Controlled Differentiation of Foliar Sclereids in *Fagraea fragrans*

Occurrence of foliar sclereids in different dicotyledonous species is well documented in the standard reference works on plant anatomy[1,2]. FOSTER, in his pioneering studies, traced the ontogeny of leaf sclereids in several angiosperms, and subsequently many other workers have studied the variation of sclereid form and their development in certain families[3–5]. However, it is only in the last 10 years that attempts have been made to identify the causal factors that may either completely inhibit the sclereid development or modify the pattern of their distribution. Of such studies, the researches on *Pseudotsuga*, a member of Coniferales, stand out very significantly, where total inhibition of sclereid formation was induced in the developing leaves[6]. In *Camellia* leaves it was demonstrated that either high osmotic concentration of the medium could inhibit the sclereid formation, or the surgical injuries made could alter the pattern of sclereid distribution[7,8]. Certain experiments were designed to test the effect of auxins on leaf sclereid formation in *Fagraea fragrans* (Loganiaceae), a common tree in Singapore, and the results of such studies are briefly summarized here.

F. fragrans is a tall evergreen tree, with characteristic branching, sometimes reaching up to more than 100 ft. and occurring both on the coast and inland. The leaves are simple, small, emarginate and are arranged alternately on slender twigs. Each twig normally bears 8–10 leaves, and on an average the leaf measures $3\,^1/_2 \times 1\,^1/_2$ inches. Even in very young leaves measuring $^3/_4 \times {}^1/_3$ inch, formation of sclereids is common, and in the mature leaves they are more abundant (Figure 1). As regards to their origin with reference to the other leaf tissues, they develop mostly from upper spongy parenchyma cells and they are both terminal and diffuse in their distribution. In form, they are polymorphic, each sclereid cell showing a central axis with a number of spicules on it, and the cells have thick, lamellated, lignified cell walls[9] (Figure 3).

Several growing end branches or twigs of approximately equal size were selected for experimentation. They were defoliated and plain lanolin was applied to the apical bud and to the leaf scars left after defoliation. These formed the control group. The other defoliated branches were smeared with lanolin paste to which indole acetic acid (IAA) was earlier added at concentrations of 0.125%, 0.25%, 0.5% and 1%. More than 20 twigs were used for each treatment and each experiment was repeated at least twice. In the twigs of the control group, after defoliation the first leaf primordium appeared after 10–12 days. The cleared preparations of such leaves showed that the distribution and development of sclereids were normal. In such twigs, where IAA at 1% concentration was used, the buds either did not expand further or they eventually died. Again at the 0.5% level, very few small leaves ($^1/_2 \times {}^1/_3$ inch) were formed and they prematurely dropped. In those twigs treated with IAA at 0.25% or lesser concentration, the early leaf development and expansion was normal, the leaves attaining the same size as those of the control group. Leaf clearing and macerated preparations of such experimental leaves were obtained and studied. Complete inhibition of sclereids was observed in the small incompletely developed leaves of the 0.5% group, or of well developed leaves of the 0.25% group (Figure 2). But the venation pattern and aereole formation was regular in such leaves, comparing well with those of the control leaves. In the leaves formed under the influence of 0.125% IAA, the sclereid development was normal, but striking structural variations could be noticed in the sclereid cells. In them the cell walls were thin, lamellations absent and the cell lumen was very broad and prominent (Figure 4). When the standard

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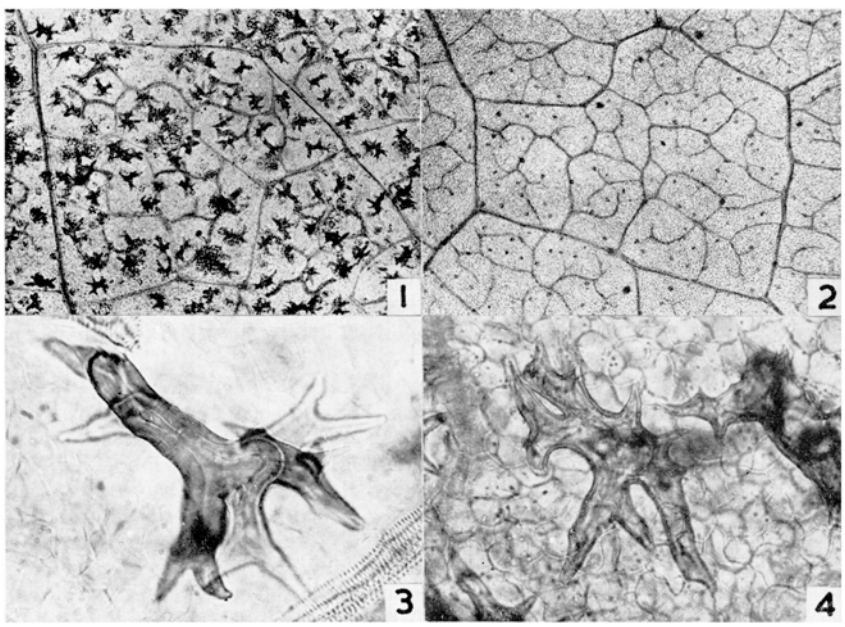

Fig. 1. A portion of the cleared lamina of the control group showing distribution of sclereids. × 36. Fig. 2. Cleared lamina of the 0.25% IAA group showing absence of sclereids. × 36. Figs. 3–4. Single sclereids enlarged from the control and 0.125% IAA groups respectively. Note the differences in cell wall thickness and dimensions of the lumen. × 126.

phloroglucinol-HCl test was employed to test the lignin content, no positive stain reaction was observed, indicating the non-ligniferous but cellulose nature of such cell walls. It may be concluded that sclereid formation was not inhibited at a lower IAA concentration like 0.125%, but the sclereids formed showed a number of intracellular structural variations. The influence of IAA and other antioxidants that promote cell growth but inhibit lignin synthesis, and the general role of auxins in cell wall synthesis have recently been discussed by SIEGEL[10] and LEOPOLD[11]. The cytochrome oxidase activity was identified in sclereid initials of *Rauwolfia* stem by histochemical studies[12]. The total suppression of sclereid development under the influence of a high concentration of IAA in the species presently investigated is well comparable with the observations made in *Pseudotsuga*. The other auxins NAA and 2,4-D were also tried in similar concentrations as mentioned above, and these inhibited further leaf development. Other details of sclereid differentiation, under the influence of IAA, as well as the effects of NAA, and 2,4-D, will be published in subsequent papers.

Zusammenfassung. Die Blätter von *Fagraea fragrans* enthalten polymorphe Sklereide. Indolyl-Essigsäure (IES) in einer Konzentration von 0,125% hemmt die Verholzung der Sklereiden, höhere IES-Konzentrationen unterdrücken die Bildung der Sklereiden vollständig.

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Bacteriophage Typing of *Vibrio eltor*

Studies on the phage-typing of *Vibrio eltor* have been described by NICOLLE and his group[1,2], GALLUT and NICOLLE[3] and EISENTARK[4]. The phage-typing scheme of NICOLLE and his group did not prove to be of much practical value[5]. GALLUT and NICOLLE examined a total of only 14 strains of *V. eltor*, and their scheme provides only a broad classification into 3 groups. The scheme of NEWMAN and EISENTARK is yet to be assessed. A tentative scheme of phage-typing of *V. eltor* was proposed by MUKERJEE[6], which has been discarded by the author himself because serious discrepancies had been observed on repetition of the test.

In 1965 a phage-typing scheme for epidemiological identification of El Tor strains was developed using 5 groups of phages, 3 being lytic mutants of temperate phages and 2 isolates from stool samples of cholera El Tor patients. Nine phage-types of *V. eltor* could be identified (Table I).

However, it was found subsequently that the host-range of the group V typing phage had changed, and it had to be discarded. It was replaced by a stool phage, H74/64, and a new scheme adopted by which 6 phage-types of strains could be identified (Table II).

This new group V phage, as may be seen from Table II, did not help in diversifying type determination as it lyses all El Tor strains isolated from cholera El Tor patients; but it helps to differentiate between the classical and the El Tor types of vibrios. Vibrios of phage-types 1 and 2 are invariably found to be non-lysogenic, whereas the other types are lysogenic.

Using the new scheme, 3464 strains of *V. eltor* isolated from the different epidemics between 1937 and 1966 have been typed. Celebes was found to have all the 6 phage-types of strains. It was also observed that the number of phage-types in the outbreaks in the course of the spread of the pandemic decreased progressively with time and distance from the original endemic focus in Celebes. In the initial phase, when cholera El Tor spread outside Celebes during 1961–62, large numbers of people carried the infection from Celebes to Hong Kong and the Philippines, and, as would be expected along the lines of

Table I

Phage-type of vibrio	Lysis by phage group				
	I	II	III	IV	V
1	+	+	+	+	+
2	+	+	+	+	−
3	+	+	+	−	−
4	+	+	−	−	−
5	+	−	−	−	+
6	+	+	−	−	+
7	+	+	−	+	+
8	+	+	+	−	+
9	−	+	−	−	+

Table II

Phage-type of vibrio	Lysis by phage group					Classification according to previous scheme
	I	II	III	IV	V	
1	+	+	+	+	+	1 and 2
2	+	+	+	−	+	3 and 8
3	+	+	−	+	+	7
4	+	+	−	−	+	4 and 6
5	+	−	−	−	+	5
6	−	+	−	−	+	9

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