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Physiological and biochemical changes during banana ripening and finger drop

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Abstract

Fruit drop of banana is due to breaking at the junction of the pedicel and pulp, and we found no true abscission zone. The breakage seems therefore due to weakening of the peel. We investigated pectin hydrolysis and some properties at the rupture zone, using 'Hom Thong' (*Musa acuminata*, AAA Group) and 'Namwa' (*Musa x paradisiaca*, ABB Group) fruit, which show massive drop and no drop, respectively. During the period of finger drop, the water content of the peel in 'Namwa' was similar to that of 'Hom Thong' and thus water content does not account for the high breakage in 'Hom Thong'. The peel thickness at the rupture area in the two cultivars was not significantly different. During the period of finger drop, the level of water-soluble pectin in the peel at the rupture area of 'Hom Thong' was higher than that of 'Namwa', indicating pectin degradation. CDTA soluble pectin and insoluble pectin was lower in 'Hom Thong', also indicating more pectin breakdown in this cultivar. Polygalacturonase activity in the peel at the rupture area of 'Hom Thong' bananas rapidly increased, but not clearly more than in 'Namwa' bananas. Pectinesterase activity in the peel at the rupture area of 'Hom Thong' was much lower than that of 'Namwa' bananas, and thus does not account for the breakage. Pectate lyase in the peel of 'Hom Thong' was considerably higher than in that of 'Namwa'. The present data indicate that the much higher pectate lyase activity in 'Hom Thong' might be responsible, at least partially, for the finger drop.

Keywords: Banana; Finger drop; Polygalacturonase; Pectinesterase; Pectate lyase

1. Introduction

Finger drop has been defined by Baldry et al. (1981) as the physiological softening and weakening which causes the individual fruit in a hand to separate from the crown. Hands of banana with fingers missing cannot be sold to consumers and individual fruit that have dropped have no pedicel and cannot be marketed. Finger drop has been reported in a diploid cultivar (Prayurawong, 1999), in the triploid Cavendish AAA Group (Semple and Thompson, 1988), and in tetraploid cultivars (Marriott, 1980). Susceptibility varies widely. For example, among the triploid cultivars 'Valery' is considerably more prone to finger drop than 'Gros Michel' (New and Marriott, 1983).

Banana finger drop is stimulated by high relative humidity and high ripening temperature (Semple and Thompson, 1988), and by ethylene (Paull, 1996). In addition, more mature hands are apparently more sensitive to finger drop (Paull, 1996).

Finger drop seems to be due to localized weakening of peel at the pedicel (New and Marriott, 1983; Semple and Thompson, 1988). Softening of banana peel, as that in other tissues, has been suggested to be due to depolymerization of pectic substances in the primary cell wall and the middle lamella (Seymour, 1993). Banana peel softening during ripening might therefore involve a number of cell wall hydrolases.

Banana pulp ripening has been associated the activities of both endo-polygalacturonase (endo-PG) and exo-PG (Wade et al., 1992), with that of pectinesterase (PE, also called pectinmethylesterase; Wade et al., 1992), and pectate lyase

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(PL; Marin-Rodriguez et al., 2003; Payasi and Sanwal, 2003; Lohani et al., 2004). It is not known if the same hydrolytic enzymes are active in banana peel. The activity of PL in the peel was below the detection limit (Marin-Rodriguez et al., 2003), although a peel PL gene was reportedly expressed (Dominguez-Puigjaner et al., 1997; Marin-Rodriguez et al., 2003). Several other hydrolytic enzymes genes might be involved in pectin degradation.

It is not known if the activity of any of these hydrolytic enzymes is associated with banana finger drop. We here report on two cultivars, one with high and one with no finger drop. We determined the weight of the fruit, the firmness of the fruit, the diameter of the stalk at the abscission zone, the thickness and the water content of the peel at the abscission zone, a number of pectin fractions and the activities of PG, PE and PL.

2. Materials and methods

2.1. Plant material

Banana fruit of the cultivars 'Hom Thong' (*Musa acuminata*, AAA Group) and 'Namwa' (*Musa x paradisiaca*, ABB Group) were harvested at commercial maturity. The fruit were placed in corrugated cardboard boxes and transported by refrigerated truck (25 °C) to the laboratory within 3 h of harvest. In the laboratory, hands were selected for size and color and cleaned in a solution of 100 $\mu l\,l^{-1}$ chlorine (Clorox). The hands were then dipped for 2–3 min in 500 $mg\,l^{-1}$ ethephon for uniform ripening and then dried at ambient temperature (29–30 °C). Ripening occurred at 25 °C and 85–90% RH. The hands were monitored daily for finger drop, pedicel rupture force, resistance to finger drop and enzyme activity. The peel at the middle of the fruit and at the pedicel in the rupture area was sampled at intervals. Peel from five hands of each treatment was pooled and frozen at −80 °C until further use.

2.2. Finger drop in hands of banana

The method was modified from Semple and Thompson (1988). A hand of banana was held at 15 cm above a table for 10 s, and the number of dislodged fingers was recorded, and expressed as a percentage of total number of fingers on the hand.

2.3. Pedicel rupture force

Pedicel rupture force was measured by pressing down a wedge probe at the pedicel until it separated from the fruit. The required force was expressed in Newtons (N). Twenty fruit were measured in each treatment, at each time point.

2.4. Resistance to finger drop

Banana fruit, attached to a hand, was inserted in a hole and held by a big clip, connected to a spring weight. As the pedicel of banana was pulled, the piston of the spring weight and a marker moved together. The marker on the spring weight stopped when the pedicel broke. The force at the moment of rupture was indicated on the marker. The resistance to finger drop was expressed in kilograms (kg).

2.5. Peel color and fruit firmness

Peel color was measured in the middle of the fruit, using a Chroma-meter CR-300 series (Minolta, Japan) with 10 mm viewing aperture. The instrument was calibrated using a white reference tile and *a* values were recorded.

Firmness was measured on one side, in the middle of a fruit. The peel was left on the fruit. The penetrometer (Chatillon and Sons, Kew Gardens, NY) was equipped with a 5 mm probe penetrating 5 mm into the fruit.

2.6. Peel water content at the rupture area, peel thickness, diameter of the pedicel, and fruit fresh weight

Peel at the rupture area (1 cm^2) of banana fruit was collected, weighed and oven-dried at 60 °C for at least 5 days. It was weighed daily until the weight did not further change. The percentage water content was calculated. The diameter of the pedicel and the thickness of the peel at the rupture area were measured on day 3. Fingers were separated at the rupture zone and weighed to determine fresh weight (FW).

2.7. Pectin fractions and enzyme assays

The extraction method for the pectin fractions was modified from Martin-Cabrejas et al. (1994). Briefly, 10 g of peel tissue was extracted for alcohol insoluble solids (AIS), then 30 mg of AIS were used for pectin extraction. The AIS was placed in water and shaken at 150 rpm for 16 h and centrifuged at 15,000 rpm, 4 °C for 30 min. After that the pellets were extracted with water again, shaken at 150 rpm for 6 h and centrifuged at 15,000 rpm, 4 °C for 30 min. The water-insoluble pellet was suspended in 0.05 M CDTA (in 1 M amidazole, pH 7), twice extracted (16 and 6 h) and centrifuged as described above. The supernatant (CDTA-soluble pectin) from the two extractions were pooled. The remaining cell walls were twice extracted with 0.05 M Na_2CO_3 (16 and 6 h). Finally, the suspension was centrifuged as above. The pectin in each fraction was assayed as uronic acid units, as described by Blumenkrantz and Asboe-Hansen (1973).

Extraction and assay methods for PG were as described by Yoshida et al. (1984) and those for PE as by Hagerman and Austin (1986). Extraction for PL used the method of Payasi and Sanwal (2003) and PL activity was determined by the method of Collmer et al. (1988), with slight modification. Briefly the assay was done in a mixture of 0.5 ml 0.36% (w/v) polygalacturonic acid in 0.05 M Tris–HCl buffer, pH 8.5, 0.3 ml 4 mM $CaCl_2$, 0.6 ml enzyme and 0.6 ml water. The reaction mixture was incubated at 37 °C for 3 h. The PL activity was determined by following absorbance at 232 nm.