

## •Short Communication•

## Vegetative Storage Proteins in Meliaceae

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**Key words:** vegetative storage proteins; ultrastructure; immuno-correlation; Meliaceae

Vegetative storage proteins (VSPs) in the vegetative organs of woody plants are defined by their vacuolar localization, relative abundance in the dormant shoot and mobilization during the regrowth of trees in spring<sup>[1-5]</sup>. Available evidence showed that they occurred widely in different taxonomic trees<sup>[6-9]</sup> and varied in ultrastructure and immuno-correlation between families<sup>[7,9,10]</sup> and were highly homologous and commonly distributed in Salicaceae species<sup>[4,8,11]</sup>. Little was known of the VSPs of different taxonomic units within a family except for Salicaceae which only contains *Populus*, *Chosenia* and *Salix*.

Meliaceae, a general tropical family subdivided into Swietenioideae, Melioidae and Cedreloideae, consists of about fifty genera<sup>[12]</sup>. As several Meliaceae species abounded with VSPs<sup>[13,14]</sup>, we further investigated more systematically the VSPs in Meliaceae and in this paper, evidence for the diversity in distribution, ultrastructure and immuno-correlation of the VSPs in the family was presented.

**1 Materials and Methods**

Meliaceae species and varieties listed in Table 1 were obtained from the Botanica Garden of South China University of Tropical Agriculture and Tropical Tree Garden in Jian Feng Mountains on Hainan Island. Samples of 1 to 2-year-old shoots were collected in two different periods: (1) the leaf-absent period for deciduous trees or the dry season for evergreen trees; and (2) the period after new shoot was fully developed for both deciduous and evergreen trees.

Light and electron microscopy and SDS-PAGE were carried out referring to Tian *et al.*<sup>[9]</sup> and Western blotting to Tian and Hao<sup>[5]</sup>.

**2 Results and Discussion**

Vacuolar protein inclusion, stained clear blue with mercury-bromophenol in the sections, was recognized under the light microscope. The vacuolar protein inclusion was abundant in the leaf-absent period and decreased remarkably during the first growth flush (data not shown) and therefore was suggested to be VSPs (Table 1).

**Table 1** Distribution of the VSPs in Meliaceae, based on the recognizable vacuolar protein inclusion

Subfamilies	Genera	Species	Distribution
Swietenioideae	<i>Swietenia</i>	<i>S. macrophylla</i> King	+++
		<i>S. mahagoni</i> (L.) Jacq.	+++
	<i>Khaya</i>	<i>K. senegalensis</i> (Desr.) A. Juss.	+++
	<i>Chukrasia</i>	<i>C. tabularis</i> A. Juss.	+++
		<i>C. tabularis</i> A. Juss. var. <i>velutina</i> (Wall.) King	+++
Melioidae	<i>Melia</i>	<i>M. azedarach</i> L.	+++
		<i>A. odorata</i> Lour.	+
	<i>Aglaia</i>	<i>A. tsangii</i> Merr.	+++
		<i>Cipadessa</i>	<i>C. cinerascens</i> (Pell.) Hand-Mazz.
	<i>Walsura</i>	<i>W. cochinchinensis</i> (Baill.) Hams	+
	<i>Dysoxylum</i>	<i>D. hongkongense</i> (Tutch.) Merr.	+
	<i>Heynea</i>	<i>H. trijuga</i> Roxb. var. <i>microcarpa</i> Pierre	-
	<i>Aphanamixis</i>	<i>A. polystachya</i> (Wall.) R. N. Parker	-
		<i>A. grandifolia</i> Bl.	-
	Cedreloideae	<i>Amoora</i>	<i>A. dasyclada</i> (How et T. Chen) C. Y. Wu ( <i>Aglaia dasyclada</i> How et T. Chen)
<i>Toona</i>		<i>T. sinensis</i> (A. Juss.) Roem.	-
		<i>T. microcarpa</i> (C. DC.) Hams	-
	<i>Cedrela</i>	<i>C. maxicana</i> Roem.	-

+++ , rich; + , poor; - , none.

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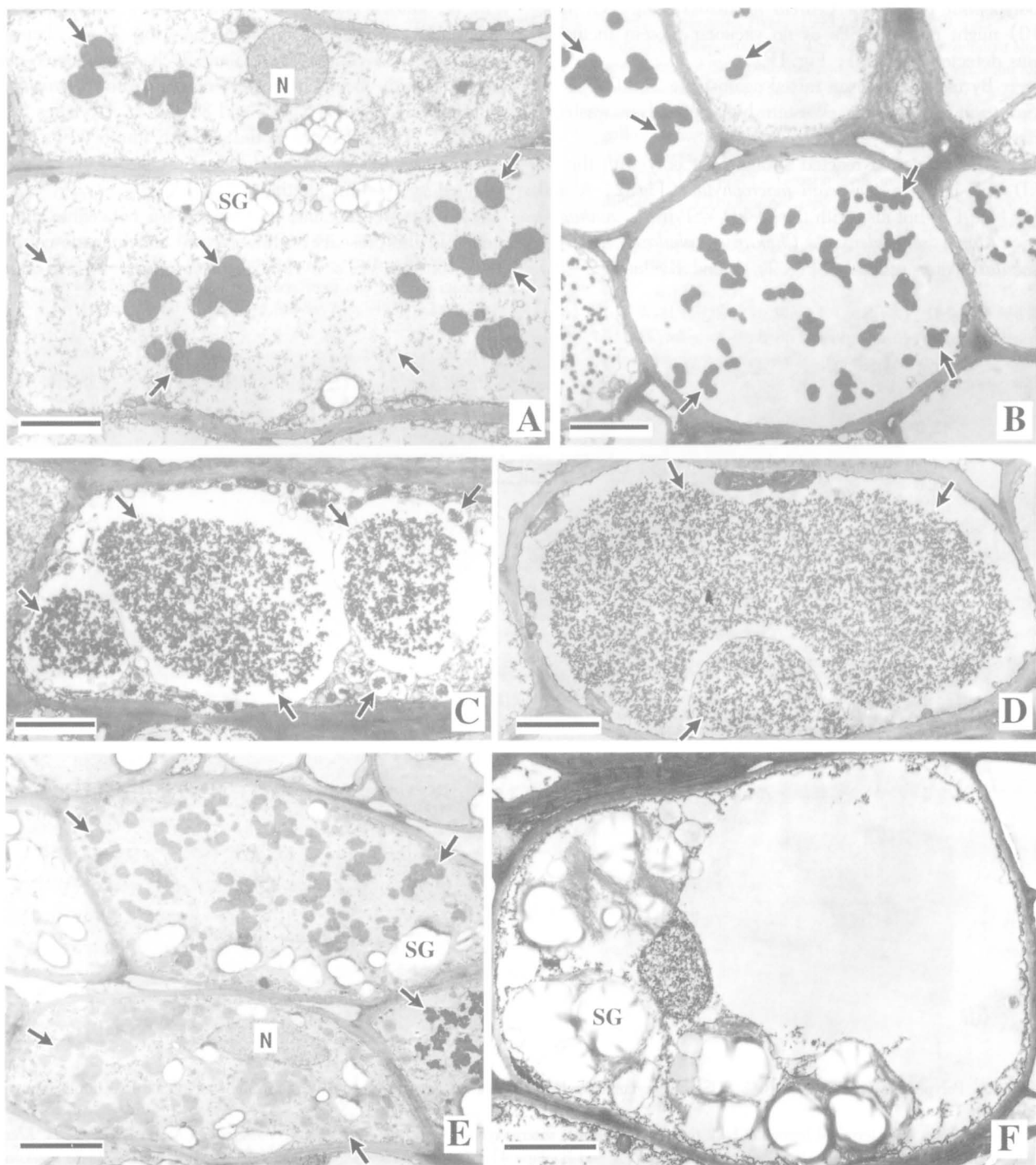
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The distribution of VSPs varied in the three subfamilies of Meliaceae. They were present in all the species of Swietenioideae, most species of Melioideae and absent in Cedreloideae (Table 1). The occurrence of VSPs in a genus seemed to be either universal or entirely absent (Table 1). Thus, the distribution of VSPs in Meliaceae

could be considered as a taxonomic character.

The protein inclusion observed by light microscopy appeared to be in different forms under the electron microscope (Fig.1, A – E) and the form of VSPs in different species of a genus was identical as shown in Fig.1C and 1D.



**Fig. 1.** Electron micrographs of branchlet in cross-section, showing different forms of VSPs in the large central vacuoles of parenchyma cells in the leaf-absent period.

Samples were taken from *Swietenia macrophylla* (A), *Khaya senegalensis* (B), *Chukrasia tabularis* (C), *C. tabularis* var. *velutina* (D), *Melia azedarach* (E) and *Cedrela maxicana* (F). Black arrow, VSPs in the vacuoles of phloem parenchyma cells. Bar = 5  $\mu$ m.

By SDS-PAGE, several prominent proteins with molecular masses of 15 to 45 kD were detected in the samples which were rich in vacuolar protein inclusion (Fig. 2, A and C). These proteins decreased when new leaves were fully expanded, paralleling with the changes of the vacuolar protein inclusion which should be the VSPs. But the two notable proteins in the SDS-PAGE polypeptide profiles of *Cedrela mexicana* (Fig. 2C, lane 10) might not be VSPs as no vacuolar protein inclusion was detected (Table 1; Fig. 1F).

By using antiserum raised against the 21 kD VSP in *Sweetenia macrophylla*, Western blotting analysis was carried out (Fig. 2).

The antiserum reacted strongly not only with the 21 kD VSP itself in *Sweetenia macrophylla* (Fig. 2, A and B, lane 1), but also with the 21 kD VSP in *S. mahagoni*, *Khaya senegalensis*, *Chorasia tabularis* and *C. tabularis* var. *velutina* (Fig. 2, A and B, lanes 2-5)

and the 18 kD VSP in *Sweetenia macrophylla*, *S. mahagoni* and *Khaya senegalensis* (Fig. 2, A and B, lanes 1-3), suggesting that these proteins were highly homologous, whereas it gave a much weaker immuno-reaction with the 19 and 20 kD VSPs in *Melia azedarach* (Fig. 2, A and B, lane 6). The 30 kD protein, a main VSP in *Chorasia tabularis* and *C. tabularis* var. *velutina* (Fig. 2, A, lanes 4 and 5) and the VSPs in the six species except *Melia azedarach* in Meliaceae (Fig. 2, A, lanes 7 and 8; Fig. 2, C, lanes 5, 6 and 7) had no immuno-correlation with the 21 kD VSP in *Sweetenia macrophylla* (Fig. 2, B, lanes 4, 5, 7 and 8; Fig. 2, D, lanes 5, 6 and 7). The difference in the ultrastructure of VSPs might account for the varied biochemical properties of VSPs revealed by Western blotting.

Thus we conclude that there is a noticeable difference in the presence, ultrastructure and immuno-correlation of the VSPs in Meliaceae, which was in sharp

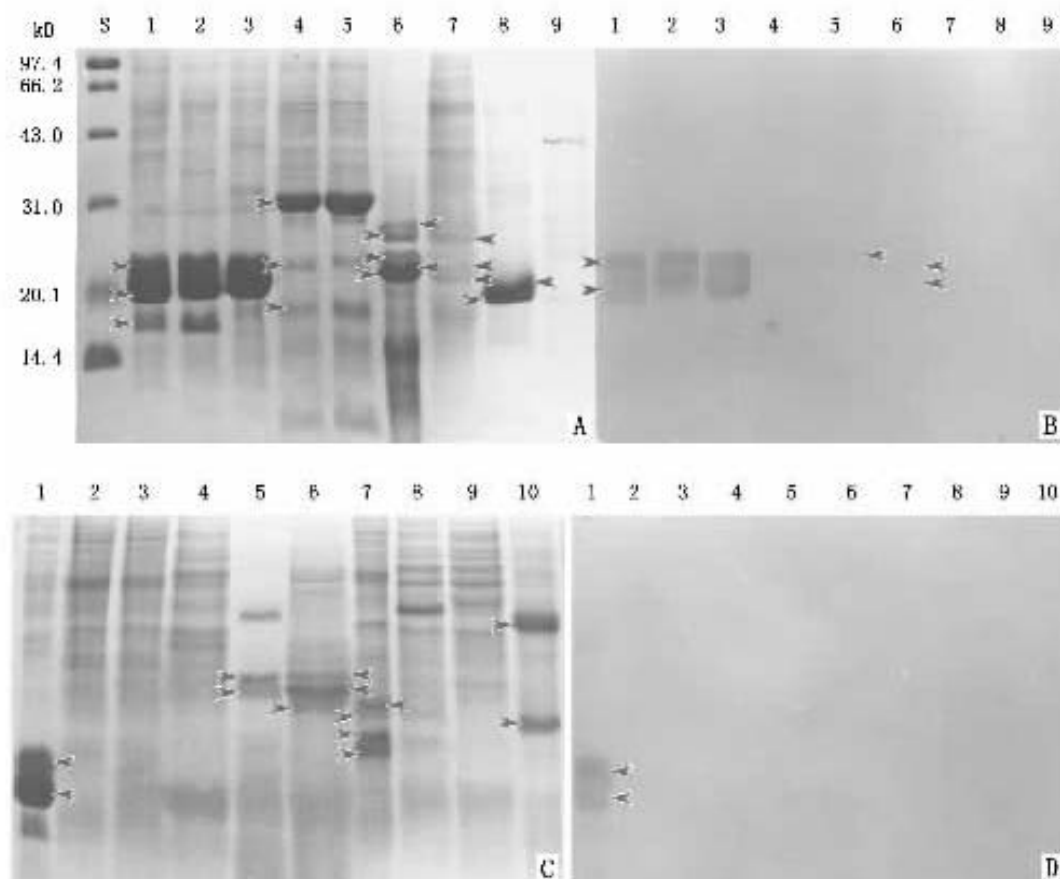


Fig. 2. Polypeptide profiles from 14% SDS-polyacrylamide gel electrophoresis (A and C) and Western-immunoblots of their duplications respectively (B and D).

Soluble proteins from terminal branchlet bark tissues of *Sweetenia macrophylla* (A and B, lane 1), *S. mahagoni* (A and B, lane 2), *Khaya senegalensis* (A and B, lane 3), *Chorasia tabularis* (A and B, lane 4), *C. tabularis* var. *velutina* (A and B, lane 5), *Melia azedarach* (A and B, lane 6), *Aglaia odorata* (A and B, lane 7), *A. zangii* (A and B, lane 8), *Amoora dasydada* (A and B, lane 9), *Sweetenia macrophylla* (C and D, lane 1), *Aphananixis polyzachya* (C and D, lane 2), *A. granatifolia* (C and D, lane 3), *Heynea trijuga* (C and D, lane 4), *Cipadessa cinerascens* (C and D, lane 5), *Walalra cochinchinensis* (C and D, lane 6), *Dysoxylum hongkongense* (C and D, lane 7), *Tona sinensis* (C and D, lane 8), *T. microcarpa* (C and D, lane 9) and *Cedrela mexicana* (C and D, lane 10). Forty  $\mu$ g protein were loaded per lane. S, Standard proteins. Arrowhead, except that in Fig. 2C, lane 10, VSPs in A and C and the protein bands detected by the antiserum in B and D.

contrast with the case in Salicaceae<sup>[4,8,11]</sup>. A further study is needed to demonstrate the relationship between the taxa of Meliaceae and the diversity in VSP distribution, ultrastructure and immuno-correlation.

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# 楝科树木营养贮藏蛋白质的研究

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**摘要:** 采用光学和电子显微镜技术及 SDS-PAGE 研究了营养贮藏蛋白质(VSPs)在楝科树木中的分布和超微结构; 并采用免疫印迹技术, 以大叶桃花心木(*Swietenia macrophylla* King)的 21 kD VSP 的抗血清检测楝科树木 VSPs 的免疫相关性。结果表明, 在桃花心木亚科(Swietenioideae)的树木中普遍存在 VSPs, 但楝亚科(Melioidae)仅部分属的树木有 VSPs 而椿亚科(Cedreloideae)树木没有 VSPs。VSPs 在同一属树木中的分布是一致的。桃花心木亚科和楝亚科树木之间的 VSPs 几乎没有免疫相关性, 但是在桃花心木亚科中, VSPs 具有相对高的同源性。楝科树木 VSPs 的超微结构存在一定程度的差异, 这可能与 VSPs 的种类不同有关。VSPs 的分布、超微结构和免疫相关性可能是楝科树木的一个分类特征。

**关键词:** 营养贮藏蛋白质; 超微结构; 免疫相关性; 楝科

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