quadrifida* it is paracytic on the adaxial surface and absent on abaxial surface (Figure 1). The stomata were oriented at a right angle to the midrib of the leaf in case of *P. grandiflora* and *P. quadrifida* but in *P. oleracea* it was irregularly scattered. The length and width of stomata is more on abaxial surface than adaxial surface for *P. grandiflora* and *P. oleracea* (Table 1). For both the adaxial and abaxial surfaces, the length of stomata are more in *P. grandiflora* followed by *P. oleracea* and *P. quadrifida* (Table 1). Similar pattern is depicted in width of stomata for both of the surfaces. Stomatal frequency per mm^2 on the adaxial surface is higher in *P. quadrifida* (54.60) while it is similar in *P. grandiflora* and *P. oleracea*. On the abaxial surface, the stomatal frequency per mm^2 is more in *P. grandiflora* than *P. oleracea*. Mean stomatal index percent on the adaxial surface is again higher in *P. quadrifida* (35.55%) followed by *P. grandiflora* and *P. oleracea,* while on the abaxial surface *P. grandiflora* has a high mean stomatal index percent (Table 1).

Micromorphological features of *P. grandiflora* and *P. oleracea* revealed that both the abaxial and adaxial surfaces have ordinary epidermal cells with polygonal sinuous anticlinal walls (Figures 1(a), 1(b), 1(c), and 1(d)). In *P. quadrifida*, on the adaxial surface, the cells are polygonal while the cells on abaxial surface are almost isodiametric (Figures 1(e) and 1(f)). The mean length of epidermal cells in all the three species does not show much variation with their respective adaxial and abaxial surfaces. On the adaxial surface, the mean length of epidermal cells is higher in *P. grandiflora* and similar in *P. oleracea* and *P. quadrifida* (Table 2). On the abaxial surface the mean length of ordinary epidermal cells is higher in *P. grandiflora* than in *P. oleracea* and *P. quadrifida* (Table 2). Besides this, *P. grandiflora* and *P. oleracea* were found to be

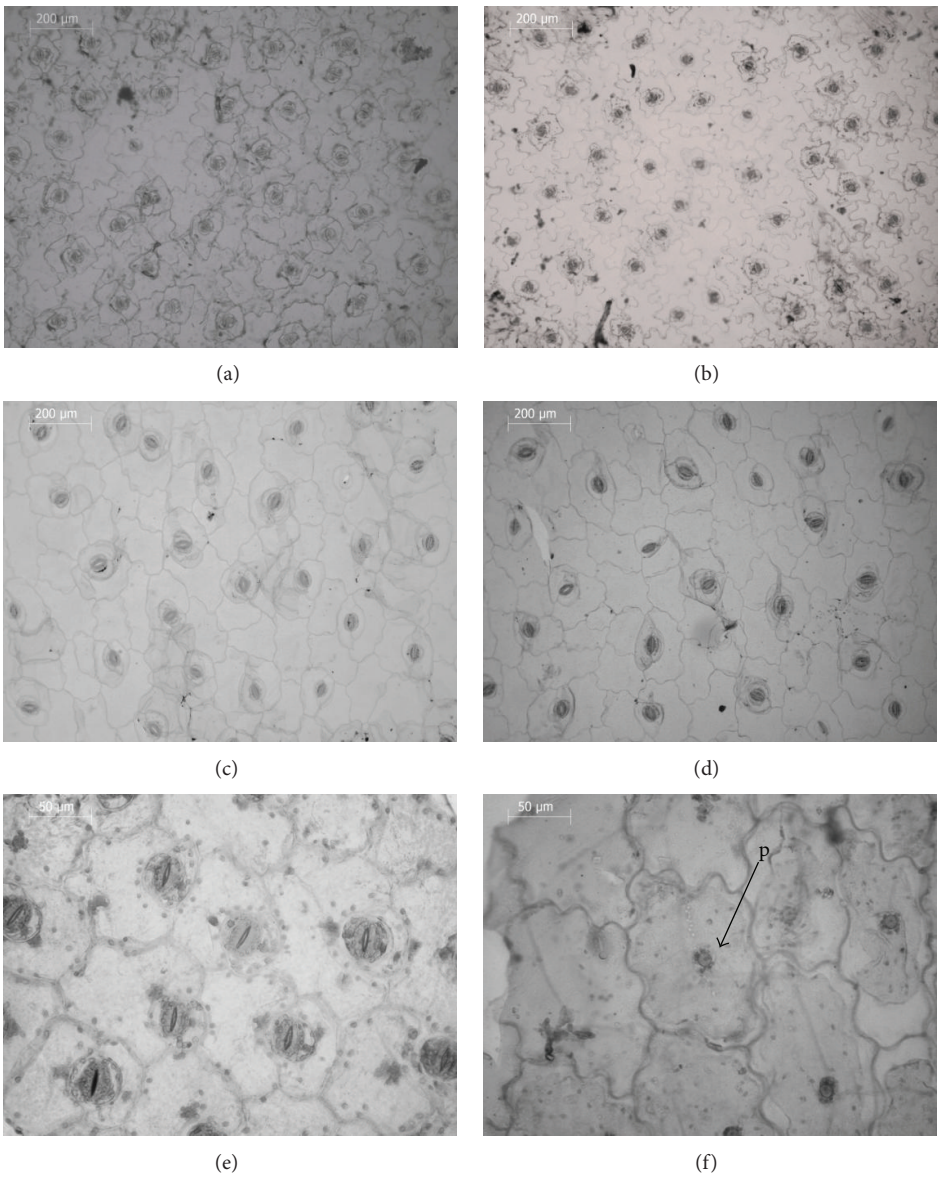

Figure 1: Foliar epidermal peels of: (a) and (b) adaxial and abaxial surface of *P. grandiflora* with sinuous anticlinal epidermal cell walls and paracytic stomata, (c) and (d) adaxial and abaxial surface of *P. oleracea* with undulating anticlinal epidermal cell walls and paracytic stomata, (e) adaxial surface of *P. quadrifida* with undulating anticlinal epidermal cell walls and paracytic stomata, and (f) abaxial surface of *P. quadrifida* with no stomata showing papillae (p).

glabrous while *P. quadrifida* was pubescent having papillae trichome.

4. Discussion

The present study elucidates the micromorphological features of three species of *Portulaca,* namely, *P. grandiflora, P. oleracea,* and *P. quadrifida*. *Portulaca* species of Nigeria has been studied by Kadiri [22], however its Indian counterpart shows micromorphological variations. Kadiri has reported that the three species are amphistomatic and the epidermal cells of *P. grandiflora* on the adaxial surface are to be isodiametric but the present investigation revealed that *P. grandiflora* has polygonal cells while *P. quadrifida* is epistomatic with its epidermal cells on the abaxial surface to be isodiametric. Besides this, the size of stomata is larger in the Nigerian species as compared to its Indian counterpart. Observations which were similar to Kadiri [22] are the presence of paracytic stomata, larger and higher number of stomata on abaxial surface in all the three species, and uniformity of stomatal distribution in *P. oleracea*. The stomata are paracytic type, flanked on either side by unequal girdling epidermal cells in all the three species. The subsidiary cells are surrounded by an additional tier of cell where the inner wall forms an arc around the subsidiary cells while the outer wall is sinuous like an ordinary epidermal cell. Similar epidermal

Table 2: Characteristics of leaf epidermis of *Portulaca* spp. (Mean ± S.E) (*μm).

Traits		*P. grandiflora*	*P. oleracea*	*P. quadrifida*
Length of epidermal cell*	Adaxial	22.12 ± 1.36	12.95 ± 0.44	12.53 ± 0.60
	Abaxial	23.94 ± 0.97	15.89 ± 0.81	13.30 ± 0.45
Pattern of anticlinal wall	Adaxial	Sinuous	Sinuous	Sinuous
	Abaxial	Sinuous	Sinuous	Sinuous

cell pattern is also reported in *Calandrinia* and *Montopsis* of family Portulacaceae [24] and in *Cinnamomum schaeffer* [25]. In this study, the stomata were paracytic type which is also reported in *Cistanthe* [26] and *Talinum* of Portulacaceae [27]. There is not much difference between adaxial and abaxial surfaces for the parameters such as stomatal index which is also reflected in the study of *Cistanthe* [26].

Significant observations were made in *P. quadrifida* where the stomata were found to be oriented at right angle to the midrib of the leaf which is in agreement with the studies of Rajagopal [28]. The leaves were found to be epistomatic in nature, and Salisbury [29], Weaver and Clements [30] have noted that the frequency as well as surface distribution of foliar stomata is correlated with plant habit. Rao and Ramayya [31] reported 35.9% plants as poor epistomatic or functional hypostomatic in the order Malvales. These plants are essentially xeric in habit adapted for conditions of stress. This seems to be true in case of *P. quadrifida* where the leaves are epistomatic and have xerophytic features such as succulent and leathery nature of leaves with thick cuticle. Trichomes in the form of papillae were found in *P. quadrifida* while *P. grandiflora* and *P. oleracea* were glabrous. Papillate trichomes have also been reported in *Talinum* [27]which is a member of Portulacaceae.

5. Conclusion

The present study revealed that change in geographical region could lead to variations within the species. Differences observed in micromorphological features of *Portulaca* species found in India are smaller size of stomata, ordinary epidermal cell to be polygonal with sinuous anticlinal walls, and *P. quadrifida* to be epistomatic with isodiametric epidermal cells on the abaxial surface. The species can be distinguished from one another as *P. oleracea* and *P. grandiflora* are amphistomatic while *P. quadrifida* is epistomatic. Micromorphological features revealed that *P. grandiflora* can easily be distinguished from *P. oleracea* as its size of stomata (length and width) and length of epidermal cell are larger as compared to *P. oleracea*. Stomatal index percent and stomatal frequency per mm^2 are higher in *P. quadrifida* while *P. grandiflora* has larger stomatal size and length of epidermal cell within the species.

A diagnostic identification key for the three species is prepared as follows:

(1) leaves are epistomatic and pubescent—*P. quadrifida,*

(1) leaves are amphistomatic and glabrous—2,

(2) mean stomatal size, larger length of epidermal cells, and stomata arranged at a right angle to the midrib—*P. grandiflora,*

(2) mean stomatal size, smaller length of epidermal cells, and stomata irregularly scattered with to respect to midrib—*P. oleracea.*

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