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RESEARCH PAPER

Structure and Composition Analysis of Oil Palm (*Elaeis guineensis* Jacq.) Pollen - *Dura, Tenera* and *Pisifera* var.

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ABSTRACT

In an oil palm plantation huge amount of pollen is produced. The results of the present investigation showed that oil palm pollen is a good reserve of proteins, sugars, lipids, minerals and vitamins A and E. The fine structure of the pollen under SEM showed a trichotomous appearance with scaly and smooth surfaces on ventral and dorsal sides respectively. The ultra structural differentiation of pollen wall into tectum, columella and foot layer was visible under TEM. The structural features under EM (SEM and TEM) supports its affinity towards biotic pollination.

Key words: Oil palm pollen, reducing sugars, β - carotene, lipids, soluble proteins, tocopherol.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a tropical oleaginous crop with highest edible oil yield potential. In all, there are three fruit types. They are (i) *dura* (thick shelled), (ii) *pisifera* (shell-less) and (iii) *tenera* (thin shelled, commercial hybrid - *dura* x *pisifera*). These three kinds of palms look morphologically more or less alike.

Oil palm is a monoecious palm with male and female inflorescence borne in separate leaf axils. In the axils of each leaf, there is a bud, which may develop into a male or female inflorescence. Each inflorescence primordium is a potential producer of both male and female organs. Each inflorescence is a compound spike carried on a stout peduncle. Spikelets are arranged spirally around a central axis. An inner and outer spathe, tightly encloses the inflorescence up to six weeks before anthesis. Afterwards both the spathes are split open and inflorescence pushes its way out.

In a male Inflorescence, the spikelets are long finger-like and is about 10-20 cm long arising from a central stalk bearing between 600-1200 male flowers, which are yellow in colour having a distinct aroma and mature from bottom to top. A mature flower is 3-4 mm long and 1.5 to 2.0 mm wide and is completely enclosed in a triangular bract. It has an outer whorl of three hard and an inner whorl of soft perianth together with six stamens fused to form a tube in the centre. The anthers are bilobed and release pollen grains through the lateral slits. The amount of pollen produced by a single inflorescence is around 50 g and liberated over a period of 2-3 days (Pillai and Ponnamma, 1996). There are distinct male and female flowering cycle in oil palm. Flowering in oil palm is influence by management practices. Stress condition of any kind is likely to induce male flower production, It is estimated that and average of 28 kg of pollen grains are produced in an acre of oil palm plantation annually. The quality of pollen grains required for normal fertilization of female flowers is minimal, thus, leaving large quantity of pollen being unutilized in a plantation. Despite this, no attempt has ever been made to characterize them in all the three types of oil palm with a view to find out the possibility of utilizing them in any beneficial manner especially with regards to their nutritional potentialities. Hence the present study was undetaken to understand the proximal composition of suitable constituents and the structural details of the oil palm pollen.

MATERIALS AND METHODS

Plant material: Mature pollen grains were collected from *dura*, *pisifera* and *tenera* palms identified from oil palm plantation of NRCOP (RS), Palode, Thiruvananthapuram. Healthy male inflorescence was identified and bagged a week before the opening of flowers by a sterilized canvas bag. After one week the spathe of the mature inflorescence was cut and pollen grains were collected by shaking the inflorescence. Subsequently fresh pollen grains were oven dried for removing the moisture at a temperature of 70°C, sieved and stored in

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desiccator for further biochemical and analytical studies.

Electron Microscopy: The fine structure of the pollen was analyzed by fixing the pollen in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 3 hrs. The fixed pollen was dehydrated and coated for SEM observation (Falk, 1980). For the ultra structural study, TEM processing was done by the method of Honetslegrova *et al.* (1996).

Biochemical Analysis: Moisture was determined by IUPAC method of analysis of oil, fats and its derivatives (Paquot and Hautfenne, 1987). A known quantity of fresh pollen was ground in mortar and pestle using 0.1 M PO_4 buffer (pH 7) with 1 mM EDTA. The brei was filtered through two-layered cheesecloth and squeezed gently. The crude filtrate was centrifuged at 10,000 *g* for 15 min and the supernatant was collected for the estimation of proteins. The protein was estimated by Bradford method (1976). The surface protein of the fresh pollen was isolated by using non-ionic detergent Triton X-100 (Nagao, *et al.*, 1987). Freshly collected pollen was moisturized and kept closed for 3 hrs at room temperature. The incubated pollen was treated with 0.1% Triton X-100 in 0.1 M phosphate buffer and vortexed for 15 min. The suspension was centrifuged at 12,000 *g* for 10 min. The supernatant was extensively dialysed against PO_4 buffer and finally against distilled water and lyophilized for further study.

Fresh pollen was put in boiling 80% methanol and refluxed for 10 min. The extract was cooled and homogenized with a pestle. The homogenate was filtered and centrifuged at 10,000 *g* for 10 min. The supernatant was taken for the estimation of free aminoacids using ninhydrin reagent (Malik and Singh, 1994). Total carbohydrates of the methanolic extract of pollen and the reducing sugars of the aqueous extract were determined by Anthrone and DNS method respectively (Southgate, 1976). The soluble phenol of the pollen was extracted in aqueous methanol and the amount was determined colorimetrically at 650 nm using the standard of catechol (Mayr, *et al.*, 1995). The total lipid was quantified from the dry pollen using hexane as solvent by soxhlet method (Paquot and Hautfene, 1987). The minor soluble constituents of the pollen *viz.*, tocopherol and β - carotene was determined according to George and Arumughan, (1992). Mineral analysis of pollen was done by atomic spectroscopy (Bhargava and Raghupathi, 1993).

SDS PAGE: SDS-Polyacrylamide gel electrophoresis was carried out in a Genei Mini model slab gel apparatus following the method of Laemmli (1970) and Fairbanks *et al.*, (1971). Protein was resolved on 10% acrylamide in the separating gel and 4% in the stacking gel.

RESULTS AND DISCUSSION

In Table 1, the quantitative level of soluble constituents present in oil palm pollen of three varieties *dura*, *pisifera* and *tenera* is summarized. The moisture content of pollen was found in the range of 30 - 50%. From the data, it could be seen that oil palm pollen contains a good amount of total proteins at a level of 25 to 40mg/g pollen of three varieties studied, indicating the nutritive value of the pollen sample. Higher amount of protein was seen in the pollen of parentals than the hybrid *tenera*. The surface protein contents in the pollen of *dura*, *pisifera* and *tenera* were 23.3, 24.4 and 15.5 mg/g respectively, which was 64% of total protein. This high amount of protein in surface of pollen suggest the physiological activeness. The pollen of the three varieties showed appreciable amount of glucose, lipids and free amino acids. The

Table 1: Soluble constituents of oil palm pollen

Chemical composition	Dura	Pisifera	Tenera
Moisture (%)	44.5	30.5	51
Total protein (mg/g tissue)	36.30	41.00	24.00
Surface protein (mg/g tissue)	23.30	26.40	15.50
Reducing sugars (mg/g tissue)	77.50	60.90	75.50
Total carbohydrates (mg/g tissue)	77.50	78.79	98.50
Free amino acids (mg/g tissue)	43.00	47.8	27.00
Lipid (mg/g tissue)	32.00	52.0	42.00
Tocopherol (mg/g tissue)	3.90	4.35	4.00
β-carotene (mg/g tissue)	8.70	10.00	7.80
Phenols (mg/g tissue)	5.12	4.78	4.14

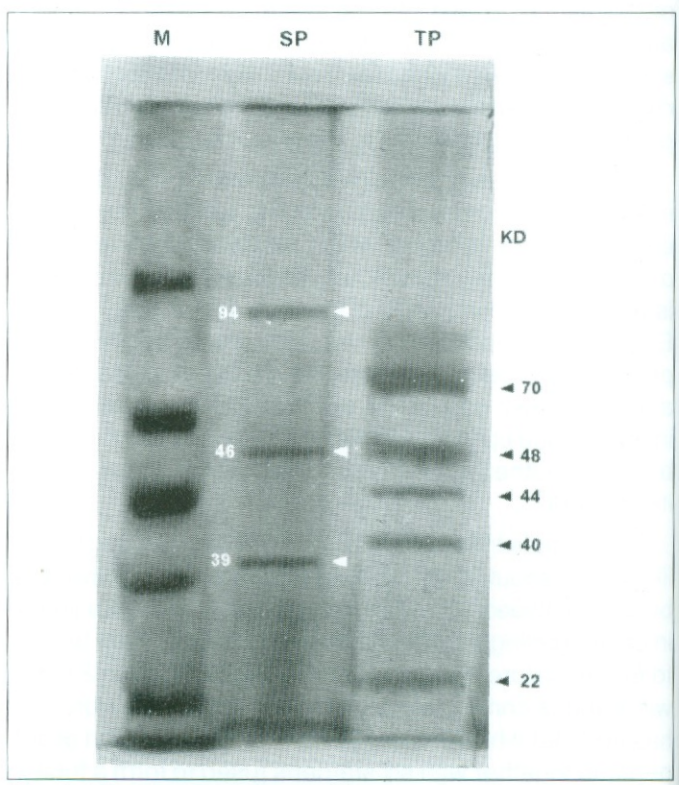

Fig. 1: Electrophoretic profile showing the polypeptide pattern of surface protein (SP) and total protein (TP) M- Marker

average composition of these three constituents glucose, lipids and amino acids in the proportion of 71, 42 and 39 mg/g clearly indicates the affinity of this pollen towards biotic pollination. The tocopherol and β - carotene content was also quantified. The average amount of tocopherol and β - carotene in the pollen of three varieties were 4 and 9 mg/g respectively. Though in micro quantities, the content of tocopherol (vitamin E) and β - carotene (vitamin A) was also promising in the pollen. Apart from the primary constituents, the phenol content showed a value of 4 to 5 mg/g pollen indicating the defensive role and the antioxidant property of this biomolecule. Thus, the vital nature of the pollen is quite convinced from the analytical data.

The polypeptide pattern of total protein and surface protein was compared by SDS PAGE. The electrophoretic profile of the samples is presented in Fig. 1. Total protein yielded a pattern of five thick polypeptide bands with molecular weight corresponding to 22, 40, 44, 48 and 70 kD whereas surface protein showed three prominent bands of molecular weight 39, 46 and 94 kD. The polypeptide bands of surface protein revealed a pattern not comparable with that of total protein indicating surface protein are quite different in their origin.

Electron Microscopy of Pollen: The fine structure of pollen under SEM supports the entomophilic nature and the storage ability. The architecture of the pollen showed a triangular structure with a broad slit appeared trichotomously. The ventral and dorsal surfaces of the pollen appeared as scaly and smooth. The surface area of the pollen and the width of the slit was calculated from the scale as 85 to 95 m and 2 to 3 m respectively (Fig. 2). The SEM of the incubated pollen showed the nature of exudates filled in the pollen slit and the pattern of pollen leaching through the slit (Fig. 3 and 4). Thus the SEM structure of oil palm pollen clearly indicated the proximity of pollen towards biotic pollination.

Fig. 5 and 6 presents the ultrastructure of the pollen under TEM. The outer wall sexine was differentiated into three distinct regions – tectum, columella and foot layer. The outermost roof tectum of sexine appeared uneven due to projections. The columella connects the tectum and foot layer. Beneath the foot layer was the unsculptured inner wall exine. At the slit region the outer wall exine was partially dissolved and inner wall intine appeared as a projection through the slit. Various inclusions like mitochondria, oleosomes, vacuoles are visible in the cytosol indicating the cytochemical nature of the pollen.

As a support of vital components, the mineral content of the fresh pollen is given in the Table 2. In the macronutrients nitrogen content was found between 4.5 to 5% as the major constituent in the pollen followed by calcium, potassium, phosphorus, manganese and sulphur.

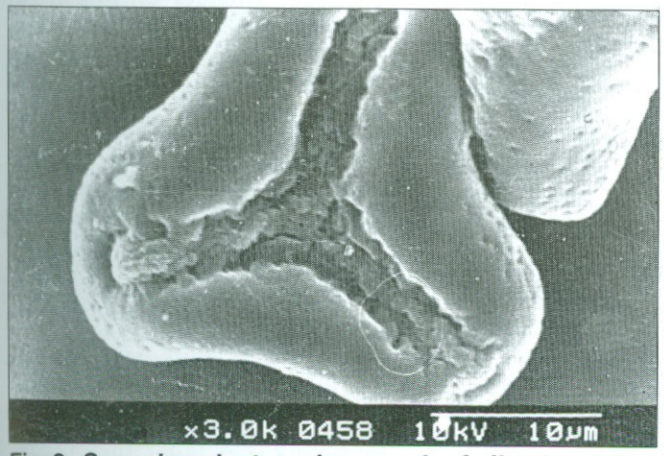

Fig. 2: Scanning electro micrograph of oil palm pollen showing the trichotomous nature and slit

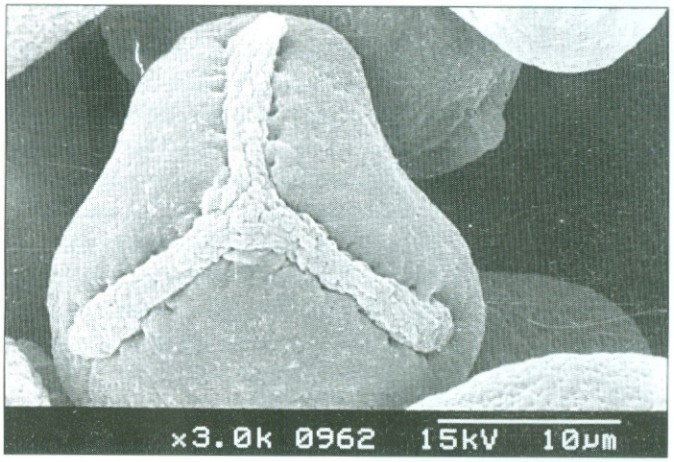

Fig. 3: SEM view of incubated pollen showing exudates

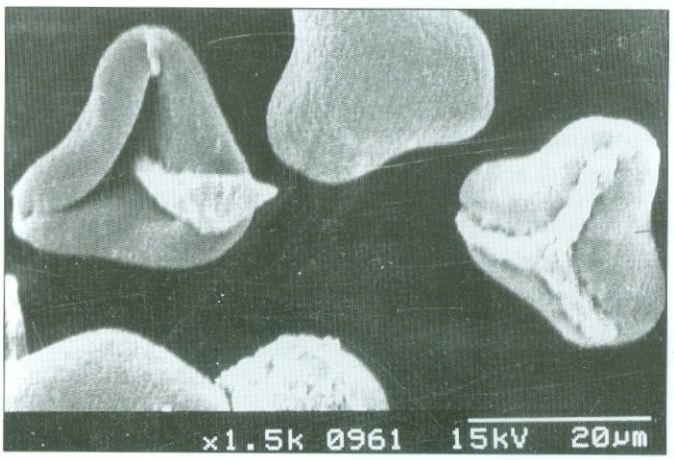

Fig. 4: Fine structure of incubated pollen showing the pattern of exudation

Among the micronutrients, the manganese content (200-500 ppm) exceeded all the other constituents. The composition of macro and microelements of the pollen further supplements the calorific value of pollen. Since the chemical characteristics of oil palm pollen highlights the vital qualities, more nutritional analysis and feeding trials are needed to use these biomolecule as a food supplement.

Table 2: Mineral elements of oil palm pollen

Mineral	*Pisifera*	*Dura*	*Tenera*
Nitrogen %	4.960	4.55	5.13
Phosphorus %	0.465	0.500	0.546
Potassium %	0.860	0.900	0.910
Calcium%	1.510	1.410	1.660
Magnesium %	0.330	0.280	0.300
Sulphur %	0.320	0.310	0.260
Manganese (ppm)	108	111	144
Iron (ppm)	231	445	480
Zinc (ppm)	106	114	118
Copper (ppm)	45	46	44

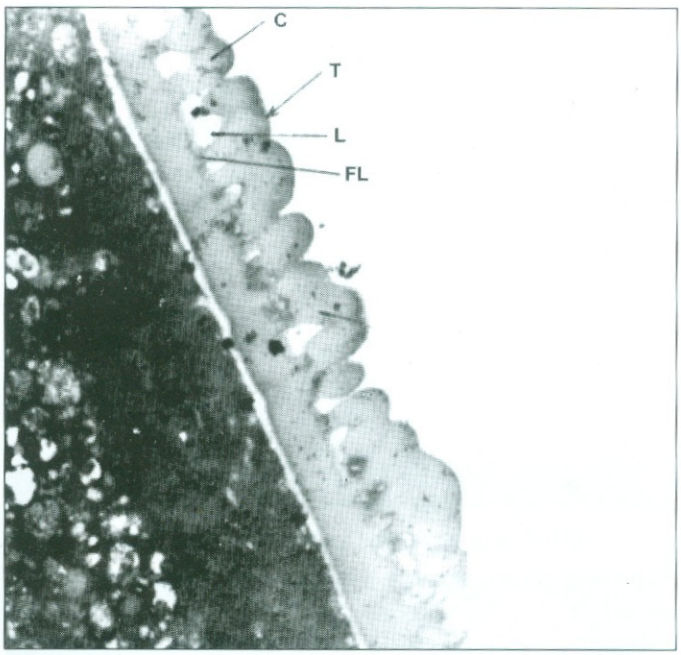

Fig. 6: Magnified view of pollen under TEM showing sculpturing pattern of pollen wall. C-columella, T-tectum, L-Lacunae, Fl-Foot layer

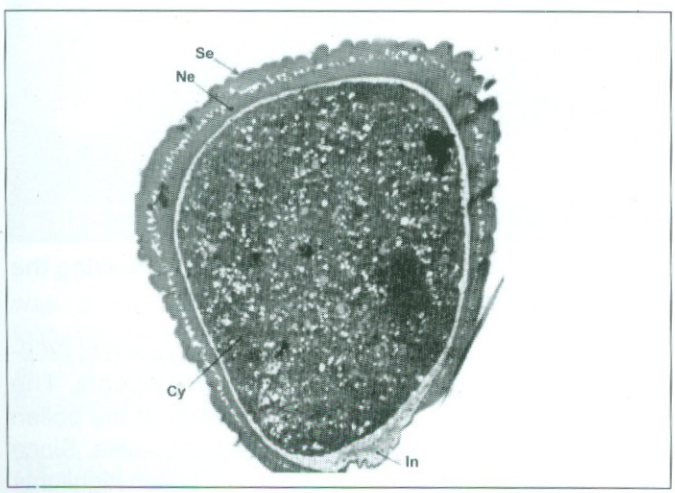

Fig. 5: Transmission electron micrograph of oil palm pollen showing the ultra structure. Se-sexine, Ne-nexine, Cy-cytosol, In - intine

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