

STOMATAL STUDIES OF SOME SELECTED MEDICINAL PLANTS OF POLYGONACEAE

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Abstract

The study reports variation in the structure and distribution of stomata in some members of Polygonaceae viz., *Rumex hastatus* D. Don, *Rumex dentatus* Linn, *Rumex nepalensis* Spreng, *Rheum australe* D. Don, *Persicaria maculosa* S.F. Gay and *Polygonum plebejum* R. Br. The type of stomata, density, frequency, stomatal index, size of stomatal pore (average length and width), size of guard cells (average length and width) and percentage of the close and open stomata were determined. The upper epidermises of the six plants contain anomocytic, paracytic, anisocytic, tetracytic and hemiparacytic stomata while the lower epidermises contain tetracytic, anisocytic, anomocytic and paracytic stomata. The statistical evaluation of the stomata and epidermis included mean, standard deviation, variance, coefficient of variance, standard error and difference of standard error were carried out. The study indicates the taxonomic utility of the stomatal type.

Introduction

The stomata allow gases exchange. In green leaves they occur either on both surfaces (amphistomatic leaf) or on only one, either the upper (epistomatic leaf) or more commonly on lower that is hypostomatic leaf (Perveen *et al.* 2007). Stebbins & Khush (1961), Stace (1969a, 1973a) and Dilcher (1974) believed stomatal type to be taxonomically significant and provide an efficient tool for separating and in some cases linking at least the higher level of taxonomic hierarchy. Due to inconsistency of stomatal type in different taxa, Pant & Kidwai (1964) and Sen & Hennipman (1981), however, did not feel so sure about the effectiveness of stomata as a tool in taxonomy. Zahur & Parveen (1982) and Siddiqi *et al.*, (1991) have utilized the stomatal characteristics in the study of taxonomic relationships in different families with some success. On the basis of arrangement of the epidermal cell neighboring the guard cell, more than 25 main types of stomata in dicots have been recognized (Metcalf & Chalk, 1979. Silva *et al.*, (1988) reported stomatal density in the *Phaseolus vulgaris*. Petrova (1988) described a difference in the stomatal number and distribution of stomata in *Glycine clandestine*, *G. canescens*, *G. tomentella* and *G. tabacina*. Ferris *et al.*, (2002) reported co-efficient of variance, stomatal density, stomatal index, epidermal cells area and number of epidermal cells per leaf of poplar. Kong (2001) reported various types of stomata in the genus *Ficus*. Little information is available on stomata of plants from Pakistan (Abid *et al.* 2007). Stomata studies of the some medicinal plants, of the family Polygonaceae is reported.

Materials and Methods

Persicaria maculosa S.F. Gay, *Rumex hastatus* D. Don, *Rumex dentatus* Linn., *Rumex nepalensis* Spreng, *Polygonum plebejum* R. Br were collected from Peshawar University Campus and *Rheum australe* D. Don was collected from Gharam Chasma (Chitral) in March – November 2005. These plants were identified with the help of Flora of Pakistan (Ali & Qaiser, 2007). The fresh leaves were immersed in water to prevent desiccation to procure epidermal cells. Peels from the abaxial surfaces were obtained with the help of the razor and mounted in Canada balsam for microscopic examination (Chaudhary & Imran, 1997). The parameters studied were presence and absence of stomata on each epidermis, type of stomata, density, frequency, stomatal index, size of stomatal pore (average length and width having 10 readings), size of guard cells (average length and width having 10 readings) and percentage of the close and open stomata (Wallis, 1985). The statistical evaluation of the stomata and epidermis included mean, standard deviation, variance, coefficient of variance, standard error and difference of standard error (Choudhary & Kamal, 2004).

Results

The type of stomata in the upper epidermis of *R. hastatus* is hexacytic and paracytic. The density and frequency of hexacytic stomata of the upper epidermis is 137.5 and 13.75, respectively. Density and frequency of the paracytic stomata of the lower epidermis is 378.5 and 37.85, respectively. Stomata in the lower epidermis are tetracytic, anisocytic and hexacytic. Densities of these stomata are 3650, 362.5 and 137.5. Frequencies of these stomata are 365, 36.25 and 13.75. Index of the stomata of the upper and lower epidermises is 24.78 and 48.52. Stomata in both upper and lower epidermises of *R. dentatus* are anomocytic. The density and frequency of anomocytic stomata of the upper and lower epidermises are 600, 860, 60 and 860, respectively. Index of the stomata of the upper and lower epidermises is 15.61 and 17.99. Stomata in the upper epidermis of *R. nepalensis* are paracytic and anisocytic and in the lower epidermis are anisocytic. The density and frequency of anisocytic stomata of the upper epidermis are 87.5; 2378 and 8.75 and 237.8, respectively. The density and frequency of anisocytic stomata of the lower epidermis are 512.5 and 51.25. Index of the stomata of the upper and lower epidermises is 19.21 and 33.39. Stomata in the upper epidermis of *R. australe* are anisocytic and tetracytic. The density and frequency of anisocytic stomata of the upper epidermis is 250; 300, 25 and 30, respectively. Stomata in the lower epidermis are paracytic, tetracytic and hexacytic. Density and frequency of these stomata are 1125; 305; 250 and 11.25; 30.5 and 25, respectively. Index of the stomata of the upper and lower epidermises is 11.25 and 39.89. Stomata in both upper and lower epidermises of *P. maculosa* are paracytic. The density and frequency of paracytic stomata of the upper and lower epidermises are 1875; 3650 and 187.5 and 365, respectively. Index of the stomata of the upper and lower epidermises is 12.01 and 54.52. Stomata in the upper epidermis of *P. plebejum* are hemiparacytic and paracytic. The density and frequency of these stomata of the upper epidermis is 235.5; 337.5 and 23.55 and 33.75, respectively. Stomata in the lower epidermis are anisocytic and paracytic. Density and frequency of these stomata are 250; 695 and 25 and 69.5, respectively. Index of the stomata of the upper and lower epidermises is 7.801 and 18.32 (Table 1).

Table 1. Stomatal study of some species of Polygonaceae.

Species	Stomatal											
	Type		Density		Frequency		Index					
	U	L	U	L	U	L	U	L				
<i>R. hastatus</i>	Anomocytic	Tetracytic	137.5	3650	13.75	365.0	24.78	48.52				
	(Hexacytic)	Anisocytic		362.5	37.85	36.25						
	Paracytic	Hexacytic	378.5	137.5		13.75						
<i>R. dentatus</i>	Anomocytic	Anomocytic	600.0	860.0	60.0	86.0	15.61	17.99				
<i>R. nepalensis</i>	Paracytic	Anisocytic	87.50	2378.0	8.75.0	237.8	19.21	33.39				
	Anisocytic		512.5		51.25							
<i>R. australe</i>	Anisocytic	Paracytic	250.0	1125.0	25.0	112.5	11.25	39.89				
	Tetracytic	Tetracytic		305.0		30.5						
		Hexacytic	300.0	250.0	30.0	25.0						
<i>P. maculosa</i>	Paracytic	Paracytic	1875.0	3650.0	187.5	365	12.01	42.52				
<i>P. plebejum</i>	Hemiparacytic	Anisocytic	235.5	250.0	23.55	25.0	7.89	18.32				
	Paracytic	Paracytic	337.5	695.0	33.75	69.5						
	Average						% Age of open and close stomata					
	Size of stomata pore				Size of guard cell							
	U		L		U		L		U		L	
	L(μ)	W(μ)	L(μ)	W(μ)	L(μ)	W(μ)	L(μ)	W(μ)	C	O	C	O
<i>R. hastatus</i>	15	9	25	12	26	18	28	16	35	65	45	55
<i>R. dentatus</i>	18	7	27	13	29	15	37	24	22	88	40	60
<i>R. nepalensis</i>	20	9	22	8	42	22	33	15	42	58	30	70
<i>R. australe</i>	14	8	18	10	26	16	30	13	40	60	55	45
<i>P. maculosa</i>	22	10	25	13	43	23	38	21	52	48	49	51
<i>P. plebejum</i>	24	15	20	11	34	22	35	25	80	20	40	60

Key: U= Upper; C= Close; L= Length; L= Lower; O= Open; W= Width

Average length and width of the stomatal pore of the upper epidermis of *R. hastatus* is 15 μm and is 9 μm, respectively. Average length and with of the stomatal pore of the lower epidermis of *R. hastatus* is 25 μm and 12 μm, respectively. Average length and width of the stomatal pore of the upper epidermis of *R. dentatus* is 18 μm and 7 μm, respectively. Average length and width of the stomatal pore of the lower epidermis of *R. dentatus* is 27 μm and 13 μm, respectively. Average length and width of the stomatal pore of the upper and lower epidermises of *R. nepalensis* are 20 μm; 22 μm and 9 μm and 8 μm, respectively. Average length and width of the stomatal pore of the upper and lower epidermises of *R. australe* are 14 μm; 18 μm and 8 μm and 10 μm, respectively. Average length and width of the stomatal pore of the upper and lower epidermises of *P. maculosa* are 22 μm; 25 μm and 10 μm and 13 μm, respectively. Average length and width of the stomatal pore of the upper and lower epidermises of *P. plebejum* are 24 μm; 20 μm and 15 μm and 11 μm, respectively. Average length and width of the guard cells of the upper and lower epidermises of *R. hastatus* are 26 μm; 28 μm and 18 μm and 16 μm, respectively. Average length and width of the guard cells of the upper and lower epidermises of *R. dentatus* are 29 μm; 37 μm and 15 μm and 24 μm, respectively. Average length and width of the guard cells of the upper and lower epidermises of *R. nepalensis* are 42 μm; 33 μm and 22 μm and 15 μm, respectively. Average length and width of the guard cells of the upper and lower epidermises of *R. australe* are 26 μm; 30 μm and 16 μm and 13 μm, respectively. Average length and width of the guard cells of the upper and lower epidermises of *P. maculosa* are 43 μm; 38 μm and 23 μm and 21 μm, respectively. Average length and width of the guard

cells of the upper and lower epidermises of *P. plebejum* are 34 μm ; 35 μm and 22 μm and 25 μm , respectively (Table 1).

Percentage of the open and close stomata in the upper epidermis of *Rumex hastatus* is 35 and 65 and that of the lower epidermis is 45 and 55. Percentage of the open and close stomata in the upper epidermis of *Rumex dentatus* is 22 and 88 and that of the lower epidermis is 40 and 60. The percentage of the open and close stomata in the upper epidermis of *Rumex nepalensis* is 42 and 58 and that of the lower epidermis is 30 and 70. The percentage of the open and close stomata in the upper epidermis of *Rheum australe* is 40 and 60 and that of the lower epidermis is 55 and 45. Percentage of the open and close stomata in the upper epidermis of *Persicaria maculosa* is 52 and 48 and that of the lower epidermis is 49 and 51. Percentage of the open and close stomata in the upper epidermis of *Polygonum pelbejum* is 80 and 20 and that of the lower epidermis is 40 and 60 (Table 1).

The statistical evaluation of the stomata of the upper epidermis of *R. hastatus* shows 7.80 mean, 0.56 standard deviation, 1.12 variance, 1.78 co-efficient of variance, 1.51 standard error and the lower epidermis have 12.97 mean, 0.95 standard deviation, 5.62 variance, 7.89 co-efficient of variance and 5.56 standard error. The stomata of the upper epidermis of *R. dentatus* shows 10.78 mean, 0.34 standard deviation, 2.25 variance, 4.56 co-efficient of variance, 5.23 standard error and the lower epidermis have 13.12 mean, 0.78 standard deviation, 7.54 variance, 9.54 co-efficient of variance and 20.56 standard error. The difference of standard error of the upper and lower epidermis of *R. hastatus* and *R. dentatus* found to be 3.72 and 15.00 respectively. Stomata of the upper epidermis of *R. nepalensis* shows 11.39 mean, 0.75 standard deviation, 2.56 variance, 5.21 co-efficient of variance, 2.31 standard error and the lower epidermis have 14.78 mean, 0.92 standard deviation, 3.61 variance, 7.14 co-efficient of variance and 7.51 standard error. The stomata of the upper epidermis of *Rheum australe* shows 5.47 mean, 0.93 standard deviation, 5.59 variance, 8.18 co-efficient of variance, 5.51 standard error and the lower epidermis have 8.87 mean, 1.56 standard deviation, 7.89 variance, 10.15 co-efficient of variance and 17.59 standard error. The difference of standard error of the upper and lower epidermis of *R. nepalensis* and *R. australe* found to be 3.2 and 10.08 respectively. Stomata of the upper epidermis of *P. maculosa* shows 15.95 mean, 0.59 standard deviation, 1.18 variance, 4.51 co-efficient of variance, 2.23 standard error and the lower epidermis have 16.71 mean, 0.89 standard deviation, 1.73 variance, 7.21 co-efficient of variance and 11.56 standard error. The stomata of the upper epidermis of *P. plebejum* shows 4.89 mean, 0.38 standard deviation, 3.21 variance, 5.89 co-efficient of variance, 3.56 standard error and the lower epidermis have 7.89 mean, 0.63 standard deviation, 5.19 variance, 8.89 co-efficient of variance and 17.12 standard error. The difference of standard error of the upper and lower epidermis of *Persicaria maculosa* and *P. plebejum* found to be 1.33 and 5.56 respectively (Table 2).

Epidermal cells of the upper epidermis of *R. hastatus* shows 55.05 mean, 1.26 standard deviation, 1.6 variance, 2.29 co-efficient of variance, 1.17 standard error and the lower epidermis have 44.05 mean, 9.72 standard deviation, 94.6 variance, 22.61 co-efficient of variance and 3.98 standard error. The epidermal cells of the upper epidermis of *R. dentatus* shows 59.97 mean, 2.46 standard deviation, 6.07 variance, 4.17 co-efficient of variance, 2.07 standard error and the lower epidermis have 24.87 mean, 5.75 standard deviation, 33.12 variance, 7.77 co-efficient of variance and 63.04 standard error. The difference of standard error of the upper and lower epidermis of *R. hastatus* and *R.*

dentatus found to be 0.9 and 59.06 respectively. Epidermal cells of the upper epidermis of *R. nepalensis* shows 35.32 mean, 1.76 standard deviation, 3.12 variance, 5.05 co-efficient of variance, 1.74 standard error and the lower epidermis have 59.8 mean, 2.17 standard deviation, 4.75 variance, 3.69 co-efficient of variance and 49.18 standard error. The epidermal cells of the upper epidermis of *R. australe* shows 46.67 mean, 3.83 standard deviation, 14.7 variance, 8.33 co-efficient of variance, 3.87 standard error and the lower epidermis have 83.25 mean, 6.53 standard deviation, 42.75 variance, 10.71 co-efficient of variance and 65.09 standard error. The difference of standard error of the upper and lower epidermis of *R. nepalensis* and *R. australe* found to be 2.13 and 15.91 respectively. Epidermal cells of the upper epidermis of *Persicaria maculosa* shows 61.07 mean, 1.45 standard deviation, 2.12 variance, 2.38 co-efficient of variance, 2.34 standard error and the lower epidermis have 35.42 mean, 5.29 standard deviation, 28.02 variance, 8.67 co-efficient of variance and 3.46 standard error. The epidermal cells of the upper epidermis of *P. plebejum* shows 21.07 mean, 2.01 standard deviation, 4.07 variance, 9.61 co-efficient of variance, 5.97 standard error and the lower epidermis have 52.05 mean, 6.75 standard deviation, 35.97 variance, 9.67 co-efficient of variance and 6.07 standard error. The difference of standard error of the upper and lower epidermis of *Persicaria maculosa* and *P. plebejum* found to be 3.63 and 2.61 respectively (Table 2).

Discussion

Different parameters were observed in this study such as presence or absence of stomata on each epidermis, type of stomata, density, frequency, stomatal index, size of stomatal pore (average length and width), size of guard cells (average length and width) and percentage of the close and open stomata. The statistics evaluation of the stomata and epidermis included mean, standard deviation, variance, coefficient of variance, standard error and difference of standard error, the stomatal type can be singled out as being the most significant in relation to the taxonomic separation of the taxa.

The type of stomata in the upper epidermis of *R. hastatus* was hexacytic and paracytic. Stomata in the lower epidermis of *R. hastatus* were tetracytic, anisocytic and hexacytic. Stomata in both upper and lower epidermis of *R. dentatus* were anomocytic. Petrova (1988) described the leaf anatomy in *Glycine clandestine*, *G. canescens*, *G. tomentella* and *G. tabacina*. There was found a difference in the stomatal number and distribution of stomata. Stomata in the upper epidermis of the *R. nepalensis* were paracytic and anisocytic and that were in the lower epidermis were anisocytic. Stomata in the upper epidermis of the *R. australe* were anisocytic and tetracytic and that were in the lower epidermis were paracytic, tetracytic and hexacytic. Fadeyi *et al.*, (1989) reported anomocytic and anisocytic stomata in *B. erecta*, *B. diffusa*, *B. repens* and *B. coccinea*. Stomata in both upper and lower epidermis of *P. maculosa* were paracytic. Stomata in the upper epidermis of the *P. plebejum* were hemiparacytic and that were in the lower epidermis were anisocytic and paracytic. The density of stomata of the upper epidermis was highest 1875 in *P. maculosa* and was lowest 87.5 in *R. nepalensis*. Density of stomata of the lower epidermis was highest 3650 in *R. hastatus* and *Persicaria maculosa* and was lowest 137 in *R. hastatus*. Silva *et al.*, (1988) reported stomatal density in the *Phaseolus vulgaris*. The frequency of stomata of the upper epidermis was highest 187.5 in *P. maculosa* and was lowest 8.75 in *R. nepalensis*. Neo & Bonini (1996) reported stomatal frequency in the leaves of *Vaccinium corymbosum*. Frequency of stomata of the lower epidermis was highest 365 in *R. hastatus* and *Persicaria maculosa* and was lowest 13.7 in *R. hastatus*.

Table 2. Statistical evaluation (Stomata and Epiderms) of some species of Polygonaceae

Species	Stomatol											
	M		S.D		V		C.V		S.E		D.S.E	
	U	L	U	L	U	L	U	L	U	L	U	L
<i>R. hastatus</i>	7.80	12.97	0.56	0.95	1.12	5.62	1.78	7.89	1.51	5.56	-	-
<i>R. dentatus</i>	10.78	13.12	0.34	0.78	2.25	7.54	4.56	9.54	5.23	20.56	3.72	15.00
<i>R. nepalensis</i>	11.39	14.78	0.75	0.92	2.56	3.61	5.21	7.14	2.31	7.51	-	-
<i>R. australe</i>	5.47	8.87	0.93	1.56	5.59	7.89	8.18	10.15	5.51	17.59	3.2	10.08
<i>P. maculosa</i>	15.98	16.71	0.59	0.89	1.18	1.73	4.51	7.21	2.23	11.56	-	-
<i>P. plebejum</i>	4.89	7.89	0.38	0.63	3.21	5.19	5.89	8.89	3.56	17.12	1.33	5.56

Species	Epiderms											
	M		S.D		V		C.V		S.E		D.S.E	
	U	L	U	L	U	L	U	L	U	L	U	L
<i>R. hastatus</i>	55.05	44.05	1.26	9.72	1.6	94.6	2.29	22.61	1.17	3.98	-	-
<i>R. dentatus</i>	59.97	24.87	2.46	5.75	6.07	33.12	4.17	7.77	2.07	63.04	0.9	59.06
<i>R. nepalensis</i>	35.32	59.8	1.76	2.17	3.12	4.75	5.05	3.69	1.74	49.18	-	-
<i>R. australe</i>	46.67	83.25	3.83	6.53	14.7	42.75	8.33	10.71	3.87	65.09	2.13	15.91
<i>P. maculosa</i>	61.07	35.42	1.45	5.29	2.12	28.02	2.38	8.67	2.34	3.46	-	-
<i>P. plebejum</i>	21.07	52.05	2.01	6.75	4.07	35.97	9.61	9.67	5.97	6.07	3.63	2.61

Key: U= Upper; L= Length; M= Mean; S.D= Standard deviation; V= Variance; C.V= Co-efficient of variance; S.E= Standard error; D.S.E = Difference of standard error

The stomatal indices have a wide range of variation. Although stomatal indices have been given considerable importance for making comparison in different taxa, it does not seem to be of any significance in the present study. This is because their values vary inconsistently in different taxa. The stomatal index of the upper epidermis was highest 24.78 in *R. hastatus* and was lowest 11.25 in *R. australe*. Stomata index of the lower epidermis was highest 48.52 in *R. hastatus* was lowest 17.99 in *R. dentatus*. Berlinger & Jauregui (1999) reported stomatal index in the leaf of *P. erosus* and *P. tuberosus*. Average length of the stomatal pore of the upper epidermis was highest 24 μ m in *P. plebejum* and was lowest 14 μ m in *R. australe*. The average width of the stomatal pore of the upper epidermis was highest 15 μ m in *P. plebejum* and was lowest in 7 μ m in *R. dentatus*. Average length of the stomatal pore of the lower epidermis was highest 27 μ m in *R. dentatus* and was lowest 18 μ m in *R. australe*. Average width of the stomatal pore of the lower epidermis was highest 13 μ m in *R. dentatus* and *P. maculosa*. Average length of the guard cells of the upper epidermis was highest 43 μ m in *P. maculosa* and was lowest 26 μ m in *R. hastatus* and *R. australe*. Average width of the stomatal pore of the upper epidermis was highest 23 μ m in *P. maculosa* and was lowest 15 μ m in *R. dentatus*. Average length of the stomatal pore of the lower epidermis was highest 38 μ m in *P. maculosa* and was lowest 28 μ m in *R. hastatus*. Average width of the guard cells of the lower epidermis was highest 25 μ m in *P. plebejum* and was lowest 13 μ m in *R. australe*. Percentage of the close stomata in the upper epidermis was highest 80 in *P. plebejum* and was lowest 22 in *R. dentatus*. Percentage of the open stomata in the upper epidermis was highest 88 in *R. dentatus* and was lowest 20 in *P. plebejum*. The percentage of the close stomata in the lower epidermis was highest 55 in *R. australe* and was lowest 30 in *R. nepalensis*. The percentage of the open stomata in the lower epidermis was highest 70 in *R. nepalensis* and was lowest 45 in *R. australe*. Chaudhary & Imran (1997) reported size of the stomatal pore, size of the guard cells, type of stomata and percentage of the close and open stomata (Table 1).

The mean of the number of stomata of the upper epidermis was highest 15.98 in *P. maculosa* and was lowest 4.89 in *P. plebejum*. The mean of the number of stomata of the lower epidermis was highest 16.17 in *P. maculosa* and was lowest 7.89 in *P. plebejum*. The co-efficient of variance of stomata of the upper epidermis was highest 8.18 in *R. australe* and was lowest 1.78 in *R. hastatus*. The co-efficient of variance of stomata of the lower epidermis was highest 10.15 in *R. australe* and was lowest 7.14 in *R. nepalensis*. Ferris *et al.*, (2002) reported co-efficient of variance, stomatal density, stomatal index, epidermal cells area and number of epidermal cells per leaf. The mean of the number of epidermis of the upper epidermis was highest 61.07 in *P. maculosa* and was lowest 21.07 in *P. plebejum*. The mean of the number of epidermis of the lower epidermis was highest 83.25 in *R. australe* and was lowest 24.87 in *R. dentatus*. The co-efficient of variance of epidermis of the upper epidermis was highest 9.61 in *P. plebejum* and was lowest 2.29 in *R. hastatus*. The co-efficient of variance of stomata of the lower epidermis was highest 22.61 in *R. hastatus* and was lowest 3.69 in *R. nepalensis*. Carvahlo *et al.*, (2001) reported type, number and dimensions of stomata in the leaf epidermis of *C. canephora* and *C. vermelho* (Table 2).

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