ANATOMICAL CHARACTERS OF FEW INFRASPECIFIC VARIANTS OF *AQUILARIA MALACCENSIS* LAMK.

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ABSTRACT

Anatomical characters of the stems of three infraspecific variants of *Aquilaria malaccensis* Lamk. have been evaluated from a taxonomic point of view. The results revealed that the variants could be differentiated from one another with the help of their colour of woods, pore abundance, percentage of solitary pores, number of pores in radial pore chains, ray types, nature of fibre walls and fibre lengths. The studied characters are described using symbolic key. A key to the taxa based on these characters has also been provided.

INTRODUCTION

Aquilaria malaccensis Lamk. (syn. *Aquilaria agallocha* Roxb.) (Thymelaeaceae) commonly known as Agar wood, *Aloe* wood or Eagle wood is an evergreen medium to large sized tree found in eastern Himalaya from Bhutan to North-east India up to an altitude of 1200 m and in Myanmar and Malaysia. *A. malaccensis* is one of the most important species of commerce and valued for its fragrant resinous darker coloured wood known in trade as agar which is used as incense (Anon, 1948) and also for distilling a kind of oil called agar attar, a vulnerable perfume retainer much priced by European perfumer for mixing their best grade scents (Baruah *et al.*, 1982). Agar wood and its products are also described as aphrodisiac, alterative, anodyne, antidiarrhoeal, antiasthmatic, astringent, carminative, cordial, diuretic, laxative, stomachache and tonic (Dey, 1980; Hussein *et al.*, 1992) and enter in to the preparation of several pharmaceutical and cosmetic products (Kirtikar and Basu, 1991).

In a recent work on foliar micromorphology and the occurrence of free amino acids in the leaves of *Aquilaria* species native to Northeast India, we recognized three infraspecific variants of *A. malaccensis* (Saikia *et al.*, 2000). The work also indicated the necessity of acquiring further evidence from other taxonomic characters. Thus, as a part of an ongoing research activity on the species, an investigation based on wood characters of three variants have been conducted from a taxonomic point of view.

MATERIAL AND METHODS

The study materials consisted of the apical stem portions of the three variants (Table I). The specimens were preserved in a mixture of 70% ethanol, acetic acid and formaldehyde at a ratio of 90 : 5 : 5 v/v separately. The stem portions were softened by boiling water for few minutes as described by DiSapio *et al.* (1997). The transverse and longitudinal sections of the processed stems were made by free hand using sharp blade and stained with safranin and fast green (DiSapio *et al.*, 1997). The sections were then mounted in canada balsam and observed under the compound microscope (Olympus OIC KH model), and Camera Lucida sketches were drawn. Ten random measurements were used for calculating a mean value of each element and for the statistical analysis.

The wood characters of each variant of the species were also studied using SEM. The preserved stem samples processed in the form of sections were mounted on copper stabs using double side sticking tape coated by gold in a Sputter Coater (EMS 550) and examined at 15 KV using JEOL 100 CX ASID 4D Scanning Electron Microscope.

The anatomical characters of the three variants of the species were then used for calculating their similarity percentages. This was estimated using the following formula.

$$S = \frac{NS}{NS + ND} \times 100$$

Where S stands for similarity value expressed in percentage; NS stands for positive indication i.e. presence (+) shared by any two; ND stands for number of positive indication in one taxa and negative indication (-) in the other. Negative indication in both taxa were ignored.

RESULTS

Presentation of result :

The result were presented using a symbolic key of characters as presented by Dutta and Samanta (1983), for wood anatomy of some Indo-Malayan Maliacese.

Symbolic key for microscopic characters :

Wood:

r+ Ring-porous, ro Diffuse-porous.

g+ growth-rings distinct, go growth-rings indistinct.

gn number of growth-rings per cm.

Vessels:

A_1 Pore abundance (Dutta and Samanta, 1983) extremely numerous (> 80/ mm²), A_2 very numerous (40   80), A3 numerous (20   40), A_4 moderately numerous (10   20), A_5 moderately few (5   10), A_6 few (2   5), A_7 very few (2).

B Solitary pore percentage.

C_n Radial pore-chain length in pore number. C_{a+} pore mostly attached to rays, C_a-pore mostly away from rays. Cm8 radial pore pair mostly 8-shaped, Cm radial pore pair mostly O-shaped.

D_1 Pore size (Dickson, 1967) extremely small (25µ), D_2 very small (25　50 µ), D_3 moderately small (50　100 µ), D_4 medium (100　200 m), D_5 moderately large (200　300 µ), D_6 very large (300　400 µ), D_7 extremely large (> 400 µ).

E_1 Length of vessel members (IAWA, 1937) extremely long (1900 µ), E_2 very long (1100 - 1900 µ), E_3 moderately long (800　1100 µ), E_4 medium (350　800 µ), E_5 moderately short (200　350 µ), E_6 very short (175　200 µ), E_7 extremely short (< 175 µ).

F_1 Tails prominent, F_2 short, F_3 indistinct.

G_1 Perforation plate foraminate, G_2 scalariform, G_3 simple.

H_1 Perforation plate extremely inclined (150 -180°), H_2 very inclined (130-150°), H_3 moderately inclined (110 - 130°), H_4 transverse (90　110°).

I_1 Wood non-stratified, I_2 nearly stratified, I_3 stratified.

J_1 Intervascular pits opposite with coalescent apertures of a few scalariform; J_2 pits circular, bordered, apertures round or transversely elongated, opposite; J_3 circular, bordered, apertures transversely elongated, alternate; J_4 circular, bordered, apertures round, alternate; J_{f+} pits to fibres present; J_{fo} pits fibres absent.

K_1 Pits minute (2 µ), K_2 very small (3 - 5 µ), K3 small (5 - 7 µ), K_4 moderately large (7 - 9 µ), K5 large (9 - 11 µ), K_6 very large (> 11 µ).

L_1 Vessel parenchyma pits scalariform simple, L_2 scalariform half bordered, L_3 scalariform bordered, L_4 round simple, L_5 round half bordered, L_6 round bordered.

M_1 Gummy contents present in vessel, M_2 pigmented, M3 absent.

N Distribution pattern represented by symbol formula proposed by Hess (1950).

O_1 Mucilage cells or canals or latex cells or vessels present, O_2 absent.

P_1 Parenchyma cells contain gummy or coloured substance, P_2 crystals, P_3 starch, Po clear.

Ray:

Q_1 Ray type hetero I (Kribs, 1935), Q_2 hetero II, Q_3 hetero III, Q_4 homo I, Q_5 homo II, Q_6 homo III.

R_1 Ray height (Chattaway, 1932) extremely low (0.5 mm), R_2 very low (0.5　1 mm), R_3 low (1 - 2 mm), R_4 rather low (2　5 mm), R_5 moderately high (5　10 mm), R_6 high (1 - 2 cm), R_7 very high (2　5 cm), R_8 extremely high (> 5 cm).

S_1 Ray width (IAWA, 1937) extremely fine (15 μ), S_2 very fine (15   25 μ), S_3 moderately fine (25   50 μ), S4 medium (50   100 μ), S_5 moderately broad (100   200 μ), S_6 very broad (200   400 μ), S_7 extremely broad (< 400 μ).

T_1 Ray frequency (Chattaway, 1932) very few (3 / nn), T2 few (3   4), T_3 moderately numerous (5   7), T_4 numerous (8   10), T_5 very numerous (> 10).

U Proportion between uniseriate and multiseriate rays.

V_1 Contents of ray cells gummy, V_2 crystals (in marginal cell), V_3 starch, V_4 oil globules, Vo clear.

Fibre:

W_1 Septate, W2 non-septate

X_1 Fibre length (IAWA, 1937) extremely long (3000 μ), X_2 very long (2200   3000 μ), X_3 moderately long (1600   2200 μ), X_4 medium (900   1600 μ), X_5 moderately short (700   900 μ), X_6 very short (500   700 μ), X_7 extremely short (< 500 μ).

Y_1 Thickness class of fibre wall (Chattaway, 1932) very thin (lumen much greater than thickness of wall), Y_2 thin (lumen greater than thickness of wall), Y_3 thick (lumen less than thickness of wall), Y_4 very thick (lumen almost completely closed).

Z_1 Fibre-pit bordered and pits few; Z_2 pits bordered and many; Z_3 pit bordered indistinct, apertre slit-like and many; Z_4 pit bordered indistinct, apertre slit-like and few.

Wood parenchyma pattern symbols (N) based on Hess (1950)

A. *Apotracheal*   Ad. diffuse, At. tangential lines or bands, Ac. concentric lines or bands.

P. *Paratracheal*   Pd. scanty paratracheal or paratracheal-diffuse, Pv. vasicentric, Pj. vasicentric confluent Pa. aliform, Pc. confluent.

(n. = narrow, b. = broad, S = widely spaced, Nc. = closely spaced, C. = continuous, B. = broken lines, Mt. = marginal terminal, Mi. = marginal initial, U. = unilateral.)

Symbol formula (N) = Major parenchyma types.

$$\text{Symbol formula (N)} = \frac{\text{Major parenchyma type}}{\text{Minor parenchyma type}}$$

Description of woods of the variants of *A. malaccensis*.

VARIANT I (Plate 1, Fig. 1A, 2A):

Wood: Pale yellowish white, r^+, g_o, $g_n = 0$.

Vessel : A_3, B = 15 %, $C_n = 3$ 4, C_{a+}, C_m 8, D_4, E_5, F_1, G_3, H_3, I_2, J_4, J_{fo}, K_3, L_4, M_3.

Table 1: Macro and micro morphological characters of the three variants of *A. malaccensis.*

Taxa	Reference of specimens cited at RRLJ* Herbarium	Salient characters
Variant I	*RRLJ - 2729*	Oblong-lanceolate leaf with caudate-acuminate leaf apex, acute leaf base, epidermal cells and stomata on lower surface of leaf 2165.35 and 131.57 respectively, stomatal size 15mm X 7mm, stomatal index 8.77, leaf area 2175mm2, secondary vein No 36, absolute vein-islet No and absolute vein-let termination No 3755.25 and 7532.02 respectively (Saikia *et al.,* 2000).
Variant II	RRLJ 2726	Obovate-lanceolate leaf with aristate-acuminate leaf apex, elliptic leaf base, epidermal cells and stomata on lower surface of leaf 1574.80 and 98.68 respectively, stomatal size 15mm X 9mm, stomatal index 6.78, leaf area 2375mm2, secondary vein No 24, absolute vein-islet No and absolute vein-let termination No 2854.90 and 5056.77 respectively (Saikia *et al.,* 2000).
Variant III	RRLJ 2731	Lanceolate leaf with acuminate leaf apex, acute leaf base, epidermal cells and stomata on lower surface of leaf 3277.95 and 164.47 respectively, stomatal size 15mm X 8mm, stomatal index 12.50, leaf area 2650mm2, secondary vein No 32, absolute vein-islet No and absolute vein-let termination No 2184.25 and 5600.00 respectively (Saikia *et al.,* 2000).

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$$Parenchyma: N = \frac{Pd., Ac., n., S}{Pv., Nc., C.} O_2, P_o.$$

Ray : Q2, R2, S4, T5, U = 71.6 : 28.5, Vo.
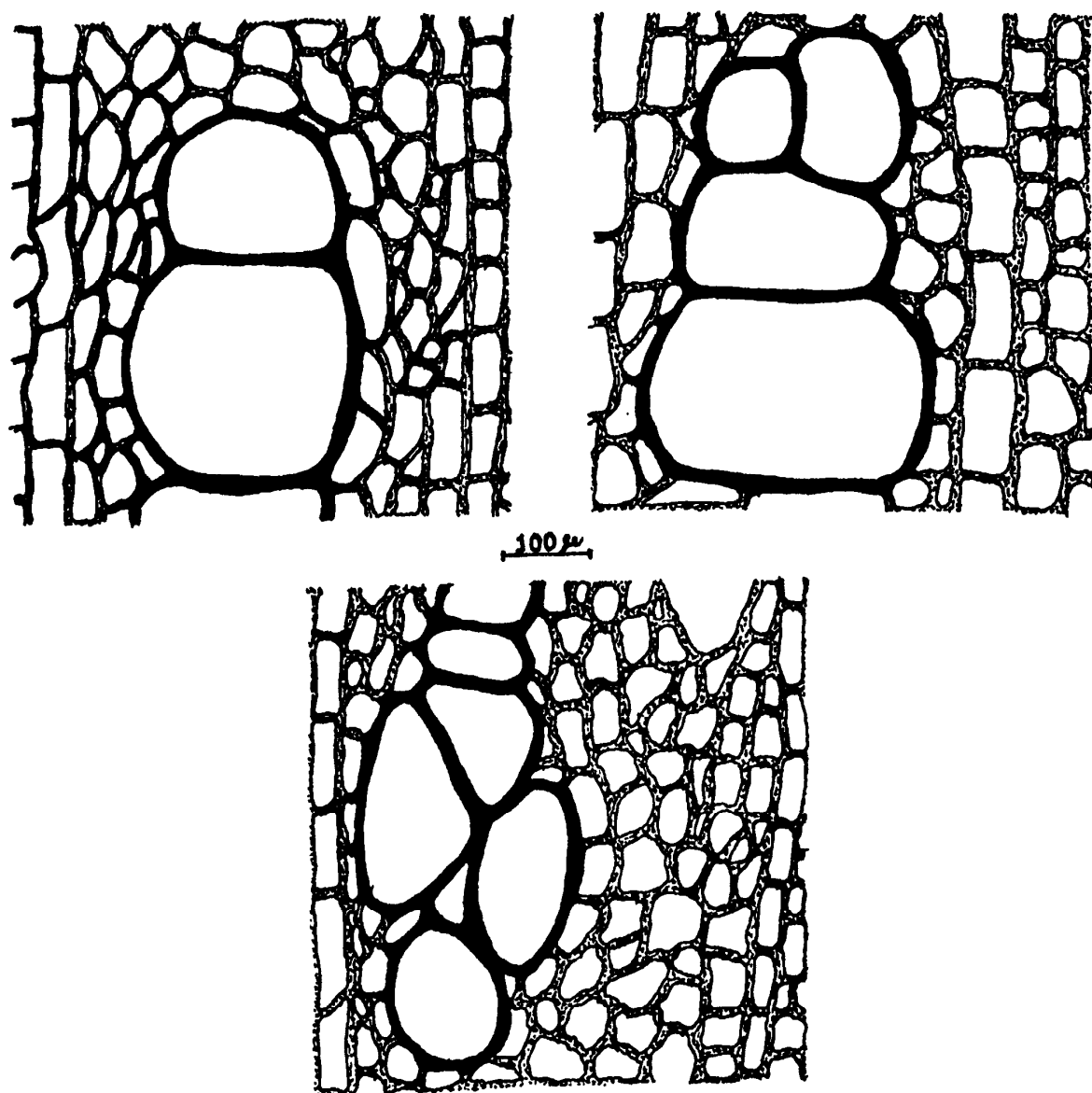*Fibre :* W1, X5, Y2, Z2.

Fig. 1 : Transverse sections of the woods of the varients of *Aquilaria malaccensis*. A. Variant I, B. Variant II and C. Variant III.
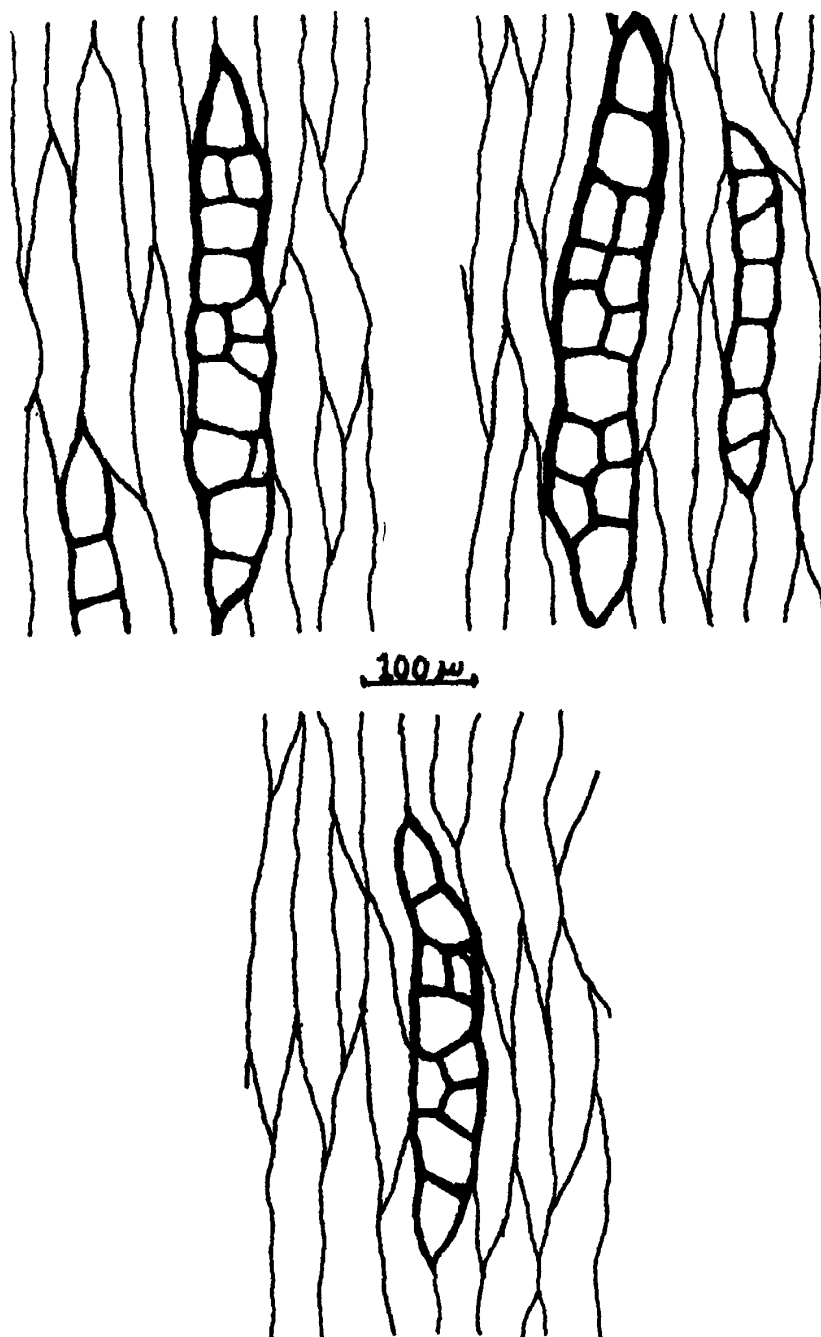
Fig. 2 : Longitudinal sections of the woods of the varients of *Aquilaria malaccensis*. A. Variant I, B. Variant II and C. Variant III.
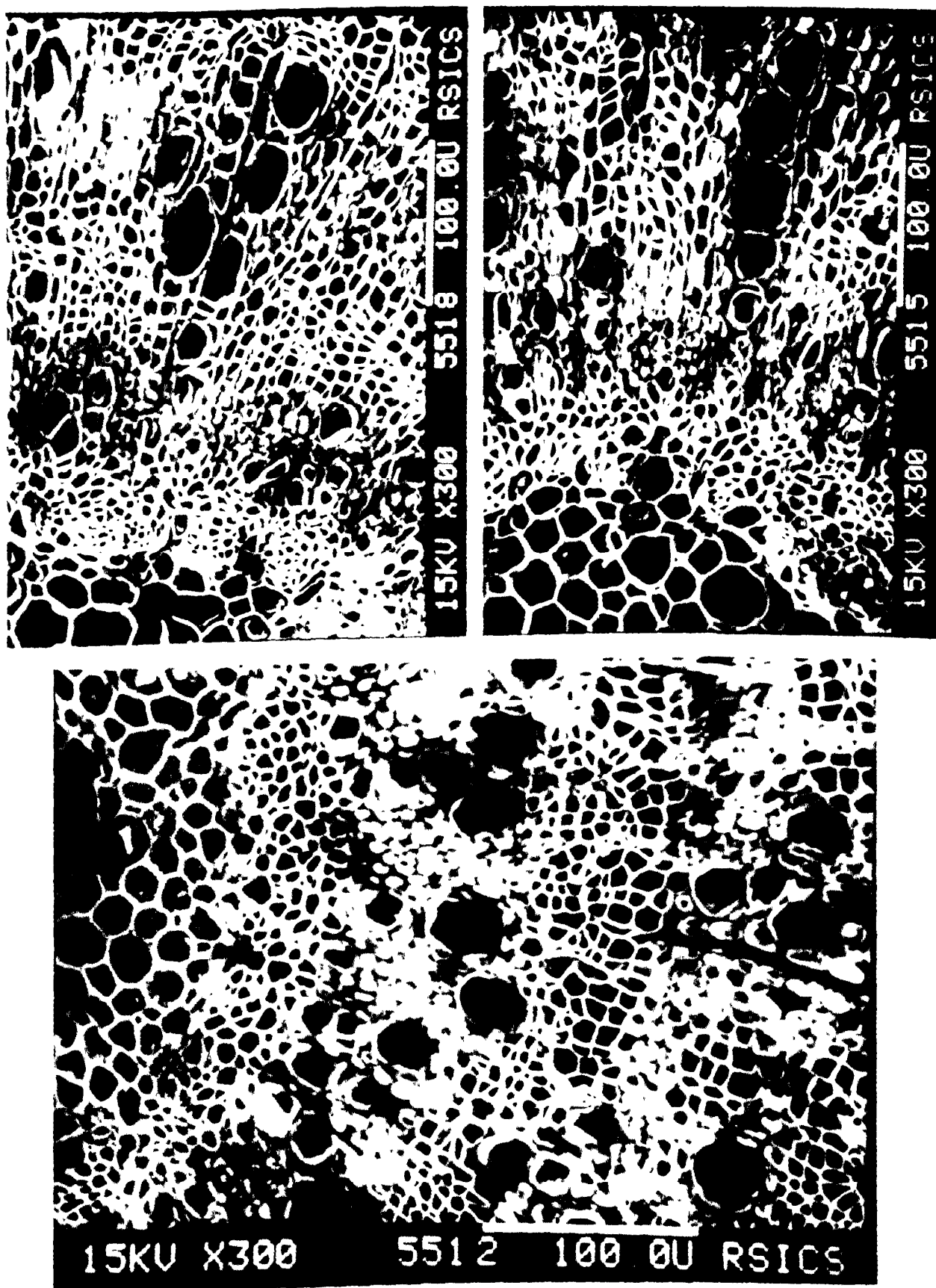
Plate 1 : SEM photographs of the variants of *Aquilaria malaccensis*. A. Variant I, B. Variant II and C. Variant III.

VARIANT II (Plate 2, Fig. 1B, 2B):

Wood : Yellowish white, r^+, g_0, g_n = 0.

Vessel : A_4, B = 27 %, C_n = 4 - 5, C_{a+}, C_m 8, D_4, E_4, F_1, G_3, H_4, I_2, J_4, $\cdot J_{fo}$, K_3, L_4, M_3.

Parenchyma: N = $\dfrac{\text{Pd., Ac., b., S}}{\text{Pv., Nc., B.}}$ O_2, P_0.

Ray : Q_3, R_2, S_4, T_5, U = 92.8 : 7.1, V_o.
Fibre : W_1, X_4, Y_3, Z_2.

VARIANT III (Plate 3, Fig. 1C, 2C) :

Wood : White, r^+, g_0, g_n = 0.

Vessel : A_3, B = 64.7 %, C_n = 2 - 3, C_a, C_m 8, D_3, E_6, F_1, G_3, H_3, I_2, J_4, J_{fo}, K_3, L_4, M_3.

Parenchyma: N = $\dfrac{\text{Pv., Ad., b, Nc.}}{\text{Pd., B.}}$ O_2, P_0.

Ray : Q_4, R_1, S_3, T_5, U = 29.6 : 70.4, Vo.
Fibre : W_1, X_6, Y_2, Z_3.

Based on these anatomical characters a similarity matrix among the variants was calculated and presented below.

Table - 2 : Percentage similarity among the variants

Taxa	Variant I	Variant II	Variant III
Variant I	100%	61%	46%
Variant II		100%	41%
			100%

From the similarity matrix, similarity percentages observed among the variants were at 41 - 61 % levels. The Variant I showed more similarity with the Variant III (61 %) and lesser with the Variant II (41 %).

DISCUSSION

The anatomy of wood characters which provides types of cells are reported to be useful in taxonomic discrimination of taxa up to varietial entities (Dutta and Samanta,

1983; Shah and kachroo, 1980 and Dickson, 1975). The wood characters of the variants presently studied not only exhibited their difference in colour but also the variants showed both qualitative and quantitative (Table 3 and Table 4) differences of their vessel, paranchyma, ray and fibre cells. A similarity matrix shown in Table 2 determined on the basis of presence or absence of characters of the woods reflects that the differences amongst the taxa exist at 39-59 %. The Variant I and the Variant II are comparatively more closer (61%) than that of the Variant I with the Variant III (46%) and the Variant II with the Variant III (41%) indicating that the variants could be classified in to two distinct groups having only 43.5% similarity between the two groups. This result is also in agreement with that

Table 3 : Quantitative data for vessels of the variants of *A. malaccensis*.

Taxa Plate (μm)	Nos. of pore / angle		Member length mm2			Vessel diameter (μm)		
	Mean	Range	Mean	S. D.	S. E.	Mean	S. D.	S. E.	Mean
Variant I	31	25-37	332.4	80.3	11.3	103.2	32.9	6.5	119.1
Variant II	17.5	16-19	427.2	105.2	15.6	120.3	38.6	7.6	104.3
Variant III	61	56-67	212.0	57.6	8.4	93.7	31.5	6.3	134.5

Table 4: Quantitative data for fibre and ray parenchyma of the variants of *A. malaccensis*.

Taxa	Fibre length	Length of multi-rays (μm)		Breadth of multi-rays (μm)		Proportion (%) of uni and multi rays	Rays / mm
	Mean	Mean	Range	Mean	Range		Mean
Variant I	857.1	0.61	0.54-0.72	53.3	51.4-55.2	71.6:28.5	12.4
Variant II	1194.4	0.85	0.79-0.91	57.6	54.1-60.5	92.8:7.2	15.6
Variant III	679.3	0.46	0.42-0.50	42.5	37.0-48.0	29.6:70.4	10.6

of the amino acid characters reported by the same authors (Saikia and Nath 2001). Thus, the two groups described the rank of varietial status. A taxonomic key to the taxa formulated on the basis of studied characters was as follows-

1.1 Wood ring porous, growth ring absent, vessel with circular, bordered, small pit, parenchyma clean, fibre septate   .....   *A. malaccensis* Lamk.

    2.1 Wood yellowish-white, pore size medium, ray height very low, ray width medium, fibre pit bordered and many :

        3.1 Wood pale yellowish-white, pore abundance numerous, solitary pore percentage 15%, radial pore-chain length in pore number 3-4, wood parenchyma narrow and continuous, ray heterotype II, ratio of uniseriate and multiseriate ray 71.5 : 28.5, fibre length moderately short with thin fibre wall

            .....   Variant I (*A. malaccensis*).

        3.2 Wood yellowish-white, pore abundance moderately numerous, solitary pore percentage 27%, radial pore-chain length in pore number 4   5, wood parenchyma broad and broken line, ray heterotype III, ratio of uniseriate and multiseriate ray 92.8 : 7.1, fibre length medium with thick fibre wall

            ....   Variant II (*A. malaccensis*).

    2.2 Wood white, pore abundance numerous and size moderately small, solitary pore percentage 64.7%, radial pore-chain length in pore number 2   3, ray width moderately fine, fibre length very short with thin fibre wall

        ....   Variant III (*A. malaccensis*).

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