Journal of Plant Biology, December 2001, 44(4) : 220-223

Comparative Macro- and Micro-Morphological Studies on Varieties of *Parthenium argentatum* Gray (Guayule)

Marimuthu Jayabalan[1], Kaniappanadar Rajarathinam[1], Muthukrishnan Jayakumar[2], and Bong-Seop Kil[3]*

[1]Research Department of Botany, VHNSN College, Virudhunagar 626 001, India

[2]Department of Plant Sciences, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

[3]Division of Life Science, Wonkwang University, Iksan 570-749, Korea

We investigated the leaf shape, venation pattern, trichomes, stomata, and branching of the inflorescence axes in 15 varieties of guayule (*Parthenium argentatum* Gray) growing in India. Working from our study objective, we were unable to identify any specific correlation between these macro- and micro-morphological characteristics and rubber content.

Keywords: Anomocytic stomata, guayule, *Parthenium argentatum*, rubber content, trichomes, venation pattern

Parthenium argentatum Gray, commonly called guayule, is a rubber-producing member of the Asteraceae. Although its variations in leaf form, branching of the inflorescence axis, and other morphological characteristics have been studied extensively (Mehta et al., 1979; Waln et al, 1983; Healey et al., 1986), only. Mehta et al. (1979) has attempted to compare a group of such morphological characters (e.g., leaf shape, trichomes) in guayule, with those found in rubber-bearing plants growing in the Mexican desert. Morphological studies enable researchers to determine rubber content without sacrificing the entire plant. Our objective here was to identify any correlation between specific rubber content and the macro- and micro-morphological characteristics of guayule varieties recently introduced in India by the National Botanical Research Institute (NBRI), Lucknow.

MATERIALS AND METHODS

Plant Material

Fifteen varieties of guayule (*P. argentatum* Gray) were collected from the Botanical garden at NBRI, Lucknow. Their morphological characteristics are listed in Table 1.

Stomatal Patterns

Leaf-sample material was placed, with the adaxial surface facing downward, on a glass plate, then flooded with parazone (domestic bleach). It was then scraped with a blade until the epidermal layer was reached (Ogundpie and Olatunji, 1991; Lee et al., 2000). The tissue was then washed in clean water and temporarily mounted in glycerin. The same process was repeated for samples from the abaxial surface. We examined 10 to 20 peelings from each variety. In addition, inflorescence lengths were measured with a standard mm scale.

*Corresponding author; fax +82-63-857-8837
e-mail bskil@wonkwang.ac.kr

Leaf Clearing

To study venation patterns, an entire leaf was collected from each of the 15 guayule varieties. Trichomes on both sides of the leaf were scraped because their density would have hindered viewing of the venation. Leaves were cleared with a mixture of trichloroacetic acid and phenol, then stained with kores pad ink (Ravindranath and Inamdar, 1982, 1985; Kim et al., 1998).

Description of Leaf Shapes

We collected 100 to 200 leaves from each guayule variety to determine their most common leaf shapes.

Trichome Study

Leaf samples were stained with Delafield's haematoxylin or Kores pad ink, then washed gently and thoroughly in tap water. Trichomes present on the adaxial and abaxial regions were scraped with a fine-edged razor blade and mounted on separate slides with 50% glycerin jelly. Their dimensions were then measured by projecting their images on a vesopan attached to a Zeiss microscope. An average value for each parameter was calculated from 100 measurements. Likewise, mean lengths, diameters, and leaf areas were recorded (using sheets of graph paper) for 100 leaves from each variety. The dried material was directly mounted on metallic stubs and a 400- Å coating of a gold/palladium alloy was applied. The coated samples were observed under an SEM (Philips 505) for our trichome study.

Estimating Rubber Content

We dried leaf samples from each variety and determined their rubber content using the extraction method of Buchanan et al. (1978).

RESULTS AND DISCUSSION

Each variety of guayule had a mixture of leaf types. Their shapes included elliptic, oblanceolate, and ovate, with entire margins or with two, six, or more toothed margins (Table 1). The pinnately dissected leaves had small or large teeth. For all varieties, the highest % frequency of leaf-type occurrence was with oblanceolate to elliptic leaves (Table 1).

All parameters of leaf size were directly proportional to rubber content (Table 2). Therefore, we sug-

Table 1. Micro-morphological studies on leaves of 15 varieties of *P. argentatum*.

Variety	Frequency of leaf shape (%)			Frequency of leaf margin (%)			
	Oblanceolate-elliptic	Oblanceolate	Ovate	Entire	2 - 4 teeth	4 - 6 teeth	6-many teeth
DP	54	21	25	7	45	22	26
10576	33	33	34	12	37	23	28
10576	44	28	22	14	36	23	27
A48118	42	27	33	12	40	22	26
G-39	38	36	26	13	37	23	27
G-39	39	34	27	9	38	24	29
11591	45	33	22	14	37	24	27
11653	47	42	11	13	39	19	29
11600	46	29	25	16	35	23	27
G-97	40	24	26	11	36	23	30
N-593	42	31	27	9	34	26	31
11604	48	39	13	12	38	22	28
N-396	46	33	21	18	36	22	32
11604	41	36	23	14	34	23	29
11488	43	31	26	16	39	20	28

Table 2. Leaf characteristics for 15 varieties of *P. argentatum*.

Variety	Rubber content (%)	Length (cm)			Width (cm)			Length width	Average leaf area (mm^2)
		Min	Max	Average	Min	Max	Average		
DP	0.37	1.3	3.8	2.8	0.4	1.6	1.2	1:2.3	216
10576	0.43	2.3	4.4	3.4	0.6	1.2	1.0	1:3.4	224
10576	0.53	2.7	5.3	4.1	0.5	1.2	1.1	1:3.7	259
A48118	0.62	2.1	4.9	3.9	0.7	1.8	1.1	1:3.5	244
G-39	0.64	1.6	3.5	2.6	0.3	0.9	0.7	1:3.7	266
G-39	0.71	2.2	5.6	3.8	0.2	1.1	1.0	1:3.8	267
11591	0.73	1.2	1.8	3.8	0.3	1.8	1.1	1:3.8	273
11653	0.99	1.6	5.9	4.3	0.4	1.2	1.1	1:3.9	284
11600	1.04	1.4	4.9	3.6	0.3	1.4	0.8	1:4.0	286
G-97	1.13	1.1	5.6	4.8	0.4	1.8	1.2	1:4.0	291
N-593	1.23	1.8	4.4	3.6	0.6	1.3	0.9	1:4.0	286
11604	1.36	1.4	6.4	5.3	3.7	1.6	1.2	1:4.4	294
N-396	1.53	1.2	4.8	4.1	0.6	1.0	0.9	1:4.5	297
11604	1.71	1.2	5.3	4.3	0.4	1.6	0.9	1:4.8	306
11488	1.98	1.7	5.8	3.9	0.2	0.9	0.8	1:4.9	314

gest that leaf dimensions (e.g., leaf area, width/length ratio) can be somewhat reliable predictors of the capacity for rubber production in a particular guayule plant.

The venation pattern of *P. argentatum* noted in the present study is similar to that described by Gilliland et al. (1984) and Ravindranath and Inamdar (1982, 1985). Among the 15 varieties studied here, few differences were seen in the number of secondaries on one side of the primary veins, the size and shape of the areoles, and the number of vein terminations in the areoles. Therefore, venation pattern cannot adequately predict rubber content.

Acentric trichomes occurred more frequently in all

Table 3. Macro-dimensional studies of trichomes from 15 varieties of *P. argentatum*.

Variety	Cap cell frequency (%)		Frequency of acentric cap cells (%) and their length gradients (µm)					
	Centric	Acentric	110 - 150	151 - 200	201 - 250	251 - 300	301 - 350	351 - 400
DP	10	90	6	14	30	26	10	4
10576	12	88	8	4	24	10	34	8
10576	14	86	5	18	36	10	12	5
A48118	10	90	18	8	56	2	6	0
G-39	16	84	6	20	34	18	2	4
G-39	12	88	12	18	36	12	8	2
11591	11	89	8	24	33	14	6	4
11653	15	85	16	14	42	8	3	2
11600	14	18	10	16	36	14	3	3
G-97	8	92	8	24	38	12	2	8
N-593	14	86	4	22	33	8	12	7
11604	17	83	10	21	38	4	8	2
N-396	13	87	18	19	31	11	4	4
11604	14	18	12	18	37	8	3	8
11488	17	83	10	10	38	8	6	3

Table 4. Dimensional studies of trichome from 15 varieties of *P. argentatum*.

Sample no.	Class value of rubber		Variety	Rubber content (%)	Trichome length (µm)	Trichome diameter (µm)	Arm ratio B:P
1		0.30	DP	0.37	221.62	18.60	1:2.36
2	A		10576	0.43	248.48	18.10	1:3.10
3			11644	0.53	184.42	23.62	1:2.10
		0.60					
4		0.61	A48118	0.62	236.16	24.78	1:1.57
5	B		G-39	0.64	227.63	26.83	1:1.65
6			G-4	0.71	180.50	22.54	1:1.46
7		0.90	11591	0.73	194.83	26.67	1:1.49
8		0.91	11653	0.99	227.67	25.07	1:1.78
9	C		11600	1.04	200.56	17.66	1:2.66
10			G-97	1.13	223.50	19.62	1:2.46
		1.20					
11		1.21	N-593	1.23	218.14	23.66	1:2.01
	D						
12				1.36	181.62	21.12	1:1.56
		1.50	11604				
13		1.51	N-396	1.53	200.00	21.14	1:1.67
	E						
14				1.71	205.00	24.98	1:1.57
		1.80	11609				
15	F	1.81	11488	1.98	177.79	22.09	1:1.56
		2.10					

(2) Varieties collected from NBRI, Lucknow. (3) Estimated at IIP, Dehradun
(6) B:P = Blunt arm of trichome : Pointed arm of trichome.

varieties. The maximum number of cap cells in a T-trichome ranged from 110 to 400 μm, with the model class of 201 to 250 μm (Table 3). Again, this micro-character was not significantly correlated with rubber content among our guayule varieties.

The overall ratio of length to diameter for cap cells in T trichomes (average of both adaxial and abaxial leaf surfaces) for all 15 varieties was >150 μm/18 μm. However, among these varieties, no consistent decrease or increase was found in cap cell dimensions (Table 1). Based on these results of varieties growing in India, therefore, we cannot substantiate the conclusions of Mehta et al. (1979), who suggested that cap-cell length decreased with an increase in rubber content.

Regardless of guayule variety, cap cells on both leaf surfaces comprised a mix of arm shapes, both ends of the arms being blunt, or else one being short and blunt with the other having a long, pointed arm with either straight or wavy cell walls all along the pointed arm or curved at the tip. For all varieties, blunt arms occurred less frequently than did pointed arms in the cap cell. Nonetheless, the ratio of blunt arms to pointed arms was never significantly correlated with rubber content (Table 4). This is in contrast to the conclusions of Mehta et al. (1979), who found a positive correlation between arm ratio and rubber content in the native guayule population, with arm ratio decreasing as rubber content increased.

Because anomocytic stomata were observed in all guayule varieties, this character could not be considered in our comparative study.

Inflorescence axes branched one to four times near the tips, and these branches were longer than the main axis. Length of inflorescence-axis branches was not correlated with rubber production in our Indian-grown plants.

Overall, none of the micro- and macro-morphological characteristics evaluated here are useful predictors of the rubber-bearing capacity of guayule plants growing in Indian agroclimatic conditions. Our results contradict those of Mehta et al. (1979), who reported that these characteristics could serve as indicators of the level of rubber production in guayule. Mehta et al. (1979) had reported that these characteristics could be modified by changes in climate, soil properties, or genetic makeup of the plant (Mehta et al., 1979). In the current study, only a limited number of plants (10 to 15 per variety) were available for study. These one-year-old plants had been recently introduced to India through the NBRI, Lucknow. We can only presume that the plants sampled here, possessed the mix of morphological characteristics described by Mehta et al. (1979) for three groups of plants.

ACKNOWLEDGEMENT

We are grateful to Prof. J. J. Shah for his suggestions in reviewing this manuscript. This work was supported by the Wonkwang University in 2000 Project.

Received August 30, 2001; accepted December 26, 2001.

LITERATURE CITED

Buchanan RA, Cull IM, Otey FH, Russell CR (1978) Hydrocarbon-rubber producing crops: Evaluation of US plant species. Econ Bot 32: 131-145

Gillil MG, Vanstaden J, Bruton AG (1984) Studies on translocation system of guayule (*Parthenium argentatum* Gray). Protoplasma 122: 169-177

Healey PL, Mehta IJ, Westerling KE (1986) Leaf trichomes of some *Parthenium* sp. Amer J Bot 73: 1093-1099

Kim IS, Pak JH, Seo BB, Song SD (1998) Ultrastructural aspects of leaves in *Festuca ovina* and *Poa spondylodes* (C-3 Poaceae). J Plant Biol 41: 170-177

Lee KB, Park JB, Lee SC (2000) Morphology and anatomy of mature embryos and seedlings in parasitic angiosperm *Cuscuta japonica*. J Plant Biol 43: 22-27

Mehta IJ, Dhillon SD, Hanson GP (1979) Trichome morphology as an indicator of high rubber bearing guayule (*Parthenium argentatum*) in native population. Amer J Bot 66: 796-804

Ogundpie OT, Olatunji OA (1991) Vegetative anatomy of *Brachiaria obtussiflorao*. Fedd Repert 102: 5-12

Ravindranath K, Inamdar JA (1982) Leaf architectural studies in the Asteraceae-I. Pak J Bot 14: 143-154.

Ravindranath K, Inamdar JA (1985) Leaf architectural studies in the Asteraceae-II. Kor J Bot 28: 57- 67

Waln K, Toukdariam K, Elder N, Hanson GD (1983) Inter specific hybridization, *In* III International Guayule Conference, Guayule Rubber Society, Inc, Pasadence, pp 317-326