ACTIVITIES OF SOME CELL WALL ENZYMES OF THE HAUSTORIUM OF THE GERMINATING OIL PALM (*ELAEIS GUINEENSIS*) SEED

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ABSTRACT
Studies were carried out on the quantity of total hydrolysed oil extracted from tissues around the haustorium of the germinating oil palm seed (*Elaeis guineensis*). The seedlings used ranged in age from day-old to 12 weeks. The activities of cellulase and proteinase enzymes were also determined. The study showed that the levels of hydrolysed lipids around the haustorium gradually increased from the day-old (0.1327g lipid/g of haustorium) to the highest value of 1.4626g/g for the 8th week. This was then followed by a drop to 0.8103g/g by the 12th week. The activity of cellulose (µmole cellobiose equiv./min) rose from 1.93×10^{-4} for the day-old to 16.00×10^{-4} by the 10th week, while the relative activity of proteinase (expressed as change in absorbance per minute) rose from 4.20×10^{-3} (day-old) to 19.20×10^{-3} (10th week). The results suggest an enhanced breakdown of cell wall components of the haustorium, aided by the cell wall enzymes, to facilitate maximal absorption of the stored food reserve.

INTRODUCTION

In the germinating oil palm seed there is the embryo which enlarges and forces its way out through the germ pore, to form externally a small button of tissue containing root and shoot initials. At the same time, the other end of the embryo enlarges to form a cotyledonary structure, the haustorium (Boatman and Crombie, 1958), which starts to absorb the endosperm. As development proceeds, the endosperm is progressively digested and the haustorium enlarges until it eventually fills the seed cavity. Finally the haustorium rots away and the seedling becomes independent (Boatman, 1956). This activity has also been reported in the coconut palm seed (Sugimura and Murakami, 1990). During germination the endosperm immediately surrounding the haustorium softens due to the dissolution of the middle lamellae and cellulose cell wall thickenings; the cell contents are then broken down and absorbed. About 160 days after germination, only a single layer of endosperm cells remains within the testa (Boatman and Crombie, 1958). As with other oil seeds, the oil palm seedling presumably mobilises reserve triacylglycerols during germination and channels the acetyl-CoA formed by β-oxidation into the glyoxylate cycle (Oo and Stumpf, 1983).

In mature plant seeds, the major class of lipids is triacylglycerol, which may constitute between 10 and 70% of the dry weight (Opute, 1979, Nartey *et al.*, 1974). One of the characteristics of a plant cell is the possession of a rigid cell wall which functions to counteract the physical and osmotic pressure produced by the cell contents. This pressure is the driving force of cell enlargements; cell elongation results from a yielding of the wall to this pressure (Bonner and Venner, 1976). The cell wall contains both cellulosic and non-cellulosic polysaccharides. Germinating seeds have been shown to contain cellulose degrading enzymes (Bonner and Venner, 1976, Hasegawa and Smolensky, 1971, Hinton and Pressey, 1974), including glucanases, pectin methylesterase, ATPases and various phosphatases. Proteinase activity has been reported in *Carica papaya* in which the activity declines as the fruit ripens (Paul and Chen, 1983), while cellulose activity was found to correlate directly with ripening in avocado (Pesis *et al.*, 1978), strawberry (Abeles and Takeda, 1990), guava (Abu-Goukh and Bashir, 2003) and tomato (Opiyo and Ying, 2010). However follow-up studies have been few, and consequently not much is known about proteinase activity, perhaps arising from the

fact that cell walls usually contain very small amounts of protein.

MATERIALS AND METHODS

Day-old germinated oil palm seedlings (*Elaeis guineensis*) were collected from the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria.

Gernination of Seeds

The sprouted oil palm seeds were grown in a germinating tray containing white sand and kept outside for absorption of sunlight and then watered daily until needed.

Extraction of Hydrolysed Lipids around the Haustorium

At different stages of development about 50 of the germinating seedlings were removed, washed with water and cracked manually. Care was taken to separate the haustorium from the adhering droplets of the semi-liquid endosperm (lipids) and sprayed with some quantity of acetone using a spray gun. The extract was concentrated in a rotary evaporator at 40°C. The extracted lipid was then weighed (Boatman, 1956).

Extraction of Cellulase and Proteinase

Five grammes of haustorium was homogenised in 50ml pre-cooled 0.5M NaCl solution; the mixture was refrigerated for 15 minutes and then centrifuged at 3000*g* for 20 minutes. The resulting supernatant was used for the enzyme assay.

Assay of Proteinase

The method of Miller and Hancock (1965) was used. The reaction mixture consisted of 2ml of 1% casein in 0.1M sodium phosphate buffer at pH 6.0 and 0.2ml enzyme extract. The reaction was terminated after 30 minutes by the addition of 1ml of 30% TCA and the mixture was centrifuged at 3000*g* for 10 minutes. The relative activity of proteinase was determined by the increase in absorbance of the supernatant against reagent blank at 280nm and expressed as absorbance change per minute.

Assay of Cellulase

The method of Paul and Chen (1983) was employed. To 3ml of 0.5% carboxymethyl cellulose was added 1ml each of enzyme extract and 0.04M sodium acetate buffer at pH 4.8. An identical sample heated for 3 minutes served as the blank, and 0.1ml of 0.1% chloramphenicol solution was added to each mixture to inhibit the growth of microorganisms. Both mixtures were then incubated at 30°C for 17 hours and then inactivated by heating in a boiling water bath for 3 minutes. The samples were cooled and then centrifuged at 3000*g* for 10 minutes, then 1ml of supernatant was withdrawn from each and analysed for reducing sugar content using the method of Somogyi (1944).

RESULTS

Table 1. Total oil extracted from around the haustorium.

Period of germination	Quantity of oil (g)
Day old	0.1327
2 weeks	0.7036
4 weeks	0.7928
6 weeks	0.8242
8 weeks	1.4626
10 weeks	0.8768
12 weeks	0.8103

Table 2. Cellulase activity in the germinating oil palm seedling.

Period of germination	Cellulase activity (µmole cellobiose equiv. released/min × 10^{-4})
Day old	1.93
2 weeks	1.93
4 weeks	9.97
6 weeks	10.03
8 weeks	10.64
10 weeks	16.00
12 weeks	15.54

Table 3. Proteinase activity in the germinating oil palm seedling.

Period of germination	Relative proteinase activity (absorbance change/min × 10^{-3})
Day old	4.20
2 weeks	6.50
4 weeks	8.21
6 weeks	11.93
8 weeks	15.62
10 weeks	19.20
12 weeks	19.17

DISCUSSION

The results obtained from this study show a gradual increase in the level of hydrolysed lipids extracted from the haustorium of the oil palm seed as germination progressed. The observed increase in lipid content in the degraded endosperm around the haustorium is in agreement with Alang *et al.* (1988), who reported that the proportion of total lipid in residual endosperm of the oil palm seed was about the same as that of undegraded endosperm, while observing a markedly increased level in degraded endosperm. At the early stage of germination, the growing seedling has a very small haustorium; consequently the surface area of the endosperm in contact with it is small. As a result of this limited contact surface for interaction between the haustorium and the endosperm, only a small amount of oil is likely to be liberated from the stores. The gradual increase in the amount of oil extracted from around the haustorium parallels the observed increase in the activities of the cell wall degrading enzymes, and this suggests a gradual increase in the rate of breakdown of the endosperm cell wall by the hydrolytic enzymes of the haustorium. The increase in activities of these two enzymes (cellulose and proteinase) up to the 10th week of the study could be linked to the accelerated growth being undergone by the seedling at this stage, requiring an enhanced rate of food mobilisation to meet its metabolic needs. This finding agrees with earlier reports on some cell wall hydrolysing enzymes such as α-galactosidase and β-mannosidase (Alang *et al.*, 1988). Similar increases in activity have also been reported for germinating coconut endosperm (Samonte *et al.*, 1989).

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