Towards Understanding and Controlling Abscission in the Oil Palm

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ABSTRACT

Losses in oil yield and quality are often attributed to the non-synchronous ripening of oil palm bunches and the shedding of loose fruits. Manipulation of the abscission process offers valuable opportunities for increasing the productivity of oil palm. Biotechnology or conventional breeding may be exploited to delay the onset of abscission or prevent it altogether.

The genes involved in abscission zone differentiation, ethylene production or cell wall breakdown are possible targets for genetic manipulation for achieving delayed or non-abscission.

Conventional breeding can exploit the naturally-occurring non-shedding palms. The virescens palm, the fruits of which are green when unripe and red when fully ripe may be a better candidate than the nigrescens for exploitation of this trait.

Both the biotechnology and conventional breeding approaches require an understanding of the abscission process.

INTRODUCTION

The harvesting of ripe oil palm bunches and their transport to the mills often result in losses that reduce the oil yield and quality. The non-synchronous ripening of the bunch and the shedding of loose fruits, i.e. the ripest in the bunch, are mainly responsible for these losses. The detached fruits are often bruised when they fall, resulting in the activation of lipases and hence, lowering of oil quality. Furthermore, the collection of loose fruits is a major problem in Malaysia because of the shortage of labour. Strategies for non-shedding may be beneficial to the oil palm industry. An understanding of the abscission process is therefore important in efforts to control the process.

ABSCISSION

Fruit abscission, like senescence, is not a random but a metabolically determined cell separation process. Abscission zones are precisely positionally differentiated and the organs are ultimately shed at these pre-determined locations. Morphological and biochemical studies have shown that the cells that constitute the abscission zone respond differently from their neighbours to the same hormonal signals. The separation usually takes place between cells at specific locations at the base of the organ, and,

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in most cases, the specific cells that separate from each other are differentiated early in the development process. The active cell separation process at fruit ripening depends on the secretion of specific enzymes and the loosening of chemical bonds in the middle lamella (Roberts et al., 2002; Sexton and Roberts, 1982). The level of ethylene generally rises in ripening and senescing organs prior to and during abscission. Ethylene is believed to be involved in the signaling of the cell separation processes. Auxins and, to a lesser extent, gibberellins and cytokinins have been shown to be effective in delaying the process of abscission in many plants. The balance between the hormones may be the key factor that regulates the process (Taylor and Whitelaw, 2001) with ethylene as a natural accelerator of abscission and auxin as a brake. Such a mechanism would enable abscission not to respond to elevated ethylene production should it be premature, e.g. during the ripening of climacteric fruit. Although the capacity of auxins to delay abscission has been extensively documented, the mechanism of how the cells within the abscission zone are prevented from responding to ethylene is not clearly understood.

This interactive regulatory role of auxins and ethylene in the abscission process has been substantiated in the oil palm (Somasundram et al., 1994). Pre-treatment with either the natural auxin, IAA, or synthetic auxin, 2, 4 D, was able to modify the timing of fruit detachment. As early as 1972, Chan et al. investigated the possibility of delaying fruit abscission by using plant growth regulators. They reported that treatment of ripening bunches with auxins, gibberellic acid or ethephon retarded abscission and harvesting could be delayed for up to five days without any increase in the number of detached fruits.

**ANATOMY OF ABSCISSION**

In the oil palm fruit, a rudimentary androecium surrounds the base of the ovary. Adjacent to the rudimentary androecium are an inner and outer whorl of tepals. As the fertilized fruit enlarges, the cells of the rudimentary androecium and tepal bases continue to divide thus keeping pace with the increase in diameter of the base of the fruit. This results in the fruit being attached to the spikelet at a junction with three distinct tissue types. Anatomical studies by Henderson and Osborne (1990) showed that a unique two-stage cell separation process occurs in the oil palm. Most fruits display a synchronous series of cell separations across a single plane of cells between the fruit and pedicel (stalk), resulting in immediate shedding of the fruit. The shedding of oil palm, however, is unique in that it follows a bi-phasic progression with a time lag of one to two days between the two phases. The abscission event is first induced at a pre-determined and positionally differentiated locus at the junction of the fruit and its pedicel (position 1) (Figures 1a and b). The site for this first stage of abscission (position 1) is clearly defined even before anthesis by a line of small cells with dense cytoplasmic granules (Osborne et al., 1992). Abscission and separation at position 1 only occur when the fruit is fully ripe. The fruit is loosened at this stage and can be easily picked. However, it is not shed, but still firmly held within the surrounding cup of the rudimentary androecial ring, the tepals, bracteoles and spiny floral bract.

The second stage of abscission only occurs after abscission at position 1 is complete. This usually occurs at position 2, i.e. in the parenchyma adjacent to the surrounding tissue of the rudimentary androecium so that the fully ripe fruit is detached while the rudimentary androecium and all the tepals are still attached to the pedicel in the spikelet in the form of a cup. Frequently, however, especially if a slightly less ripe fruit is mechanically disturbed, the cell separation may occur outside the ring of the rudimentary androecium at position 3. The fruit is then shed with some or all the androecial tissue attached but free of the enclosing tepals. When a fruit spikelet is removed from the bunch before the fruits are fully ripe, they will eventually detach. The separation always occurs first at position 1 followed by a second stage across the bases of the tepals, i.e either position 4 or 5. Separation at positions 2 and 3 are bypassed. Fruits separating under these conditions retain some of the rudimentary androecium and are still enclosed in the ring of tepals leaving the stump of the pedicel fully exposed. The spiny floral bract and bracteoles are never shed. Unripe fruits do not undergo cell separation at position 1 or other positions and as a consequence are not shed.

**ENZYME CASCADE**

A whole cascade of enzymes is induced during fruit abscission and this is preceded by an array of changes in gene expression such as the up-regulation of genes encoding for cell wall degrading enzymes. The β-1, 4-glucanase was the earliest enzyme suggested to be involved in
Figure 1. Longitudinal section of oil palm fruit showing a) abscission zone and floral appendages; b) the different positions where cell separations can occur.

wall loosening at the abscission site. The \( \beta-1, 4 \)-glucanases are part of a large gene family of which seven members (Cel 1 to Cel 7) have been isolated (Brummel et al., 1997; del Campillo, 1999) in tomato and Arabidopsis.

Polygalacturonase plays an important role in abscission and increases in polygalacturonase activity have been reported for the shedding of leaves, flowers and fruits (Roberts et al., 2002). In tomato, three polygalacturonase gene family members, TAPG 1, TAPG 2 and TAPG 4, are associated with abscission. Despite sharing high homology at the nucleotide level (80%-90%), their transcripts (1.5 kb) are substantially shorter than in the fruit enzyme (1.9 kb) and share a sequence identity of only 50% (Kalaitzis et al., 1997).

Although abscission is linked to the induced expression of \( \beta-1,4 \) -glucanhydrolyase (cellulase) by the separating cells of the abscission zone in dicot fruits and leaves, this is not the case for the oil palm. The abscission zone of the oil palm does not produce cellulase although ripening mesocarp tissue does. Further evidence for this is the fact that the cells of the mesocarp do not separate despite the production of cellulases (Osborne et al., 1992). At position 1 of the oil palm fruit, an active polygalacturonase is produced at abscission while high levels of a \( \beta-1,3 \) -glucanhydrolyase are induced at positions 2 and 3 (Osborne et al., 1992). These differences in the enzymes involved are obviously a reflection of the overall differences in cell wall composition of the oil palm from other plants. It also indicates that the abscission process is different in the oil palm and shows differences in the major enzymes induced in the two distinct stages of abscission.

**SIGNAL FOR ABSCISSION**

Abscission occurs as a result of loss of adhesion between highly active living cells. The process involves wall breakdown which is usually confined to two or three layers of cells in a plane across the base of the organ to be shed. All living classes of cells in this plane appear to behave in a similar way. However, the regulation of this spatial and temporal organization is not well understood.

The inductive stimulus appears to be a rise in ethylene and perhaps abscisic acid and a reduction in auxin. The induction is followed by a *lag phase* prior to weakening of the zone in which the cells that will separate become recognizable as they accumulate cytoplasm and organelles. The cells usually have increased rates of respiration and protein and RNA synthesis compared to the adjacent tissues. Protein and RNA synthesis inhibitors applied during the early lag phase effectively inhibited abscission (Sexton and Roberts, 1982). The tensile strength of the abscission zone decreases during the separation phase due to breakdown of the middle lamella. Ethylene markedly accelerates the separation phase. It increases the activity of the cellulases and polygalacturonase and stimulates their secretion into the wall.

In most crops where fruit shedding has been extensively studied, the signal for abscission is directly linked to a critical level of ethylene produced by the ripening fruit which triggers the ethylene-responsive target cells of the abscission zone. In tomato and other fruits, ethylene synthesis increases as ripening proceeds and reaches a threshold at full ripeness and induces the expressions of cell wall modifying enzymes (glucanhydrolases) that loosen adhesion at the zone cell interphase with neighbouring cells. Although the secreted enzymes may migrate considerable distances through the cell walls of adjoining tissues, the line of separation is very precise. This shows substrate specificity between the secreted enzymes and the walls of a limited number of cells which are restricted to the immediate vicinity of the zone (Osborne, 1989).

In most fruits, once the abscission cascade of enzymes is produced, separation is initiated and the fruit shed. This is quite different in the oil palm. The levels of ethylene produced remain insignificant throughout fruit ripening when lipid and carotene levels increase. It is only when the lipid content is near the maximum (and the fruit deep orange in colour) that ethylene synthesis starts to increase. This starts at the apical end of the fruit and quickly progresses towards the base. Ethylene production is closely linked to the onset of cell separation at position 1. Time-course experiments have shown that only fruits that exhibit a rise in ethylene synthesis initiate separation at position 1 (Osborne et al., 1992). Several layers of cells that synthesize neither carotene nor storage lipids form a narrow barrier between the mesocarp and the cells of the zone. It is at the junction of these barrier cells and zone cells that the first separation takes place.

The second stage separation at positions 2 or 3 is dependent on the first stage separation at position 1 and not directly on the signal of
ethylene (Osborne et al., 1992). A different signal generated by the separating cells at position 1 appears to trigger separation at positions 2 and 3. When the rudimentary androecium develops into a carpel, as in the case of mantled fruit, the cells are differentiated and no longer responsive to the abscission-inducing signals and hence cell separation processes.

**NON-SHEDDING VARIANTS**

As early as 1967, Hartley described the occasional occurrence of abnormal fruit development in the oil palm. This refers to normal crosses and should not be confused with clonal abnormality. In these fruits, the rudimentary androecium, instead of remaining a vestigial organ, enlarged and developed into six (usually) supplementary carpels around the main fruit. These parthenocarpic lobes were similar to the rest of the mesocarp in that they synthesized both lipids and carotenoids and ripened simultaneously with the fertile ovary. Some seedlings and at least one genetic line have routinely produced such fruits and offered a potential for high yield on account of the additional lipid-rich lobes. However, the potential was not realized because the fruits were not shed and hence, there was no *loose fruit* signal for harvesting. The bunches were left on the palms to rot.

Donough et al. (1995) reported three non-shedding palms in a 1987 planting of Deli *Dura* x AVROS *pisifera* material at Pamol, Sabah. A similar mutant was discovered in a 1987 planting of Deli *Dura* x Ulu Remis *pisifera* material at Pamol, Kluang (Donough et al., 1995). Bunch analysis showed that the oil content of the non-shedding bunches was comparable to those of normal palms in the same plantations. Detailed studies showed that the aberrant abscission process was due to the absence of certain enzymes.

**OPPORTUNITIES FOR CONTROLLING ABSCISSION**

Manipulation of the abscission process offers valuable opportunities for increasing the productivity of the oil palm. Biotechnology or conventional breeding may be exploited to delay the onset of abscission or prevent it altogether.

**Biotechnology**

The genes involved in abscission zone differentiation, ethylene production or cell wall breakdown are possible targets for genetic manipulation for achieving delayed or non-abscission. Attenuating the sensitivity of the abscission zone cells to ethylene or IAA may provide a mechanism for preventing abscission. Impeding the differentiation of abscission zone cells by heterologous expression of genes such as *jointless* (see below) may be another possible strategy.

A spontaneous mutant that does not abscise any organs was reported for *Lupinus angustifolius* (Clements and Atkins, 2001). Ethylene production by the different organs of the plant was similar to the wild type. The authors concluded that the mutation was specific to the abscission process and was probably due to a lack of or delay in the expression of hydrolytic enzymes(s) associated specifically with abscission zone differentiation and separation. Such spontaneous mutations may also be exploited in the oil palm.

Tomato plants transformed with an antisense endo-1, 4-beta-glucanase (cellulase) *Cel 2* produced transgenic lines, where the *Cel 2* mRNA abundance was reduced by more than 95% in the ripe fruit mesocarp and about 80% in the fruit abscission zones relative to non-transgenic controls. No differences in ethylene evolution were observed and softening of the pericarp tissue was indistinguishable from that in the non-transgenic controls. However, the suppression of *Cel 2* mRNA accumulation caused a significant increase in the force required to cause breakage of the abscission zone. Thus, the *Cel 2* gene product contributes to cell wall degradation during fruit abscission, but apparently does not play a role in the softening or textural changes associated with ripening (Brummel et al., 1999).

MADS-box proteins are a family of transcription factors that are involved in the establishment of specific sites for cell differentiation. A mutant of tomato, designated *jointless*, does not develop a pedicel abscission zone and the gene responsible for this phenotype was identified by map-based cloning to encode a MADS-box protein (Mao et al., 2000).

Ethylene biosynthesis may be inhibited by silencing 1-aminocyclopropane-1-carboxylate (ACC) synthase. ACC synthase is a regulatory enzyme and the amount of ethylene produced depends on the level of transcription of this ripening-specific gene.
Ethylene production in the oil palm is tightly linked with the onset of cell separation at position 1. However, ethylene is a hormonal signal for several metabolic processes and inhibiting ethylene synthesis may affect these processes. A more targeted approach would be to silence the ethylene-responsive genes associated with position 1. An abscission zone-specific promoter would direct expression of the relevant genes in the abscission zone. It is envisaged that the oil palm, like most other plants, has several classes of polygalacturonase. Inhibiting the activity of the polygalacturonase enzyme associated with position 1 by silencing the gene may also inhibit abscission.

Antisensing the β-1, 3-glucanhydrolase gene of positions 2 and 3 of the oil palm would result in loosening of the fruit at position 1 but shedding will not occur as the fruit will be held within the surrounding cup of the rudimentary androecial ring.

**Breeding**

The naturally occurring non-shedding palms are promising candidates for breeding for non-abscission. However, exploitation of the non-shedding character will be difficult in the current oil palm planting materials which are predominantly the *nigrescens* type and hence,
difficult to determine if a bunch is fully ripe in the absence of loose fruits. The virescens palm, the fruits of which are green when unripe and red when fully ripe (Figure 2), may be a better candidate for exploitation of the non-shedding trait. Pamol initiated a programme in which the non-shedding mutants were crossed with virescens palms in an attempt to produce virescens non-shedding progenies. Selfings and crosses of the first three non-shedding palms have been planted in Sabah in 1997 and are now fruiting (Rao, pers. comm.). The results are, however, too few to make firm conclusions on the heritability of the trait.

CONCLUSION

The powerful tools of biotechnology or conventional breeding can be exploited to control fruit abscission in the oil palm. Most of our current knowledge on oil palm abscission comes from studies carried out by Osborne and co-workers. In the efforts to produce a non-shedding palm, one must also take into account the effect of such bunches on the milling process. One technical problem that may be encountered is that of unstripped bunches. A detailed understanding of the abscission as well as milling process is important to strategise the routes for achieving non-abscission.

REFERENCES


BRUMMELL, D A; HALL, B D and BENNET, A B (1999). Antisense suppression of tomato endo-1, 4-beta-glucanase Cel2 mRNA accumulation increases the force required to break fruit abscission zones but does not affect fruit softening. Plant Mol. Biol., 40: 615-622.


