

## FOLIAR SURFACE CHARACTERS OF *NICOTIANA TABACUM* L. CULTIVARS IN RELATION TO FORENSIC SCIENCE

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An attempt has been made to identify the cultivars of tobacco used in the preparation of cigarettes based on the epidermal characters which in turn will be useful in identification of the cigarette stubs available at crime scene.

**Key Words :** Crime scene, epidermis, tobacco.

Among various physical evidences like blood, hairs, firearms, explosives, sometimes cigarette and beedi stubs encountered at the crime scene may also form an excellent physical evidence giving a clue in identifying the criminal(s) or the person(s) visited the crime scene. The epidermal characters of the tobacco cultivars are genetically controlled and are stable for a given cultivar (Bentley & Wolf, 1945; Wolf, 1946). Therefore, when the brand marks are not present on the cigarette or beedi stubs discarded at the crime scene, the identification of the brand can be determined based on the epidermal characters of the tobacco available in the butts (Prabhakar *et al.*, 1988; Bhatia *et al.*; 1988 Rao *et al.* 1992a, b,c). Presently four cultivars of *Nicotiana tabacum* used in the preparation of cigarettes is taken up to study the variation in the epidermal characters. Mature leaves of four tobacco cultivars (Table 1) used in the preparation of cigarettes were collected from the farm of central tobacco research institute, Katheru (East Godavari district, Andhra Pradesh, India) and fixed in Carnoy's fixative (Johanson 1940). Slides of epidermal peels were prepared following Prabhakar *et al.* (1988). The frequency of epidermal cells, stomata, size of stomata, trichomes and percentage frequency of trichomes from five leaves belonging to five different plants of each cultivar and 10 readings from each leaf from ten different regions were studied (Table 1-3).

**Epidermal cell complex :** The shape of the epidermal cell in *N. tabacum* are described to be anisodiametric and the anticlinal walls as sinuate (Ahmad 1964). Earlier in beedi tobacco cultivars of *N. tabacum* and *N. rustica* and tobacco obtained from different brands of beedies, the shape of the epidermal

cells were observed to be mostly polygonal anisodiametric, few polygonal linear and the anticlinal walls straight to curved in some, while in others wavy or sinuate (Prabhakar *et al.*, 1988; Bhatia *et al.*, 1988; Rao *et al.*, 1992a,b,c). Similarly in the presently studied cigarette tobacco cultivars of *N. tabacum* the shape of the epidermal cells are mostly polygonal anisodiametric on both surfaces, with few polygonal linear cells (Figs. 1A, B, E, F; 2A, B, E, F). Rarely polygonal isodiametric cells are recorded on both surfaces of the cultivars (*Cash. Haronic, Italian golden burley*) and abaxial of *Flue cured variety special* (Figs. 1A, B; 2A, B, E, F; 1F). Anticlinal walls are mostly sinuate (U-shaped) on both surfaces of *Cash, Haronic* and on abaxial of *Flue cured variety special, Italian golden burley* (Figs. 1A, B; 2A, B; 1F; 2F). The anticlinal walls are mostly wavy on adaxial of *Flue cured variety special* (Fig. 1E), while on adaxial of *Italian golden burley* the anticlinal walls are mostly curved (Fig. 2E) Surface in all is smooth and cytoplasmic contents are scanty. The epidermal cells are distributed all over except on veins and are variously oriented and irregularly arranged. The frequency of epidermal cells vary from 22,419 per cm<sup>2</sup> as on adaxial of *Italian golden burley* to 34,320 per cm<sup>2</sup> as on abaxial of cultivar *Flue cured variety special* (Table 1). Costal cells are mostly polygonal-linear, rarely polygonal-anisodiametric, rhomboidal-linear, nonlinear, trapezoidal-linear and trapezoidal-linear. anticlinal walls are straight to curved and thin. Surface is smooth. Cytoplasmic contents are scanty. They are distributed on all veins, parallelly oriented and irregularly arranged (Figs. 1C, D, G, H; 2C, D, G, H).



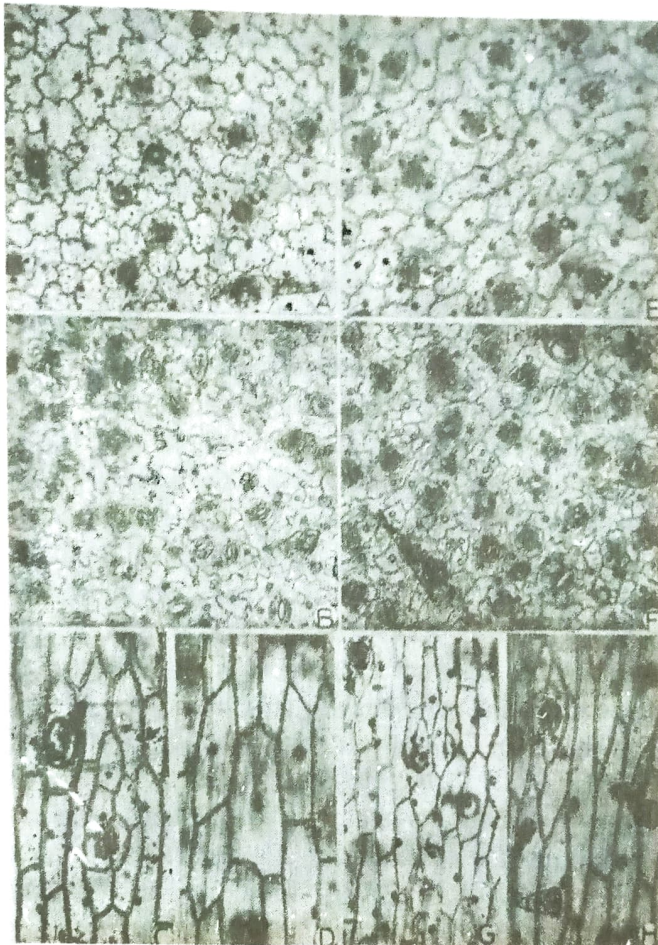


Figure 1. A-D *Nicotiana tabacum* L. cultivar *Cash*: A & B, Adaxial and abaxial epidermis (intercostal region) respectively; C & D, Adaxial and abaxial costal cells (X 240). E-H *Nicotiana tabacum* L. cultivar *Flue cured variety special*: E & F, Adaxial and abaxial epidermis (intercostal region) respectively; G & H, Adaxial and abaxial costal cells (X 240).

**Stomatal complex:** Stomata are described to be anomocytic (Ahmad 1964, 1975; Metcalfe & Chalk 1950) and anisocytic (Ahmad 1964). In addition to the above, tetracytic, cyclocytic, staurocytic and paracytic are reported by Prabahakar *et al.* (1988), Bhatia *et al.* (1988), Rao *et al.* (1992a). In the presently investigated tobacco cultivars used in preparation of cigarettes only three types of stomata are recorded viz., anomocytic (Fig. 3K), anisocytic (Fig. 3L) and tetracytic (Fig. 3J). However the anisocytic stomata is dominant. The frequency of stomata vary from 4,444 per cm<sup>2</sup> as on adaxial of *Flue cured variety special* to 10,469 per cm<sup>2</sup>

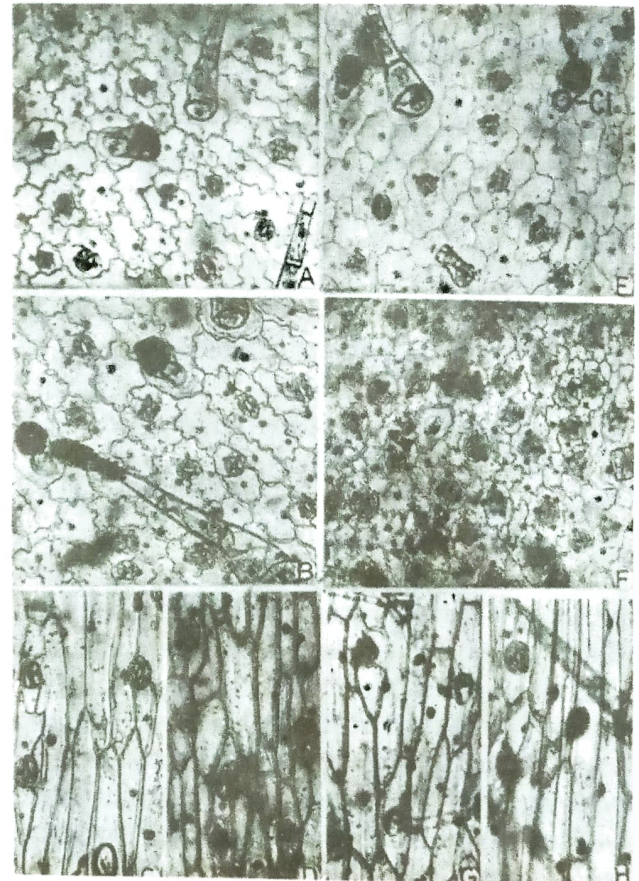


Figure 2. A-D *Nicotiana tabacum* L. cultivar *Haronic*: A & B, Adaxial and abaxial epidermis (intercostal region) respectively; C & D, Adaxial and abaxial costal cells (X 240). E-H *Nicotiana tabacum* L. cultivar *Italian golden burley*: E & F, Adaxial and abaxial epidermis (intercostal region) respectively; G & H, Adaxial and abaxial costal cells (X 240).

as on abaxial of *Italian golden burley* (Table 1). Pair of guard cells are elliptic and rarely circular in *Cash*, *Haronic*, and *Italian golden burley*. Abaxially the subsidiaries are of abutting type and adaxially they are of free types (Ramayya & Rajagopal, 1980). Distribution of stomata are all over the lamina including on primary veins. They are variously oriented and irregularly arranged throughout but parallelly oriented on veins (Figs. 1 & 2).

**Trichome complex:** Glandular trichomes with spherical or ellipsoidal head and branched multicellu-



Table 1: Mean frequency of epidermal cells, stomata, trichomes (per cm<sup>2</sup>) stomatal index, stomatal length and width in (µm) in different cigarette tobacco cultivars of *Nicotiana tabacum*.

Name of the cultivar	Epidermal cell		Stomata			Trichome frequency	
	frequency ad/ab	frequency ad/ab	Index ad/ab	length ad/ab	width ad/ab	nonglandular ad/ab	glandular ad/ab
1. Cash	33629/34172	6617/9481	16/22	37/38	26/28	420/300	715/1130
2. Flue cured variety special	23950/34320	4444/10419	16/23	41/43	29/31	260/260	777/1270
3. Haronic	23407/26074	6123/9086	21/26	38/38	32/29	60/115	1470/1514
4. Italian golden burley	22419/28444	5925/10469	21/27	38/41	26/26	120/255	1361/1325

Ad=Adaxial; Ab = Abaxial; glandular = (uniseriate capitate, clavate, ovalis capitate and ramulose conical capitate hairs); L = Length; Non glandular = (uniseriate conical hairs); W = Width.

Table 2: Mean percentage frequency of uniseriate trichome types on leaf lamina of different cigarette tobacco cultivars of *Nicotiana tabacum*

S. No.	Name of the trichomes	1 Ad/Ab	2 Ad/Ab	3 Ad/Ab	4 Ad/Ab
1.	Conical	37/21	25/17	4/7	8/16
2.	Capitate	13/30	17/16	22/29	16/26
3.	Clavate	21/4	30/14	8/22	38/19
4.	Ovalis Capitate	25/45	26/53	65/42	34/39
5.	Ramulose Conical capitate	4/-	2/-	1/-	4/-

1. Cash; 2. Flue cured variety special; 3 Haronic; 4. Italian golden burley; Ad. adaxial Ab. abaxial; . absent.

lar (Bentley & Wolf, 1945; Tanaka, 1955; Metcalfe & Chalk, 1950) were recorded in *Nicotiana*. Earlier investigation (Prabhakar *et al.* 1988; Bhatia *et al.* 1988; Rao *et al.* 1992a, b,c) on beedi tobacco cultivars of *N. tabacum* and *N. rustica* and tobacco obtained from different brands of beedies, six types of uniseriate trichomes were recorded viz., uniseriate conical hair, uniseriate capitate hair, uniseriate clavate hair, uniseriate ovalis capitate hair, uniseriate ramulose conical capitate hair and uniseriate ramulose capitate hair were recorded. Presently all the above types except ramulose capitate hairs are observed (Fig. 3A-I). However uniseriate ramulose conical capitate hair is rare and restricted only adaxial surfaces of leaf (Table 2).

Information regarding the size and the percentage frequency of the trichome types has not been given much importance (Metcalfe & Chalk 1950; Goodspeed 1954; Tanaka 1955; Ahmad 1964, 1975; Roberts *et al.*, 1981; Burk *et al.*, 1982) for it is generally regarded that these characters within a species or on a given organ varies. However it is presently observed that though

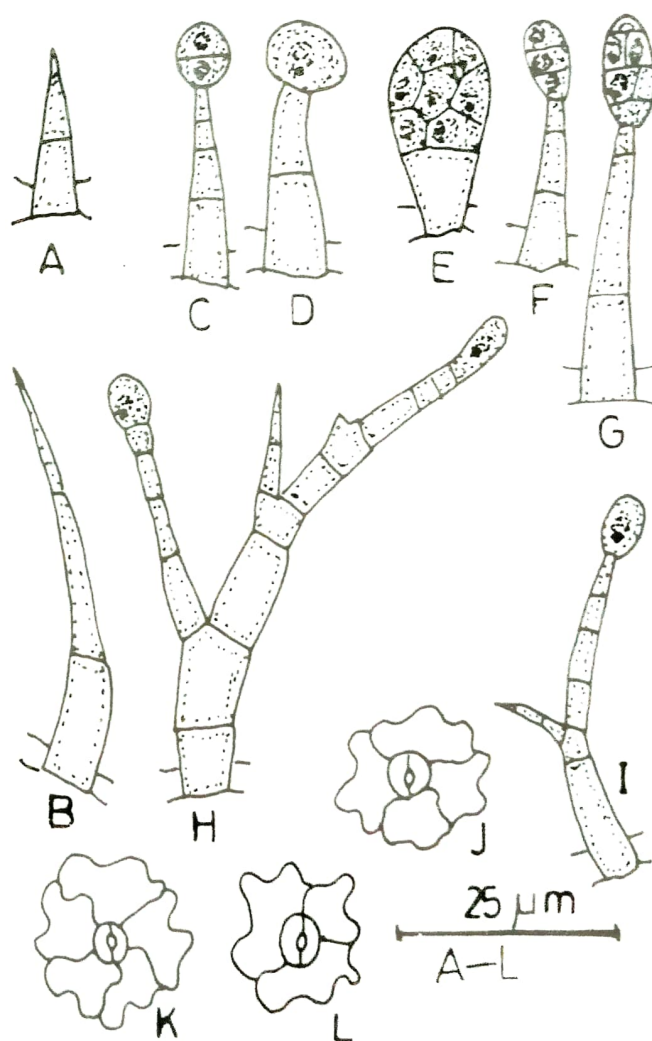


Figure 3. Types of hairs and stomata. A, B, Uniseriate conical hairs; C, D, Uniseriate capitate hairs; E, Uniseriate clavate hair; F, G, Uniseriate ovalis capitate hairs; H, I, Uniseriate ramulose conical capitate hairs; J, Tetracytic stomata; K, Anomocytic stomata; L, Anisocytic stomata.

Table 3. Length (in  $\mu\text{m}$ ) of trichome types on leaf lamina of different cigarette tobacco cultivars of *Nicotiana tabacum*

Name of the trichomes	1		2		3		4		5	
	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab	Ad	Ab
Name of the Cultivar	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max	Min/Max
1. Cash	168/224	152/192	160/440	248/336	80/112	88/96	224/1208	256/544	240/760	-
2. Flue cured variety special	72/272	64/128	272/336	176/304	64/88	72/80	256/440	304/536	264/1240	-
3. Haronic	128/192	144/224	144/256	184/280	80/88	72/88	248/760	240/800	360/1280	-
4. Italian golden burley	80/216	88/184	256/432	152/272	72/80	72/88	416/1480	272/624	544/840	-

1. Conical 2. Capitate 3. Clavate 4. Ovalis capitate 5. Ramulose conical capitate; Ad = Adaxial; Ab = Abaxial; Min = Minimum; Max = Maximum; - . absent.

they vary in the same cultivars a comparative study indicated that the variation is within a certain limit in different cultivars. For example uniseriate conical hair varies from 64 to 128  $\mu\text{m}$  on abaxial of *Flue cured variety special* and 144 to 224  $\mu\text{m}$  on abaxial of *Haronic* (Table 3). Similarly the percentage frequency of conical hair is four per cent on adaxial of *Haronic* and 37 per cent on adaxial of *Cash* (Table 2).

The smokers are usually habituated to a particular brand of cigarette or beedies, because each brand has its own characteristic aroma which depends upon the type of hair present on the tobacco leaf. Aroma of the tobacco is usually attributed to the exudate of glandular hairs (Bruchner 1936; Wolf & Jones 1944; Bentley & Wolf 1945; Wolf 1946) and quantity of the exudate depends on the frequency of these glandular trichomes. This indirectly indicates that the frequency of hairs can be used as a good character in differentiation of genuine or counterfeit cigarettes or beedies and also in identification of cigarettes and beedies discarded at the crime scene (Prabhakar *et al.* 1988; Bhatia *et al.* 1988; Rao *et al.* 1992a, b, c).

Earlier studies (Prabhakar *et al.* 1988; Bhatia *et al.* 1988; Rao *et al.* 1992a,b,c) and present observations indicated that the epidermal characters like, shape, anticlinal walls, type of stomata, size and percentage frequency of trichomes are significantly variable from one cultivar to the other, which can be of use in forensic investigation. In order to make easy for the identification of tobacco used in the preparation of cigarettes, a key based on only leaf epidermal characters is provided below:

Key for the identification of the cigarette tobacco cultivars.

1. Adaxially epidermal cells with sinuate anticlinal walls.
2. Frequency of glandular hairs are 1470/cm<sup>2</sup> on adaxial surface
3. Frequency of glandular hairs are 715/cm<sup>2</sup> and the size of the ovalis capitate hair upto 1480  $\mu\text{m}$  long
3. Frequency of epidermal cells are 34,320/cm<sup>2</sup> and the size of the ovalis-capitate hair is upto 536  $\mu\text{m}$  long flue cured variety special

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