Molecular and developmental analysis of the fruit abscission zone and shedding process in the oil palm species *Elaeis guineensis* and *Elaeis oleifera*.

### Introduction

Oil palm (*Elaeis guineensis*) belongs to the Arecaceae family and is the number one source of edible vegetable oil worldwide. Previous studies suggest that the fruit shedding process in oil palm is dependent on ethylene at the different anatomical level from other fruits (Henderson and Osborne, 1990 and 1994; Osborne et al., 1994). In most fruits, there is a synchronized series of cell separations between the fruit stalk and the fruit. In addition, both the *Cel1* and *PLL* transcripts are enhanced in response to the ethylene treatment that leads to cell separation and fruit shedding (Figures 1, 2 and 5). In the future, we plan a comprehensive histological analysis of the oil palm abscission process in the future. In addition, both the *Cel1* and *PLL* transcripts are enhanced in response to the ethylene treatment that leads to cell separation and fruit shedding (Figures 1, 2 and 5). In the future, we plan a comprehensive histological analysis of the oil palm abscission process. In the past, we have examined the abscission zones and physiological factors that affect the cell separation processes that lead to fruit shedding in two oil palm species, *Elaeis guineensis* and *Elaeis oleifera*. In addition, we also examined the shedding process in the mangosteen (*Garcinia mangostana* L.). We have identified a number of candidate genes encoding cell-wall-modifying enzymes and have examined their transcript accumulation patterns in the AZ of *E. guineensis*. Our results indicate that the fruit shedding process differs between the two species and will provide an excellent model for understanding the molecular mechanisms associated with abscission and fruit shedding, a key agronomic character of oil palm.

### Results and Discussion

#### **E. guineensis Fruit Abscission Zones**

- **Kernel**, **Tepals** (Radundary androecium (stamens))
- **Mespocarp**
- **Adjacent Zones**
- **Primary Abscission Zone**

**Figure 1.** Diagrammatic representation of a partial longitudinal section through an *E. guineensis* fruit to illustrate the primary and adjacent abscission zones (adapted from Osborne et al., 1992).

#### **Inter- and Intra-Species Differences in the Effect of Ethylene on Oil Palm Fruit Abscission**

- E. *guineensis* PO6851 and PO6925 are different lines
- E. *oleifera* II are different

**Figure 2.** (A) Treatment of spikelets of oil palm fruits with 10 ppm ethylene for 24 hours increases the number of fruit that begin to detach both from oil palm species and the marketed fruit phenotype. However, 100% of the fruit from both the marketed and *E. guineensis* PO6851 began to be or were completely detached, only 30% of the normal *E. oleifera* fruit began to detach and the remainder were attached to the adjacent zones. (B) In addition, E. *guineensis* fruit tepals remained attached to fruit, whereas the *E. guineensis* tepals remain attached to the fruit stalk after ethylene treatment. (C) Detachment of fruit from the *E. guineensis* PO6851 line was delayed by 24 hours compared with *E. oleifera* II.

- 1 budding was determined by applying physical pressure to the fruit to test for the detachment of the cell separation events. 160 days after pollination (DAP) polination date unknown, (stage II, green; stage III orange). PO6851 and PO6925 are different lines.

**Figure 4.** Naphthol Blue Black stained longitudinal sections through abscission zones from oil palm flowers and fruits. (A) *E. guineensis* normal (B) and mantled female flowers before pollination (C) and *E. guineensis* 120-DAP fruit (D), *E. oleifera* fruit before (E) and after C3H4 treatment, and (F) *E. guineensis* 120-DAP fruit and (G) mantled fruit after C3H4 treatment. Above dotted lines indicates the AZ (Primary Abscission Zone) in the female flower, MM (Stamens), DC (Cap), Br (Bracteole), P (Pedicel), T (Tepal) (Petal (Pericarp), M (Mesocarp).

**Figure 3.** An analysis of the ethylene released during 24 hours from fruits of *E. guineensis* PO6925 (A) and *E. oleifera* (B) at different stages of development. While the pollination dates of *E. oleifera* are unknown, stage II is green whereas stage III is orange indicating a more advanced stage of development.

**Figure 5.** (A) Transcript profiles of cell wall modifying genes candidates from *E. guineensis* samples collected from the field were examined in the AZ (A) and mesocarp tissue (B) during *E. guineensis* fruit development and in response to ethylene treatment in the laboratory (C). Primers for *PG, Cell1*, *PLL, PME* were designed and utilized in an ethylene and ethylene treatment. Above dotted lines indicates the AZ (Primary Abscission Zone) in the female flower, MM (Stamens), DC (Cap), Br (Bracteole), P (Pedicel), T (Tepal) (Petal (Pericarp), M (Mesocarp).

**Figure 6.** Ethylene released in g/L/gFW/h from fruits of *E. oleifera* (A) and *E. guineensis* (B) before and after C3H4 treatment. Above dotted lines indicates the AZ (Primary Abscission Zone) in the female flower, MM (Stamens), DC (Cap), Br (Bracteole), P (Pedicel), T (Tepal) (Petal (Pericarp), M (Mesocarp).

#### **Histological analysis of the oil palm abscission zone**

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- **PG-Polygalacturonase**
- **Cell1 (β-1, 4 glucanase)**
- **PLL-Pectate lyase protein**
- **PME-Pectin methyltransferase**
- **EFA- Elongation factor alpha**
- **FB- Flower Base**
- **Stamen**
- **Kernel**
- **Stamens**
- **Tepals**
- **Mantled bracts**
- **Mantled flower**
- **Mantled fruit**
- **Staminal bract**
- **Staminal flower**
- **Staminal fruit**

- **Table 1.** Transcript accumulation patterns in the AZ of *E. guineensis* and *E. oleifera*. The PG and Cell1 transcripts are enhanced in response to ethylene treatment in the laboratory (C). Ethylene treatment, and one in the AZ at 180-DAP. A PME transcript was detected in the AZ at all stages and in the mantled AZ, but was only detected in the 30 and 120-DAP mesocarp samples. Both Cell1 and PME transcript accumulation is enhanced in the ethylene treated samples, whereas no increase was seen for PG or PME. The PME transcript accumulation was lower in the samples used in the ethylene experiments and the mantled fruit samples than in field collected samples.

### Conclusions and Prospects

Here we present the first comparison between the fruit shedding process and abscission zones from the two species of oil palm, *E. guineensis* and *E. oleifera* and the marketed phenotype. Our results indicate that a 24 hour treatment with ethylene induces cell separation to begin in all fruits examined (Figure 2A and 4E, F, G). However, there appear to be differences in the abscission process between the *E. guineensis* and *E. oleifera* and the marketed phenotype given their different responses to ethylene. This differential response may be due to differences in the structure of the AZ in the three fruit types or other yet unknown molecular factors. Earlier work indicated that the abscission process in *E. guineensis* is a two-phase delayed process involving primary and adjacent abscission zones (Figure 1, Henderson et al., 1990). When treated with ethylene, *E. oleifera* fruit reveal two characteristics that are different from *E. guineensis* (1) all the fruit are shed from the spikelet after the 24 hour treatment and (2) do not remain attached at adjacent zones. There are two possible explanations, either there are no adjacent abscission zones or there are adjacent abscission zones and the AZ process could occur within the 24 hour treatment indicating differences in the signalling leading to abscission. In support of the later hypothesis, we found that *E. oleifera* fruit release qualitatively less ethylene during development which suggests differences in the signalling process that leads to abscission. In support for the former hypothesis, histological analysis indicated that the AZ of *E. guineensis* is different from *E. oleifera* given that *E. guineensis* has no pedicel with the fruit attached directly to the spikelet tissue (Figure 4D and E). In addition, the tepals remained attached to the *E. oleifera* fruit that suggests differences in the structure of the AZ in the species. 100% of the marketed fruit began to shed in response to ethylene, however, some fruit remained at adjacent zones which indicates that they still function despite the transformation of the staminal into pseudocarp near the adjacent zones (Figure 4B and G, Adam et al., 2005, 2007). One explanation for this increase in ethylene sensitivity may be due to other modifications that occur in the marketed fruit that effect the abscission process. Finally, towards understanding the molecular mechanisms involved in the abscission process of oil palm, we have identified putative cell wall modifying enzyme genes, including *PG, Cell1, PLL* and *PME* from *E. guineensis* lines.

### References
