Cell wall metabolism in gibberellin-treated persimmon fruits

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Abstract

The application of gibberellin [GA3] to persimmon fruits as an orchard spray, at least 2 weeks prior to harvest, has been shown to delay ripening of the fruit on the tree and its rate of softening after harvest. This effect persisted during and after cold storage. The delay in softening has been attributed to the effect of the phytohormone on cell wall metabolism. To examine this hypothesis, cell walls of GA3-treated fruit were compared to those of non-treated fruit. Comparison between fruit was from harvest till the termination of post-storage softening. The study included TEM examinations, assay of certain hydrolase activities and determination of compositional changes occurring in the various cell-wall carbohydrate polymers. Our findings indicate that GA3 either delays or inhibits all of the cell wall changes that were found to accompany fruit softening, including dissolution of the middle lamella, separation of the plasmalemma from the cell-wall, mitigation of the structural coherence and density of the primary cell wall, increased solubilization of pectic polymers, loss of neutral sugars, predominantly arabinose and galactose, and increased activities of exo-polygalacturonase [PG] and endo-1,4-β-glucanase [EGase]. The principal discernible compositional difference between GA3-treated fruit and control fruit at harvest was a higher total carbohydrate content in the cell wall material extracted from GA3-treated fruit, which was due chiefly to an increased amount of cellulose.

1. Introduction

Gibberellin has been shown to delay maturation and ripening of a number of fruits. With each species, however, the type of response is different, though there are some responses common to a number of species. Thus, for example, inhibition of chlorophyll breakdown and carotenoid or anthocyanin synthesis occur in tomato [13], apricot [1], citrus [25], strawberry [28] and persimmon [17]. Delayed maturation accompanied by increased growth is found in grapes [18] and cherries [26]. Increased firmness has been demonstrated in cherry [14] and in persimmon fruits [6, 23]. A delay in the onset of the climacteric respiration has been reported for banana [33], apricot [1], tomato [5] and persimmon [7]. As far as is known, there is not one fruit, which demonstrates all of these responses and the action of GA3 on ripening processes is therefore pleiotropic.

The contribution of cell wall metabolism in ripening fruits to processes other than textural changes is a relatively new concept, based on the findings that the oligomeric products of cell wall hydrolysis have a number of physiological and regulatory effects [8]. Such oligomers can induce ethylene production [32], function as elicitors for fungal resistance [30], and may be involved in the release of proteins from the cell wall during ripening [20]. As there is some evidence that the cell wall is one of the sites responsive to GA3 in some ripening fruits [25], and in vegetative tissues [22], it might be the site for initiation of ripening processes other than fruit softening. This study describes the changes incurred by GA3 in the cell wall metabolism of ripening persimmon fruits.
2. Materials and Methods

2.1 Plant material

Persimmon (*Diospyros kaki* L., "Triumph") trees were sprayed at two locations with 50 mg l⁻¹ GA₃, plus 0.025% Triton X-100, approximately 2 weeks prior to the peak of the estimated commercial harvest. Both orchards are in the southern coastal plain of Israel, about 5 km apart, planted in heavy loam on *D. virginiana* rootstock. Orchard A was planted in 1977, at a density of 330 trees/ha. Orchard B was planted in 1980 at a density of 400 trees/ha. Both orchards are irrigated and fertigated in a similar manner, orchard B receiving 20% more water than orchard A. The main difference between the two orchards is that the trees in orchard A are not pruned and reach a height of 6–7 m, whereas those in orchard B are pruned annually and maintained at a height of ca. 3 m. Sprays were applied in each orchard when the ground color of the fruit had turned from green to yellow-orange. Fruits from 5 sprayed and 5 untreated trees in each orchard were harvested 2 weeks after treatment. Sixty fruits of uniform size and color from each tree were sampled and divided into 3 subsamples for the assays described below, at harvest, after 3 months of storage at -1 °C and after a week at 20 °C, following cold storage. All fruit had the astringency removed prior to assaying, by exposure to 80% CO₂ for 24 h at 25 °C. Fruit firmness was measured with a mounted Hunter-Spring penetrometer using an 11 mm tip, on two pared cheeks of each of 20 fruits, at each examination. Five of these fruits, of approximately the average firmness value, were selected for preparation of the cell wall material, as described below.

2.2 Cell wall carbohydrates

A composite sample of 50 g pulp tissue was blended with 200 ml cold acetone (−20 °C) for 2 min. The slurry was centrifuged and the pellet was washed once in acetone and twice in 70% ethanol. The final pellet was rinsed in acetone and air-dried overnight before weighing. These acetone/ethanol insoluble solids (AEIS) included, apart from the cell wall material, the insoluble tannin, part of which had been precipitated by the CO₂ treatment to remove astringency. The insoluble tannin constituted approximately 50% of the AEIS, which ranged from 3.7% to 4.5% of the fresh weight. It was found necessary to insolubilize all the soluble tannin prior to cell wall extraction and thus include it in the AEIS, otherwise it would have been an unknown quantity, varying with the gradual softening of the fruit.

Samples of the AEIS were analyzed to determine the carbohydrate components of the cell wall. Water-soluble pectin (WSP) was extracted twice from 100 mg AEIS with 30 ml H₂O, by stirring each time for 1 h. The supernatants were combined and the pellet was extracted in a similar manner with 50 mM trans-1,2-diaminocyclohexane-N,N',N' tetraacetic acid (CDTA), pH 6.0, to collect the calcium pectate fraction (CSP). The pellet was resuspended in CDTA, brought to pH 11.0 and stirred for 30 min. to de-esterify the insoluble pectic fraction (ISP). The de-esterified pectin was solubilized with 10 mg fungal pectinase (Sigma), after reducing the pH to 5.5 with glacial acetic acid. The resulting uronic acids were measured colorimetrically [2].

Non-cellulosic neutral sugars (NS) were derivatized to alditol acetates by hydrolysis with 2N trifluoroacetic acid (TFA), reduction and acetylation [3]. The derivatives were separated and identified by gas chromatography on a Carlo-Erba chromatograph fitted with a 30 m fused silicon capillary column (DB-225, J & W Scientific). The oven temperature was 210 °C and Hz was used as a carrier gas. Quantitation was based on integration of the peak area from the flame ionization detector, using myo-inositol as an internal standard. Cellulose was measured in the TFA-insoluble fraction by the anthrone colorimetric assay [12]. Sugar component analysis of this fraction by gas chromatography, as described above, showed that 98% was glucose.

2.3 Enzyme assays

Polygalacturonase (PG). Ten g of peeled fruit tissue were diced and blended with 4% polyethylene glycol 6000 (PEG) in an Ultra-Turrax homogenizer. The slurry was centrifuged for 10 min. at 10,000 g at 0 °C and the supernatant was discarded. The pellet was washed with distilled H₂O, re-sedimented as above and resuspended [1:1 (w/v)] in 0.1 M citrate-phosphate buffer, pH 6.0, containing 1 M NaCl, and stirred in the cold for 90 min. The supernatant was collected after centrifugation at 10,000 g and dialyzed overnight against 100 volumes of distilled water at 4 °C. The final supernatant was the crude extract used for PG assay, based on the procedure developed by Honda *et al.* [21] and adapted by Gross [16]. The reaction mixture contained 0.2% polygalacturonic acid in 0.05 N acetate buffer, pH 5.2, and the incubation time was 20 h. A