

Extraction and Identification of Epicuticular Wax and its Variations in Coconut Genotypes

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Chloroform extracted more wax from the leaf surface of coconut (*Cocos nucifera* L.) than either benzene or hexane. The wax content was higher during dry season than during wet season in all the three hybrids viz., MOD × WCT, MYD × WCT and COD × WCT. The thin layer chromatography run in benzene gave better resolution and separation of wax components than other solvents tested. Genotypic variations existed in the intensity of wax components.

Key words : *Cocos nucifera* L., epicuticular wax, thin layer chromatography.

INTRODUCTION

In plants, the protective role against the infection and entry of pathogens is played by a polymer composed of lipid components namely cuticle. The soluble cuticular lipids present on the surface of various plant organs imparts resistance to water loss and infection. The long chain carbon esters of the epicuticular wax (ECW) can be extracted by using various organic solvents such as hexane, benzene, chloroform and petroleum ether. Hexane has been used for surface wax extraction from *Panicum* species (6) whereas chloroform was used to extract wax from barley (4). Similarly, wide range of organic solvents are used to separate the components of surface wax by chromatographic technique. Thus, the effectiveness of solvent for extraction and separation depends on the plant species. In the present study, an attempt has been made to select a suitable solvent for the extraction and separation of ECW of coconut palms. The changes in the wax content in plants of different age, during two seasons and varietal differences in the content and composition were also studied.

MATERIALS AND METHODS

Coconut palms (*Cocos nucifera* L.) of West Coast Tall, WCT (both four year seedling and 20 year old adult palm) and hybrid seedlings of COD × WCT (Chowghat Orange Dwarf), MOD × WCT (Malayan Orange Dwarf) and MYD × WCT (Malayan Yellow Dwarf), grown under field conditions with the normal agronomic and cultural practices were used

in the experiment. Three palms in each case with the sixth leaf from the top were taken for wax extraction.

Wax extraction

25 gms of leaf material was cut into uniform strips (3 × 1 cm) and extracted individually for 15 seconds in 30 ml each of the solvent contained in 50 ml beaker with chloroform or hexane or benzene. The contents were evaporated under vacuum. The surface wax thus extracted was estimated quantitatively using potassium dichromate colour reaction (3).

Since chloroform was found to extract wax content better than the other two solvents (Table 1), later experiments were carried out with chloroform.

The wax content from the leaves of three hybrids viz. COD × WCT, MOD × WCT and MYD × WCT was extracted during two distinct seasons i.e. non-stress period (October 1987) and stress period (March 1988) and the content estimated colorimetrically.

Table 1. Epicuticular wax content ($\mu\text{g. cm}^{-2}$) from the leaf tissues of WCT palms extracted with different solvents (Sept. 1988)

Solvent	Seedlings	Adult palm
Chloroform	8.2 ± 0.93	16.3 ± 1.05
Benzene	5.9 ± 0.46	10.0 ± 0.89
Hexane	1.9 ± 0.08	0.53 ± 0.58

Separation of wax components

The thin layer chromatographic (TLC) technique was employed to separate the wax components. 100 μ l and 50 μ l of sample consisting respectively of 20 μ g and 10 μ g wax from WCT seedlings and 40 μ g and 20 μ g from WCT adult palms were loaded on the activated silicagel G plates (Mesh size 10-40 μ).

The procedure for chromatographic separation of wax was the same as that described by Larson and Svenningson (4). The R_f values of wax components separated into various bands were compared with the durum wheat leaf wax run in benzene.

RESULTS AND DISCUSSION

From Table 1, it is evident that chloroform extracted higher quantity of ECW than the other two solvents irrespective of the age of the palm. The spectral absorbance in various solvents showed that the peak was at 590 nm irrespective of the solvent used and seedlings had less ECW content than the adult palms. Such age-related changes in wax content were reported earlier in some plants species (1).

Among the hybrids tested, MYD \times WCT contained higher amount of surface wax than MOD \times COD and COD \times WCT in both wet and dry season (Table 2). There was significant increase in the wax content during dry season in all the three hybrids, which could be attributed to changes in environmental factors. Present data thus indicate that coconut wax is prone to changes due to environmental factors, as reported in at (1).

Table 2. Epicuticular wax content (μ g. cm^{-2}) during 'wet' and 'dry' season in three coconut hybrids

Hybrid (G)	Season (S)	
	Wet	Dry
COD \times WCT	20.3	67.3
MYD \times WCT	22.9	74.0
MYD \times WCT	16.7	63.0

(G' = 7.07** S = 708.8** G \times S = Not significant)

Chromatographic separation of wax carried out by running the TLC plates initially in chloroform, hexane or benzene with samples from seedlings and adult palms showed different resolution. Benzene resolved better than chloroform and hexane. Bukovac

and Wilmeyer (2) also used benzene for TLC to separate wax components from a tree species, Afghanistan pine.

The components of wax in coconut were identified with standard durum wheat wax. The components identified in decreasing order of R_f values were hydrocarbons (0.94), esters (0.86), β -diketones (0.61), alcohols (0.45), hydroxy- β -diketones (0.31) and acids (0.12). All the three hybrids exhibited the above wax components, although the intensity of bands differed among the hybrids. For instance the hydrocarbons and alcohols were found to be more in MYD \times WCT than in COD \times WCT, which corroborates the quantitative differences in wax content in the two hybrids (Table 2). A similar variation in the pattern of wax separation was noticed in other genotypes of coconut differing in their tolerance to drought (5). The role of ECW in checking transpirational loss of water from leaf surface has been well demonstrated in many crops (3). Drought tolerant coconut genotypes had higher wax content and lower transpiration rate as compared to the drought susceptible genotypes (5).

From the foregoing it may be concluded that chloroform is an ideal solvent for extraction of wax and benzene for better resolution of wax components in coconut leaves. The study highlights the fact that changes in wax content depend on age of the plant and the environmental factors.

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