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Wax Deposition on the Fruit Surface of Satsuma Mandarin as Affected by Water Stress

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Abstract: The effect of water stress on fruit surface wax deposition during fruit maturation in'Okitsu wase'satsuma mandarin (*C. unshiu* Marc.) was observed with scanning electron microscopy (SEM). Wax deposition increased with fruit maturity. Wax deposition on fruit from water stressed fruit was less than the control. The percentage of wax coverage over stomata was higher in the control than in the water stressed fruit from 141 to 170 days after anthesis (DAA) but was similar to thereafter. These results suggest that wax deposition on the fruit surface is delayed by water stress.

Key words: fruit surface, SEM, stomata, water stress, wax deposition

INTRODUCTION

Plant cuticles contain cuticular waxes that play an important role at the interface between primary plant tissues and the atmosphere (Jetter *et al*., 2000). It is accepted that citrus leaf and fruit cuticles carry a thin film of epicuticular waxes on the surface of their cutin matrix (Baker *et al*., 1975). Citrus fruit is commonly coated with waxes that reduce the gas exchange between fruit and the atmosphere resulting in reduced weight loss of fruit, and elevated internal CO_2 concentration (Hagenmaier and Baker, 1993). Takagi *et al*. (1994a) reported that transpiration rates on the fruit peel differed with citrus cultivars and with peel surface temperatures. The wax volumes on the peel also differ with citrus cultivars, position of bearing fruit and cultural conditions (Takagi *et al*., 1994b). Fruit epicuticular wax concentration increased with maturity (Freeman *et al*., 1979). However, fruit transpiration is closely related to sugar accumulation in fruit caused by water stress (Takagi *et al*., 1994c).

It is known that water stress due to drought on the soil increases sugar content in fruit (Sugai and Torikata, 1976; Mukai *et al*., 1996; Yakushiji *et al*., 1996). Also, water stress caused increase in acid content in the central portion of the mid section whereas reduction was recorded in the outer portions compared with control in satsuma mandarin fruit (Moon and Mizutani, 2002).

However, little has been reported on the effect of water stress on wax deposition on the fruit surface. Here we report the effect of water stress on wax deposition on peel surfaces of satsuma madarin fruit with maturity.

MATERIALS AND METHODS

Plant materials and water stress treatment: Nineteen-year-old'Okitsu wase'satsuma mandarin (*C. unshiu* Marc.) trees grown in the open field at the Experimental Farm, Faculty of Agriculture, Ehime University (Hojo, Ehime, Japan) were used in 1999. Anthesis time, which was designated as 80% full flower, was May 15 (days after anthesis, 1 DAA). All trees were thinned to one fruit per 20-25 leaves in early August. The orchard floor (30m x 20m), with 12 trees, was covered with micro-perforated vinyl sheets (Tyvek, Dupont) from August 12 (87 DAA) for about five months. To determine levels of water stress, leaf water potential was measured at pre-dawn with a leaf water potential equipment (Meiwa Co. Japan). The water potential for control trees was -0.33±0.02 MPa at 140 DAA and -0.9±0.04 MPa at 190 DAA. The water potential for stressed trees was -0.61±0.02 Mpa at 140 DAA and -1.2±0.05 MPa at 190 DAA. Three fruit of similar size were randomly selected from the peripheral portions of three trees at 130 DAA (September 25), 141 DAA (October 4), 170 DAA (October 28) and 205 DAA (December 9).

SEM observation: Peel disks (3 x 6 mm) were excised from the stem end of fruit, transferred into test tubes containing 25ml of formaldehydrate-glutaraldehyde fixative buffer solution and fixed in the dark refrigerator for 12 hours. After fixation disks were dehydrated in ethanol and t-butyl alcohol, and thereafter frozen in liquid nitrogen. The samples were vacuum dried, and attached to microscope stubs and coated with gold using an ion sputter (JEC-1100, Nipon Elec. Int. Japan). The samples were observed and photographed under a scanning electron microscope (SEM, JSM-T200, Nipon Elec. Int. Japan) at an accelerating voltage of 25KV and magnificationsis x100, x500 and x2,000. Wax coverage was quantified with the help of a magnifying glass from photographs taken at x100 and x500 for both water-stressed and control fruit. Wax densities on peel were classified as ranging from medium to very high by visual analysis.

RESULTS AND DISCUSSION

Table 1 shows that the percentage of wax coverage over stomata and wax density on peel as affected by water stress. Wax density on the peel in both water-stressed and control fruit was increased with maturity. However, wax density higher in control than in water-stressed fruit at 170 DAA (Fig. 1, 2), while there was no difference in wax density for both water-stressed and control at 141 and 205 DAA. These results show that wax density on peel was affected by water stress. The amount of epicuticular wax produced is dependent on environmental growth conditions, with increased radiant energy flux or decreases in humidity and soil moisture content stimulating wax production (Baker and Procopiou, 1980; Walton, 1990). However, wax production in the peel is closely related to citrus cultivars, fruit bearing position and cultivation condition (Takagi *et al*., 1994b). Also fruit epicuticular wax was affected by plant growth regulation treatment such as gibberellic acid during maturation in 'Washington' navel orange (El-Otmani and Charles, 1985). The epicuticular wax was mostly removed by washing fruit under high pressure compared with unwashed controls in 'Hamlin' orange (*Citrus sinensis* L.) at harvested fruit (Petracek, *et al*., 1998). Baker *et al.* (1975) reported that hydrocarbon, primary alcohols, fatty acids and aldehydes are important constituents of the waxes in citrus fruit. However, aldehydes and fatty acids are dominant in citrus fruit waxes (Freeman *et al*., 1979). In water-stressed fruit, the ^{14}C activity of the ethanol soluble fraction in the fruit increased in comparison with that of the ethanol insoluble fraction (Kadoya, 1973). Thus, water-stress may decrease ratio of photosynthates meant for wax production on fruit surface.

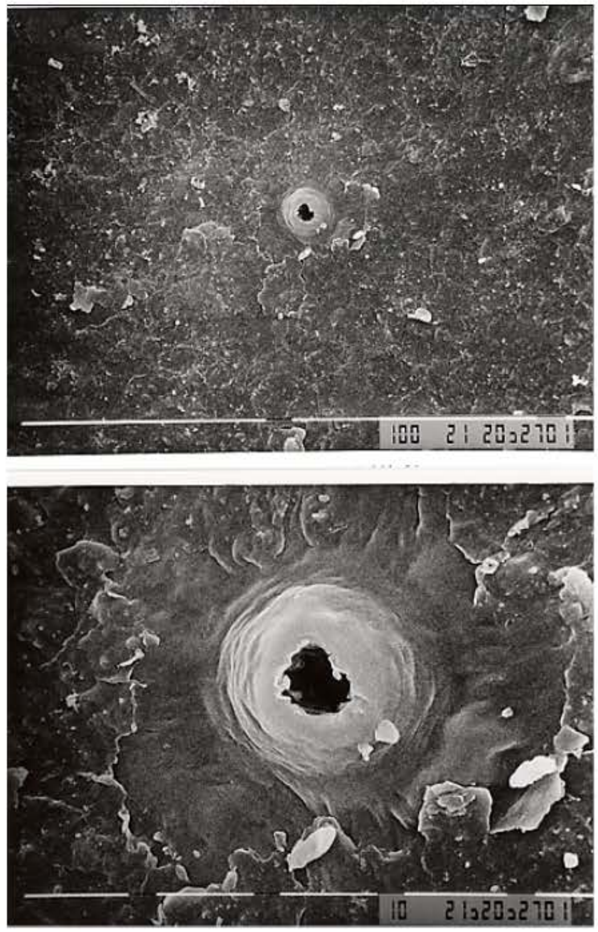

Fig. 1: SEM observation of wax and stomata on peel of 'Okitsu wase' satsuma mandarin (Citrus Unshiu Marc.) at 131 DAA (Control, A: x 500, B: x 2,000).

The percentage of wax coverage over stomata increased in both water-stressed and control fruit with maturity (Table 1, Fig. 1, 2). The percentage complete wax coverage was higher in the control than in water-stressed fruit from 140 to 170 DAA. However, there was no difference between the two treatments at 230 DAA. The waxes are effective barriers to gas permeation (Hagenmaier and Shaw, 1992). Kawano *et al.* (1977) reported that the permeability constants of gas through the peel of satsuma mandarin higher in order to CO_2, O_2

Table 1: Wax coverage (%) of stomata and wax density on peel in 'Okitsu wase' fsatsuma mandarin as affected by water stress

	Water-stressed fruit				Control fruit			
DAA	Wax	Exposed	Half covered	Completely covered	Wax	Exposed	Half covered	Completely covered
130	-[z]	-	-	-	++	87.0±4.2	8.0±2.1	5.0±2.5
141	++[y]	54.0±5.0	30.0±8.2	16.0±4.8	++	58.0±7.6	4.3±2.7	37.7±5.4
170	+++	44.3±8.5	0	55.7±8.5	++++	0	0	0
205	++++	0	0	100	++++	0	0	100

[z]: not observed, [y]: wax density (++: medium, +++: high, ++++: very high), DAA: days after anthesis

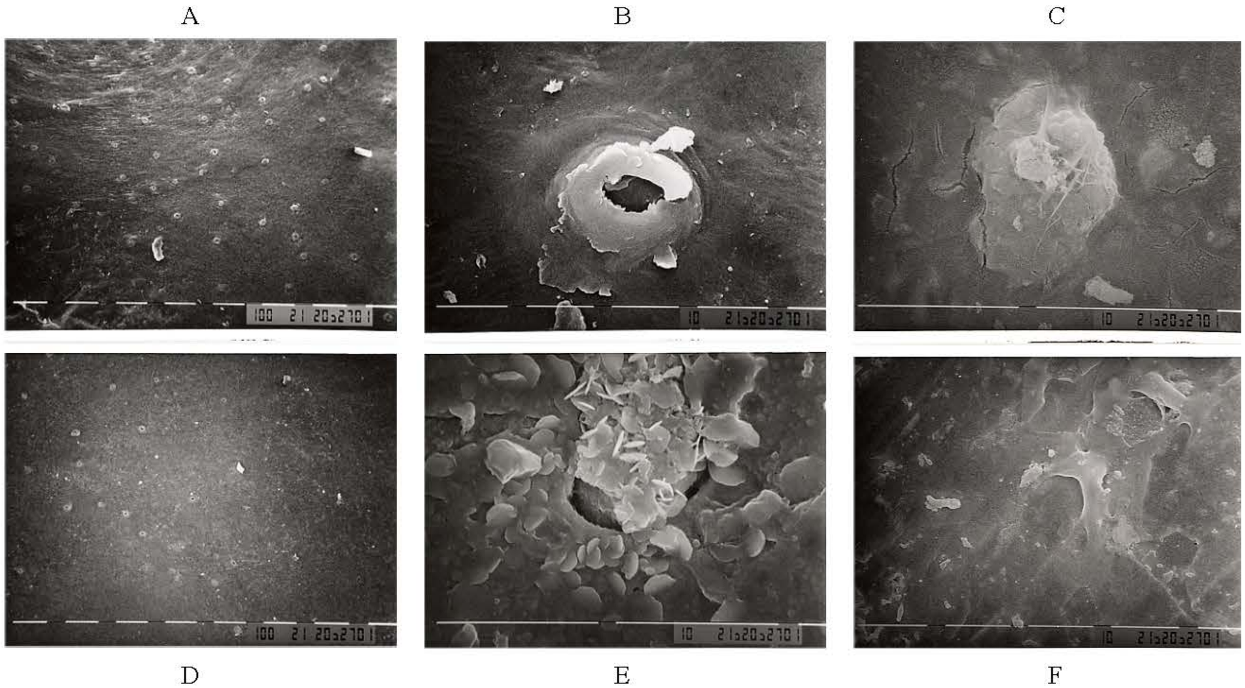

Fig. 2: SEM observation of wax and stomata on peel of 'Okitsu wase' satsuma mandarin (Citrus Unshiu Marc.) A and D, B and E, C and F correspond to 141, 170 and 205 DAA, respectively. A – C: Water-stressed fruit, D – F: Control fruit. (A and D: x 100, B, C, E and F: x 2,000).

and N_2. Citrus fruit is coated with wax during storage resulting in reduced respiration rate and acid content, weight loss, elevated internal CO_2 concentration (Hagenmaier and Baker, 1993), increased ethylene, decreased O_2 concentration (Petracek *et al.*, 1998), increased sugar content and affected flavour (Farooqi *et al*, 1988). Transpiration from peel is closely related to fruit quality (Takagi *et al.*, 1994c). The SSC and acid content was lower in the fruit by vinyl lap treatment for the inhibition of transpiration compared with the control (data not shown). If the wax on the peel inhibited fruit transpiration, the SSC and acid content may be affected by wax. Thus the wax on the peel may affect in sugar and acid content in juice. It is known that water stress due to drought increases sugar content in fruit (Sugai and Torikata, 1976; Mukai *et al.*, 1996; Yakushiji *et al.*, 1996). The SSC and acid content was increased or decreased in different portions of fruit by water-stressed compared with control in satsuma mandarin (Moon and Mizutani, 2002). Our results suggest that wax deposition on the peel is delayed in water-stressed fruit compared with the control. However, we have not detected a relationship between wax deposition on peel and sugar and acid content in juice under water-stressed condition. Thus further studies are needed to examine the effect of peel wax on internal quality during fruit development and maturation.

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