Effects of GA3 on Puffing and Levels of GA-like Substances and ABA in the Peel of Satsuma Mandarin (Citrus unshu Marc.)

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Abstract. GA3 was applied to satsuma mandarin fruit at the concn of 100 ppm 25 and 35 days before harvest. The percentage puffy fruit was greater in the untreated than in both treated plots at harvest time. Chlorophyll was more abundant in the peel of treated fruits than in those of the untreated. GA-like substances in the peel decreased rapidly in untreated fruit in early September, then leveled off toward maturity. They increased more than 40 times in the flavedo of treated fruit as compared with the control. ABA content in the untreated peel increased gradually during early fruit enlargement and then increased rapidly to a very high value during maturity. The ABA content in the peel of GA3-treated fruit did not increase at the same rate as the control. Sugar content in the peel was depressed by GA3 application.

Satsuma mandarins, which are cultivated in southwestern Japan, usually develop into puffy fruit in late autumn as harvest approaches. These fruits command lower market prices. Furthermore, they are also easily damaged in the processes of harvest, packing and transportation. Thus, practices which would reduce the disorder are of considerable economic importance.

Puffiness is caused by the disintegration of albedo tissues which have 2 characteristic phases (10). One is the transformation of the albedo to somewhat spongy structures which include large schizogenous intercellular spaces. This transformation results from a steady but continuous expansion of the epidermals during the later growing period. The second phase is characterized by swelling or shrinking of the mature peel which occurs when the flavedo imbibes and gives off water through its surface.

Puffy rind development was alleviated by application of GA3 at color break, but it also caused a delay in degradation of chlorophyll and development of carotenoid pigments (11). Additional findings on GA effects on the levels of chlorophylls, GA-like substances, ABA and sugars in the peels of satsuma mandarin are reported herein.

Materials and Methods

GA3 treatment and sampling of fruit. The experiment was conducted on the ‘Ikeda’ satsuma mandarin growing in an experimental orchard at Ehime University at Matsuyama, Japan.

Fruit was collected at 2-week intervals from August 13, 1974 until harvest on Nov. 23. GA3 was applied to the fruits at concn of 0 and 100 ppm by the dipping method on Oct. 23 and Nov. 1. Fruit on Oct. 23 were at color break; i.e., the rind color was light green with yellow spots on the styler end. The yellow color was slightly greater on Nov. 1.

On Nov. 23, the remaining fruits were harvested and the percentage of puffy fruit and specific gravity of the fruit were determined. Fruits were washed and peeled. The flavedo was then separated from the albedo, lyophylized, ground and stored in an air tight container at -20°C.

Analysis for sugars and chlorophylls. Sugars were analyzed by Somogy method (16) after extraction with 80% methanol at 0°C for 12 hr. The extracts were filtered and evaporated to the aqueous phase under vacuum at 40°C. The extracts were adjusted to pH 6.5 with NaOH and centrifuged at 17,000 g for 20 min. The supernatant liquid was adjusted to pH 7.5 with 5% NaHCO3 and partitioned 3 times with 50 ml ethyl acetate. The aqueous phase was adjusted to pH 2.5 with Na2SO4 and partitioned 3 times with ethyl acetate to obtain the free acid fraction.

The fraction was evaporated to dryness, and the residue dissolved in 1 ml ethanol. One hundred µl of the solution was spotted on Toyo-roshi 51 paper (2 x 4 cm), and then developed with solvent (iso-propanol:1 ammonia:1 water (by vol) for 20 cm. After drying, the strips were cut into 10 segments and analyzed by the barley endosperm bioassay.

Barley endosperm bioassay. Using the procedures of Coombe et al. (5), barley seeds, Sakaui Kawa #1 (hulled barley) were soaked for 3 hr in 50% H2SO4 for sterilization and husk removal. After washing with distilled water, barley seeds were halved transversely. Two endosperm pieces were incubated for 48 hr at 30°C in 50 ml vials containing 1 ml of test solution. Three replicates of each treatment were used. The produced reducing sugar was assayed by Somogy method (16).

Extraction and purification of ABA. The 5 g samples were extracted with 80% methanol at 0°C for 36 hr during which methanol was changed 3 times. The combined extracts were filtered and centrifuged. After centrifugation, the supernatant liquid was adjusted to pH 2.8 with Na2SO4 and partitioned with ethyl acetate. The ethyl acetate phase was partitioned with 8% NaHCO3. The NaHCO3 phase was adjusted to pH 2.8 with H2SO4 and then partitioned with ethyl acetate. These ethyl acetate extracts constituted free ABA.

The solvent was evaporated and the residue redissolved in 1 ml ethanol. A 100 µl aliquot was streaked on paper and chromatographed via the same procedures used for GA-like substances. The strips at Rf 0.6–0.8, to which ABA migrated, were cut to small pieces and the ABA was eluted with acetone.

Gas liquid chromatography. For gas liquid chromatographic analysis, acetone eluates were methylated with diazomethane (15) and a 2-µl aliquot injected into a gas liquid chromatograph (Hitachi 063 GLC) equipped with a 63 Ni foil electron capture detector. The chromatogram was obtained with the following operating conditions: The glass column (3 mm x 2 m) was packed with silicone SE 30 on Chromosorb W, 60/80 mesh. The N2 carrier gas rate was 60 ml/min, while the N2 scavenger gas flow was 5 ml/min. The temps were maintained as follows:

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Identification of ABA. Abscisic acid in the peel extracts was identified by 2 methods, using gas liquid chromatography: 1) the retention times between the peaks of authentic ABA and the extractives were compared, 2) MeABA in the extracts was converted to 2-trans-MeABA by irradiation with UV light and then analyzed by GLC (12).

Results and Discussion

Growth and quality of fruits. The growth curves of non-treated and GA$_3$-treated fruit obtained by measuring the diam of fruit indicated that treatment had little effect on growth (Fig. 1). This suggests that the fruit enlargement was almost completed by the time of GA$_3$ application. Application of GA$_3$, however, resulted in a decrease in the development of puffy fruit (Table 1), from 78% in the non-treated lot to 20 and 17% by the Oct. 23 and Nov. 1 treatments, respectively. The difference in puffy fruit between the 2 dates of application was not significant. The specific gravity of fruits, which is closely related to puffiness was significantly lower in the non-treated fruit in comparison to the treated fruit. Soluble solids and acid contents of the juice were not influenced by GA$_3$-treatment. Chlorophyll content was significantly lower in the controls.

The rind softness and development of orange color associated with fruit ripening are delayed by application of potassium salt of GA(1, 2, 3, 4, 6, 13, 17). It is inferred that KGA treatments delayed or reversed certain aging processes (14). Reduction of puffy peels by application of GA$_3$ might be attributed to the same reasons. Since one characteristic associated with puffy peels is the swelling or shrinking of mature rinds as water is inbibed and transpired through the fruit surface, any treatment which would delay the onset of senescence should reduce the incidence of the problem.

Identification of ABA. The retention time for an ABA-like substance in gas-liquid chromatography (Fig. 2), and its time course of isomerisation by irradiation with UV light (Fig. 3) indicated correspondence between it and authentic ABA (12).

Seasonal changes of GA-like substances and ABA. Assays for GA-like substance in acid ethyl acetate fraction in the albedo and flavedo of the peel revealed that the activities at Rf 0.5 and 0.6 were low in the former tissue and high in the latter (Fig. 4). GA-like substances in the flavedo during the fruit growing season were high in August, decreased rapidly until mid-Sept., and then leveled off toward maturity (Fig. 5). However, GA level increased more than 40 times in the 100 ppm GA$_3$-treated flavedo as compared to that of the untreated flavedo. The albedo which contained small amounts of GA-like substances in untreated fruits, increased rapidly after GA$_3$ treatment.

ABA content was low in the flavedo of the untreated fruits in mid-August (Fig. 6). During fruit growth the ABA content in the untreated flavedo increased gradually until late Oct., and then continued to increase rapidly to a very high value during fruit maturation. ABA content in the GA$_3$-treated flavedo did not increase as much as that of the control. Although the same trends were obtained on the time course change of ABA content in both albedo and flavedo, the content of ABA was lower in the former than in the latter. The later

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific gravity</th>
<th>Puffy fruit (%)</th>
<th>Soluble solids (%)</th>
<th>Acids (g/100 cc)</th>
<th>Total chlorophylls (mg/100 g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treatment</td>
<td>0.809</td>
<td>78.0</td>
<td>9.9</td>
<td>1.20</td>
<td>0.7</td>
</tr>
<tr>
<td>GA$_3$-treatment Oct. 23</td>
<td>0.854</td>
<td>20.2</td>
<td>9.4</td>
<td>1.21</td>
<td>9.1</td>
</tr>
<tr>
<td>GA$_3$-treatment Nov. 1</td>
<td>0.860</td>
<td>17.0</td>
<td>9.4</td>
<td>1.25</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Fig. 2. Gas liquid chromatograms obtained from methylated authentic ABA and from methylated fraction purified from satsuma mandarin peel extracts: 1) Me.Cis-ABA, 2) Me.Trans-ABA.

Fig. 3. Time course for the conversion of MeABA to 2-trans-MeABA obtained from satsuma mandarin peel extracts during irradiation with UV light.
the GA\textsubscript{3} treatment was made to fruits, the higher was the ABA content in the peels.

From this experiment, it is postulated that aging of peels is delayed or reversed by application of GA\textsubscript{3}. Normally, during fruit growth and ripening, GA level decreases and the ABA and sugar contents increase in flavedo tissues. The treatment resulted in a sudden rise of GA-like substances in flavedo tissues and a simultaneous reduction in ABA and sugars levels (Fig. 7). These changes were accompanied by a reduction in the chlorophyll degradation rate.

From these results, it appears that the balance or interaction between growth promoting and inhibiting substances such as GA and ABA control the maturation rate of flavedo tissues as reported by Goldschmidt et al. (7, 8, 9). These authors interject the idea that ethylene plays a major role in the complex maturation processes.

References
Irrigation and Applied Nitrogen Effects on Snap Beans and Pickling Cucumbers

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Abstract. Field studies were conducted to determine the response of snap beans (Phaseolus vulgaris L.) and cucumbers (Cucumis sativus L.) to no, intermediate, and high irrigation with 0, 65, 100, and 135 kg N/ha on beans and 56 and 112 kg N/ha on cucumbers. Intermediate irrigation increased marketable yields, but high irrigation did not. Average snap bean yields for the 3-year period by soil water regimes were 5,800, 7,000, and 6,800 kg/ha, and for cucumbers were 32,200, 35,400, and 33,000 kg/ha for no, intermediate, and high irrigation, respectively. Applied N increased yields, with the 3-year average snap bean yields being 4,600, 6,600, 7,200 and 7,700 kg/ha for 0, 65, 100 and 135 kg/ha rates, respectively, and cucumber yields being 31,900 and 35,100 kg/ha for 56 and 112 kg/ha respectively. There was a greater response to N fertilizer on the spring crop than on the fall crop.

In areas with long frost-free periods, it is possible to grow more than 1 crop per season of certain vegetables such as snap beans and cucumbers. Frequently there is a market advantage from an early and late crop of these vegetables. One problem sometimes encountered in this type of management is occurrence of drought during the plant's critical maturity state. In central Alabama, there is a period of relatively low rainfall in May-June and again in the fall (13). Snap beans and cucumbers are planted in mid-April and mid-August. Snap beans are sensitive to water stress and high temp, especially during blossoming and pod growth (3, 4, 8).

The response of snap beans to P and K fertilizer has been both positive and negative, depending on past treatment and levels of these elements in the soil (5, 6, 12, 13, 14). The response to N has been positive, but the response usually has been limited to 35 to 85 kg N/ha. Worley and Harmon (14) reported that N reduced the percent marketable pods, but the effect was overcome by increase in yield.

Studies with cucumbers showed a positive response to 30 to 60 kg N/ha (1, 2, 9, 10, 11), while rates of 100 kg N/ha or higher had an adverse effect on yield by stunting plants (1, 9). Johnson et al. (7) reported that response of pickling cucumbers to N was variable, depending on the soil type. The greatest response was on eroded, upland soil, where 90 kg N/ha appeared to be the best rate. There was no benefit from applied N on highly productive soils. Inadequate N and/or K may adversely affect fruit length and shape of cucumbers (2) and an N x K interaction has been indicated (11).

The purpose of this experiment was to determine the effect of supplemental irrigation and applied N on marketable yields of snap beans and pickling cucumbers.

Materials and Methods

'Harvester' snap beans and 'Carolina' pickling cucumbers were grown in the field in central Alabama from 1973 to 1975 on a Luvedale fine sandy loam (Rhodic Paleudult). Crops were grown in both spring and fall except fall 1975 when the snap bean stand was too poor for harvesting. Both snap beans and cucumbers were grown at 3 soil water regimes with 4 N rates on snap beans and 2 N rates on cucumbers. There were 3 replications of a split-plot design, with soil water regime as main plots and N rates as subplots.

Irrigation levels tested were a) no irrigation, b) irrigation when 70% (intermediate irrigation), and c) irrigation when 40% (high irrigation) of the available soil water had been removed from the surface 60 cm of soil. Soil water suction was 2 bars for the intermediate treatment and 0.67 bar for the high treatment. Plants were furrow-irrigated to replenish the surface 60 cm of soil to field capacity. Available water-holding capacity of the soil was approx 1 cm/dm of soil. A dike was constructed around each plot to confine all rainfall and irrigation water. Irrigations were based on readings from gypsum soil moisture blocks for cucumbers and on gravimetric soil samples for snap beans. It some cases rainfall occurred immediately after irrigation, which caused excessive soil moisture in the soil profile for short periods of time.

Nitrogen rates from ammonium nitrate were 0, 65, 100, and 135 kg/ha for snap beans and 56 and 112 kg/ha for cucumbers. All plots were fertilized with P and K according to soil test. Spring crops were planted about April 15, and fall crops about August 10. Snap beans were harvested once over, to simulate machine harvesting, for each crop at about 55 days after planting for the spring crop, and about 65 days after planting for the fall crop. All plots were harvested on the same day when approx 50% of the pods were sieve size No. 4. Cucumbers were hand harvested at 2 to 3-day intervals, for a total of 9 to 16 harvests for each planting. Plants were sprayed weekly throughout the