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CYTOCHEMICAL AND LEAF EPIDERMAL STUDIES IN THE GENUS *PIPER*

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ABSTRACT

Chromatographic separation of the methanolic leaf extract showed the presence of a common spot in all the 10 species of *Piper* and hence appears to be a chemotaxonomic marker for the genus. Besides this generic marker, species specific spots were found in *P. nigrum*, *P. betle* and *P. zeylanicum*. Further the phenolic spot designated No. 5 was found in all the diploids and No. 2 in the tetraploid species of the genus. The leaf epidermal characteristics of *Piper* species showed that a combination of two or more characters could be used to identify species such as *P. nigrum*, *P. longum*, *P. betle*, *P. chuvya* and *P. trineuron*. A high positive correlation was observed between chromosome number (ploidy level) and guard cell length, but the correlation was negative between chromosome number and stomatal index values.

INTRODUCTION

The genus *Piper* includes species varying from 500 to 1200 (Hooker, 1973; Trimen, 1895; Ridley, 1924), of which *Piper nigrum*, *P. longum*, *P. betle*, *P. sylvestre*, *P. attenuatum*, *P. trineuron*, *P. zeylanicum*, *P. thwaitseii*, *P. argyrophyllum* and *P. chuvya* are reported to occur in Sri Lanka (Samuel, 1980).

Two dimensional paper chromatography has been used for analysis of phenolic compounds in taxonomy of *Baptisia* (Alston and Turner, 1959; 1963a) *Potentilla* and *Prunus* (Bate-Smith, 1961) and *Citrus* (Das, Rahdha and Prakash, 1977). Flavanoid pigments are considered to be very useful class of secondary constituents as chemotaxonomic markers. Some phenolic spots could be specific to species and such species specific spots have been reported in many plants namely *Baptisia* (Alston and Turner, 1963b) and *Potentilla* (Bate-Smith, 1961).

Prat (1936) collected evidences to show that the epidermal characters of the leaf are of taxonomic importance. Interspecific variation in *Hevea* has been observed in abaxial foliar characteristics such as outline of epidermal walls,

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appearance of epidermal cells, stomatal guard cells, veinlets and presence or absence of epidermal hairs (Senanayake, 1969). Intra-specific variation in abaxial leaf epidermal characteristics are reported in *Areca catechu* (Bavappa, 1966) and *H. brasiliensis* (Senanayake, 1970).

High correlation between leaf epidermal characters and chromosome number has been reported in many species (Stebbins, 1971). In wheat, the diploids had the highest stomatal frequency which decreased with increasing ploidy level (Rajendra, Mujeeb and Bates, 1978). In *Briza* the diploid, Eurasian species have different set of flavanoids in the leaves from the tetraploid South American species (Christine, Williams and Murray, 1972). Variation in the phenolic constituents of the diploid and tetraploid and tetraploid species have been also reported in the genus *Lotus* (Harney and Grant, 1964). The present investigation was undertaken to study variation existing in abaxial leaf epidermal characters, chromosome number and phenolic constituents of leaves among 10 species of *Piper* from Sri Lanka.

MATERIALS AND METHODS

Materials for the study of leaf epidermal characters, of all the 24 accessions belonging to 10 species (Table I) were collected from pepper vines grown under uniform conditions at Matale Research Station, Sri Lanka. Epidermal peels were prepared and necessary measurements were made as reported by Samuel, Bavappa and Balasubramanium (1983).

For preparation of leaf extracts, 500 gm of mature green leaves in each of the accessions were collected, cut into small pieces and air dried. One hundred gm of air dried leaf samples was first extracted with petroleum ether using a Soxhlet apparatus. The remaining was subsequently extracted with chloroform and methanol successively. The extracts obtained with different solvents were evaporated using a rotary evaporation and dried overnight under vacuum. Comparable concentration of extract of accessions under test was prepared by dissolving 1 gm of the dried extract in 15 ml of methanol and 25 μl of this extract was used for chromatographic separation of the phenolic constituents. Two dimensional paper chromatography was carried out using Whatman No. 1 paper. Twenty five micro litre of the extract was applied near the left hand corner of the 30 × 30 cm Whatmann No. 1 paper and developed in the first direction using water saturated solution of butan 2-ol. After the solution had run to the edge of the paper it was taken out of the tank, dried and developed in the second direction using 2 per cent acetic acid. The chromatograms were sprayed with sulphanilic acid spray and the spots marked.

A linear regression equation was fitted for the chromosome number (X) and various abaxial leaf epidermal characteristics (y). Pure error sum of squares and lack of fit sum of squares were estimated to investigate appropriateness of the fitted model (Draper and Smith, 1966). Correlation coefficient of the two variables were computed.

Table I. *Leaf epidermal characteristics, chromosome number and phenolic spots in 10 species of* Piper

Species	Accession number	Epidermal Cell length (μm)	Guard Cell length (μm)	Stomatal Index	Chromosome numbers 2n	Phenolic Spots
P. nigrum	65, 66, 67 (cultivated)	20.26	24.26	3.6–3.9	52	2, 7, 11, 17
	39, (wild)	26.00	28.14	3.9	52	2, 7, 11
	25, (wild)	32.85	30.61	4.9	52(78)	2, 7, 11
	16, (wild)	30.83	30.16	4.4	65	2, 7, 11
P. zeylanicum	34	26.55	34.64	3.8	39	4, 7, 9
P. thwaitseii	9	31.68	32.47	3.5	39	7
	15	28.30	29.34	4 3	65	3, 7
P. trineuron	57	41.89	27.93	5.8	26	3, 5, 7
B. betle	6	45.89	28.12	10.3	52	2, 4, 7, 10
P. chuvya	24	35.59	33.31	6.2	52	2, 4, 7, 11
P. longum	3	44.06	27.64	9.1	26	4, 5, 7, 11
P. argyrophyllum	4	46.89	27.04	6.2	26	5, 7, 8
	21	45.62	26.71	6.7	26	1, 5, 7
	26	47.07	25.41	7.0	26	7
	54	46.19	24.20	6.0	39	5, 7, 8, 14
P. sylvestre	7	42.88	28.76	7.6	39	5, 7, 14
	13	32.09	22.11	6.7	26	3, 5, 7, 14
	29	44.55	28.64	8.2	39(26)	3, 5, 7
	42	44.72	25.99	7.2	26	5, 7, 8
	58	44.28	26.64	6.8	26(39)	5, 7
P. attenuatum	1	28.03	27.64	4.1	39	3, 5, 7
	32	32.23	26.54	4.7	26	3, 7, 8

Somatic chromosome numbers were determined from root tip squash technique described by Samuel and Bavappa (1981).

RESULTS AND DISCUSSION

Certain features of the leaf epidermis has been shown to be under precise genetical control and these have been used widely, in taxonomic studies (Brandham and Cutler, 1978). Investigations carried out on abaxial leaf epidermal characteristics such as shape of epidermal cell, appearance of anticlinal cell wall, guard cell length, stomatal index, presence and absence of trichomes and cell inclusions showed high variability. The epidermal cells of *P. longum*, *P. betle* and *P. chuvya* are rectangular with wavy anticlinal walls in contrast to other species where the cells are more polygonal with some what straight walls (Figs. 1 & 2). The stomatal index of two of these three species are distinct in that *P. betle* and *P. chuvya* have an index of 10.3 and 6.2 respectively. The length of epidermal cell in *P. chuvya* is less compared to *P. longum* and *P. betle* (Table I). Unicellular trichomes are present in all the three species but mucilage cavities are observed only in *P. chuvya.*

In the case of *P. nigrum*, *P. zeylanicum*, *P. thwaitseii* and *P. trineuron*, where the epidermal cell shape and stomatal index show similarities, absence of trichomes in *P. nigrum* helps to distinguish it from the rest. Mucilage cavities are present in *P. nigrum* and *P. trineuron* and this helps to distinguish *P. trineuron* from *P. zeylanicum* and *P. thwaitseii.* However the leaf epidermal characteristics of *P. zeylanicum* and *P. thwaitseii* are more or less similar. The epidermal cells of *P. sylvestre*, *P. betle*, *P. longum* and *P. argyrophyllum* are much longer (Table I) than that of *P. nigrum*, *P. trineuron*, *P. zeylanicum*, *P. thwaitseii* and *P. attenuatum.* Though the morphological features of *P. nigrum* and *P. attenuatum* are quite distinict, there are strong resemblances in their leaf epidermal characteristics. The results of the present study show that in genus *Piper* a combination of two or more epidermal characters could be used in identifying species.

It is evident from Table I that generally the stomatal indices of the diploid species (2n=26) are higher than that of the tetraploid species, though *P. betle* and *P. chuvya* do not confirm to this. Similarly the guard cell length of the diploid species are small (24–26 μm,) while that of the tetraploid species ranged from 28–33μm, except in the case of the cultivated varieties of *P. nigrum.* The linear regression analysis for two variables (chromosome number and epidermal characters) show a positive correlation between ploidy level and stomatal index.

The results of the phenolic separation of the methanol leaf extract (Fig. 3) showed that spot designated No.7 is present in all the 10 species of *Piper* investigated so far in relatively large quantities compared to other phenolics (Table I). Hence it could be used as a good chemotaxonomic marker for the genus. Spot No. 2 which turned orange with sulphanilic acid was found in *P. nigrum*, *P. betle* and *P. chuvya.* However, the relative amount of the

Fig. 1. *P. LONGUM:* RECTANGULAR CELLS WITH WAVY ANTICLINAL WALLS

Fig. 2. *P. NIGRUM:* POLYGONAL CELLS WITH STRAIGHT WALLS

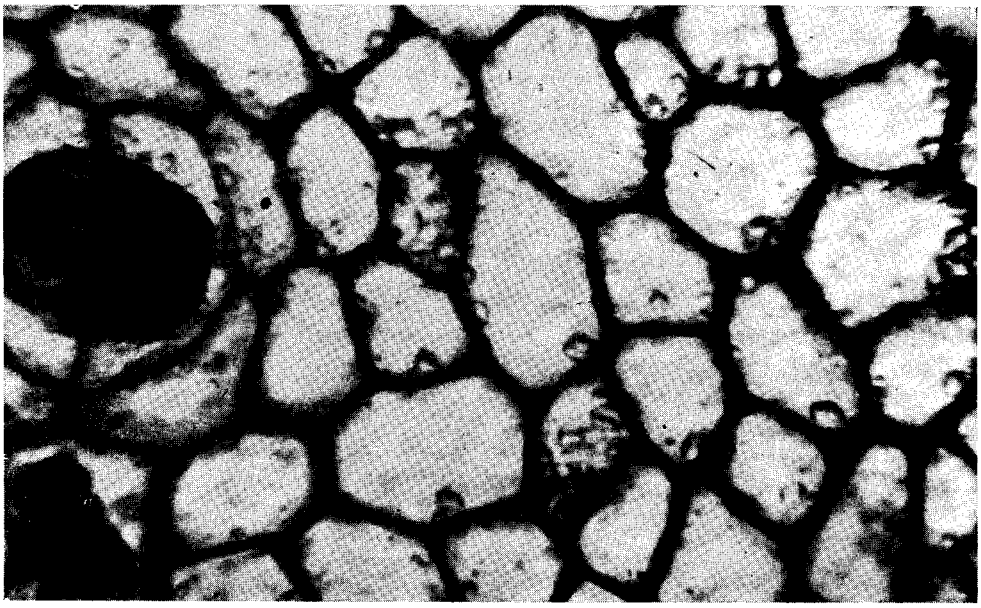
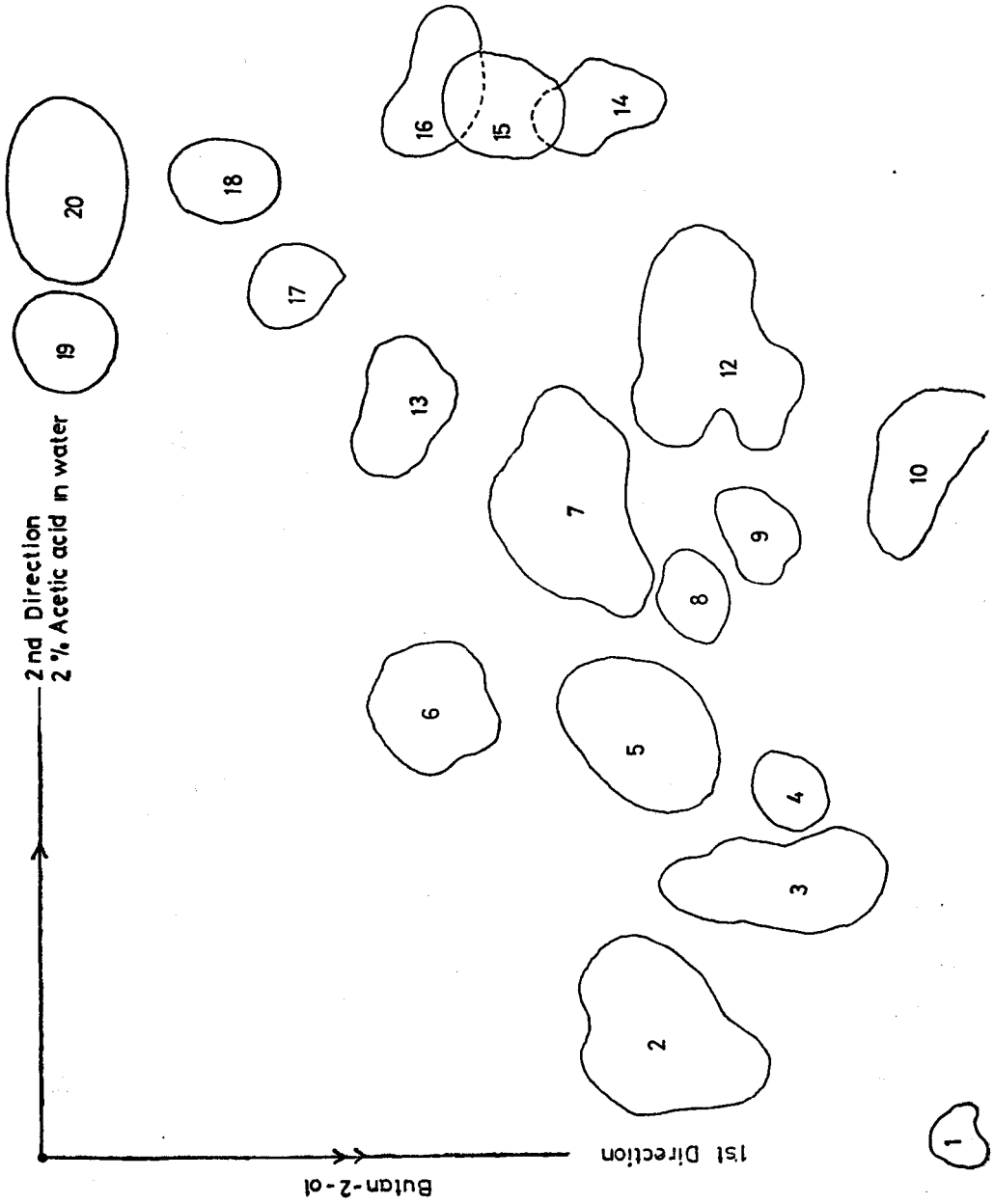


Fig. 3. POSITION OF PHENOLIC SPOTS ON THE CHROMATOGRAM



phenolic compound and its colour intensity was high in *P. nigrum* compared to others as such it could be used in the identification of this species. Spot No.9 is specific to *P. zeylanicum* and No. 10 to *P. betle.* Such species specific spots have been reported earlier for other plant species (Alston and Turner, 1963 b).

Spot No. 11 which turned pink with sulphanilic acid is found to be commonly distributed in the cultivated species such as *P. longum*, *P. chuvya* and *P. nigrum* (Table I). However spot No 17 was only in the cultivated varieties of *P. nigrum* (Panniyur I, Kuching and Local). These two spots are useful in distinguishing the cultivated species of *Piper* and the different cultivars of *P. nigrum.* Such variations in the flavanoid composition of the cultivated species and varieties have been reported in barley (Frost et al., 1978).

On the 20 phenolic spots observed in the methanolic separation, spot No.5 was found in all the diploid species and spot No.2 in the tetraploid species of the genus (Table I). Such relationships between ploidy level and phenolic constituents are reported in *Baptisia* (Alston and Turner 1959, 1963 b) and *Citrus* (Das et al., 1977).

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