LEAF CUTICULAR DYNAMICS IN DISJUNCT POPULATIONS OF *TRIFOLIUM REPENS* L.

G. K. SHARMA and CHRISTIE JONES

Biology Department, The University of Tennessee, Martin, Martin, TN 38238

ABSTRACT—Studies of leaf cuticular features were conducted on three disjunct populations of *Trifolium repens* L. to determine the degree of constancy of cuticular features in this species. Plastic cuticular imprints were prepared according to the method of Williams. Microscopic analysis of the abaxial leaf surface cuticular feature data from the three plant populations revealed that stomatal frequency and trichome length were inconsistent. However, subsidiary cell complex, length and width of the smallest and the largest stoma, and the trichome type were consistent in all three plant populations for this taxon.

Cuticular features have been used extensively in paleobotanical and phylogenetic interpretations. Furthermore, their significance in taxonomic investigations has been documented for many years (Fritsch, 1903; Sharma, 1983; Sharma and Lewis, 1987; Stace, 1965; Stebbins and Khush, 1961; Timmerman, 1927; Watson, 1962). Sinclair and Sharma (1971) suggest that leaf cuticular features, if properly interpreted, are useful taxonomic tools and are extremely important in taxonomic and phylogenetic investigations. Stace (1969) used cuticular features in the taxonomy of Combretaceae. A recent study by Paoletti and Gellini (1993) describes the stomatal density variation in beech and holm oak leaves.

The present study was conducted to explore the significance of leaf cuticular features in the interpretation of taxonomic affinity in disjunct populations of white clover (*Trifolium repens* L.), an herbaceous taxon widely distributed in many parts of the world.

MATERIALS AND METHODS

Five plants of white clover were collected from each population. Population A from Berlin, Germany (52° 30′ N longitude and 3° 24′ E latitude) was growing in a polluted habitat. Population B was representative of the clean habitat of the central Himalayas at 31° 55′ N longitude and 77° 41′ E latitude. Population C was collected from a rural habitat in Martin, Tennessee

TABLE 1. Distribution and abaxial leaf cuticular features of three *Trifolium repens* populations: Population A (Berlin, Germany), Population B (Himalayas, Ladakh), and Population C (Martin, Tennessee).

Trait	Population[1]		
	A	B	C
Stomatal frequency[2]	25.9 ± 4.8	24.7 ± 4.4	41.1 ± 7.0
Stomatal length (μm)			
Largest stoma	25.7 ± 1.7	26.1 ± 1.4	25.2 ± 1.9
Smallest stoma	18.8 ± 1.4	20.0 ± 1.9	19.2 ± 2.1
Stomatal width (μm)			
Largest stoma	15.5 ± 1.3	15.0 ± 1.1	14.1 ± 1.4
Smallest stoma	12.0 ± 1.0	12.7 ± 1.1	12.3 ± 0.9
Trichome length (μm)			
Longest trichome	68.4 ± 15.6	76.5 ± 12.5	69.8 ± 20.0
Shortest trichome	51.6 ± 16.6	67.5 ± 10.7	60.0 ± 16.3
Trichome type	Unicellular	Unicellular	Unicellular
Subsidiary cell complex (cells)	3	3	3

[1] Values are mean ± *SD*, $n = 20$ measurements.

[2] Mean stomatal frequency = Stomata of the leaf surface observed through a 40× objective and 10× oculars (field area = 0.152 mm^2).

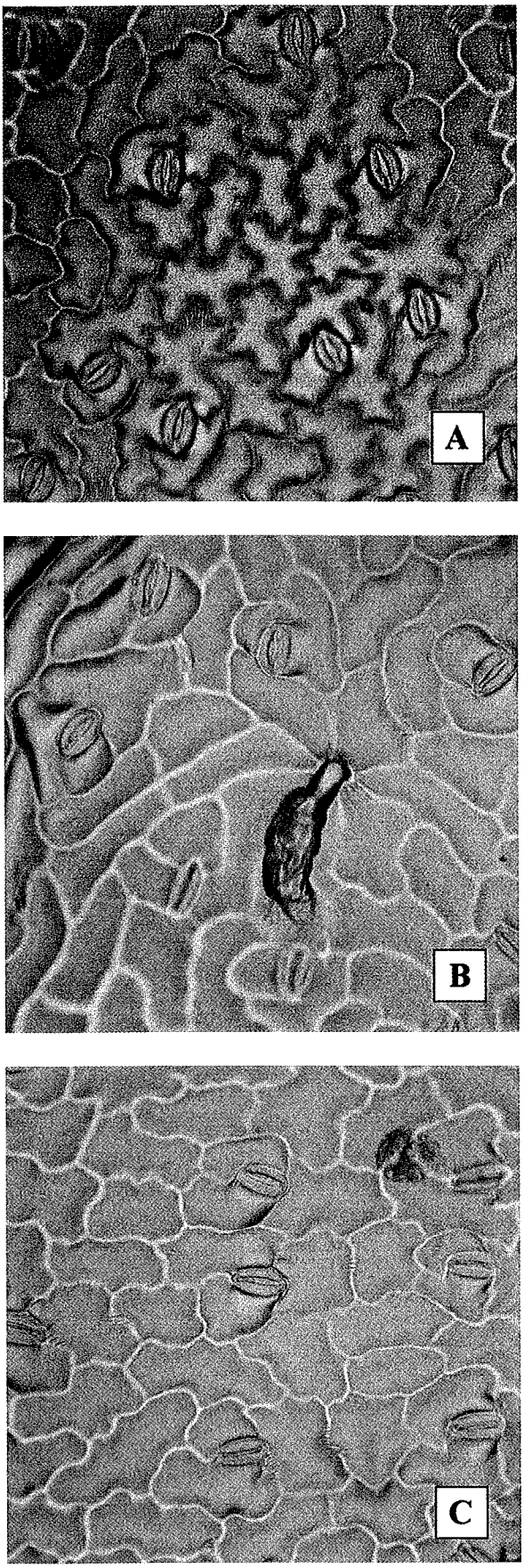

FIG. 1. Abaxial leaf surface of *Trifolium repens* L. Panel A is stomatal size variation, panel B is simple unicellular trichome, and panel C is the subsidiary cell complex. All photographs are the same magnification (267×).

(36° 21 N′ longitude and 88° 51′ W latitude). The habitats of the three populations were characterized by diverse macroclimatic and environmental conditions. Five leaves were selected from the five plants from each population and the central leaflet was used for making leaf cuticular slides. The leaflets were washed with mild detergent and distilled water, air dried, and a few drops of Duco-cement were applied to the abaxial surfaces forming a thin layer of film over the leaf surfaces. Upon drying, the films showing the leaf cuticular impressions were removed (Williams, 1973). A small portion from the central area of the leaflets was used to make study slides. Leaf cuticular data such as stomatal frequency and size, trichome length and type, and subsidiary cell complex were recorded by selecting 20 fields (n = 20) at random from each microscope slide. The microscopic analysis was done using a 40x objective and 10× oculars (field area = 0.152 mm²). Statistical analysis (mean ± *SD*) of the data is shown in Table 1. Photomicrographs of the cuticular imprints were taken.

RESULTS

The data collected are summarized in Table 1. Stomatal frequency values from the abaxial leaf surfaces of the three populations show that the plant population from Martin, Tennessee had the highest value (41.1 per unit area; 0.152 mm²), while the plant population from the Himalayas had the lowest value (24.7). Trichome length for the longest trichome varied from 68.4 μm in population A to 76.5 μm in population B. The shortest trichome had a length of 51.6 μm in population A, 60.0 μm in population C, and 67.5 μm in population B. Trichomes, though rare, were unicellular in all three populations (Fig. 1B). Stomatal width values of 14.1μm, 15.0 μm, and 15.5 μm for populations C, B, and A respectively for the largest stoma showed little variation for this trait. Similarly, stomatal width values for the smallest stoma ranged from 12.0 μm to 12.7 μm also showing little variation in this trait among the three plant populations. Stomatal length of the largest stoma in all the three populations had a range of 25.2 μm to 26.1 μm. Length of the smallest stoma ranged from 18.8 μm in population A to 19.2 μm in population C to 20.0 μm in population B (Fig 1A). Again, both the length of the largest and the smallest stoma represent fairly consistent values. The three small cells remaining beside the stoma may be interpreted as a subsidiary cell complex (Fig. 1C). This pattern was found to be consistent in all the three plant populations.

DISCUSSION

Since the purpose of this investigation was to evaluate the leaf cuticular characteristics for taxonomic use, it seems logical to suggest from the above data that some leaf cuticular characteristics of *T. repens* can be used for taxonomic purposes. It is obvious from the above discussion of the data that stomatal frequency and trichome length are unreliable traits for taxonomic purposes for this taxon. However, unicellular trichome, subsidiary cell complex, and the length and width of the largest and smallest stoma remained constant and hence can be regarded as reliable cuticular characteristics for the taxonomy of this taxon.

Additional studies are underway to determine whether leaf cuticular dynamics can be used for interpreting taxonomic affinities in other taxa.

LITERATURE CITED

FRITSCH, F. B. 1903. The use of anatomical characters for systematic purposes. New Phytol., 2:177—178.

PAOLETTI, E., AND R. GELLINI. 1993. Stomatal density variation in beech and holm oak leaves collected over the last 200 years. Acta Oecologica, 14:173–178.

SHARMA, G. K. 1983. Cuticular differentiation in *Lamium amplexicaule* L. and *Lamium purpureum* L. J. Tenn. Acad. Sci., 58: 43–45.

SHARMA, G. K., AND P. B. LEWIS. 1987. Cuticular differentiation in *Kalanchoe fedschenkoi* and *Kalanchoe daigremontiana* (Crassulaceae). J. Tenn. Acad. Sci., 62:97–98.

SINCLAIR, C. B., AND G. K. SHARMA. 1971. Epidermal and cuticular studies in leaves. J. Tenn. Acad. Sci., 46:2–11.

STACE, C. A. 1965. Cuticular studies as an aid to plant taxonomy. Bull. British Mus. Nat. Hist. Series E, 3:1–78.

———. 1969. The significance of the leaf epidermis in the taxonomy of the Combretaceae III. The genus *Combretum* in America. Brittonia, 21:130–143.

STEBBINS, G. L., AND G. S. KHUSH. 1961. Variation in the organization of stomatal complex in the leaf epidermis of monocotyledons and its bearing on their phylogeny. Amer. Bot., 48:51–60.

TIMMERMAN, T. A. 1927. Stomatal numbers: their value for distinguishing species. Pharmacol. J. Pharmicist, 118:241–243.

WATSON, L. 1962. The taxonomic significance of stomatal distribution and morphology in Epacridaceae. New Phytol., 61: 36–40.

WILLIAMS, J. A. 1973. A considerably improved method for preparing plastic epidermal imprints. Bot. Gaz., 134:87–91.