Abscisic Acid Content in Chilled Tomato Fruit

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Abstract. Free abscisic acid (ABA) was determined in pericarp of mature-green tomato fruit before and after chilling at 2C for 12 days, in chilling-sensitive and chilling-tolerant cultivars. After being ripened at 20C, these fruit were compared with field-ripened fruit. Chilled fruit contained 2 to 3 times the amount of free ABA as did unchilled. The ABA isolation method described involves the use of preparative and analytical high performance liquid chromatography followed by gas chromatography.

Tomato fruit are sensitive to chilling at the mature-green (MG) stage (Morris, 1982), but often show little visible damage until ripening, when there is uneven color development, pitting, and decay. Crooks (1985) examined various physiological characteristics to try to distinguish differences between chilling-sensitive and chilling-tolerant fruit at the chilled MG stage, before visible injury is evident. Chilling temperatures result in an ABA increase in plant tissues, e.g., tomato seedlings (Daie and Campbell, 1981). In this paper, we report on differences in free ABA content between chilled and nonchilled tomato fruit of sensitive and tolerant lines and describe a reproducible method of ABA isolation using preparative and analytical high performance liquid chromatography (HPLC) for purification and separation.

Tomatoes were grown in 1984 and 1986 with three replications in a randomized complete-block design at the Homer C. Thompson Vegetable Research Farm, Freeville, N.Y. All were *Lycopersicon esculentum* Mill., except one cross with *L. pimpinellifolium* (line 281). Of the four lines grown, two were normal large-fruited types and two were cherry types. One of each was chilling-sensitive, 'New Yorker' (NY, large-fruited) and 'Early Cherry' (EC, small-fruited). The others were chilling-tolerant lines, 79-546 (79, large-fruited) and 281 (cherry).

Fruit were harvested at the MG stage, calyces and pedicels removed, washed in tepid water, dipped in a 300-ppm chlorine solution for 3 min, and air-dried. The three replications, maintained throughout the treatments, were divided at random on trays for chilling and nonchilling treatments. Nonchilled fruit were ripened at 20C. Chilling treatment was

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2C for 12 days followed by ripening at 20C (≈ 14 days). Fruit were also picked ripe from the field and sampled immediately, coincident with those held a total of ≈ 26 days.

In 1986, only NY and 79 (large-fruited) fruit were harvested MG and subjected to chilling, but sampling was at the MG, turning, pink- and red-ripe stages. These stages

were also sampled directly from the field. In 1984, containers of water were placed in front of the fan in the storage rooms (average 90% RH at 2C and 68% RH at 20C). In 1986, tomatoes were placed in buckets laid sideways on shelves, with the open bucket draped by wet cheesecloth with a continuous drip, with an average 95% RH at 2C and 91% RH at 20C.

At the time of sampling (three single fruit per replication), the fruit were separated into pericarp and seeds, immediately frozen in liquid N₂, and held at -20C for up to 3 days until lyophilization. The freeze-dried pericarp of each single-fruit sample was ground in a Wiley mill with a 40-mesh grid.

All distilled water used in ABA extraction and isolation was millipore-filtered, and all organic solvents used were HPLC grade (Burdick and Jackson, Muskegon, Mich.). Samples of 40 mg dry weight (DW) were extracted with 4 ml 80% cold methanol, with stirring, for 1 hr in darkness at 0C (we determined this interval during preliminary tests). Sample size was determined by running a DW curve (1 to 100 mg; 100 ml methanol/DW) to optimize the level of ABA without overloading the columns (Fig. 1).

Fig. 1. Concentration of ABA isolated from tomato pericarp as a function of dry weight (average of three replications).

Fig. 2. Free ABA content in chilled and nonchilled MG tomato fruit: chilled fruit subsequently were ripened in a 20C chamber (1984). NY = 'New Yorker', large-fruited, chilling-sensitive; EC = 'Early Cherry', small-fruited, chilling-sensitive; 79 = line 79-546, large-fruited, chilling-tolerant; 281 = line 281, cherry, chilling-tolerant.

Fig. 3. Free ABA content in chilled and nonchilled MG tomato fruit, the chilled fruit subsequently were ripened in a 20C chamber (1986). NY = 'New Yorker', large-fruited, chilling-sensitive; 79 = line 79-546, large-fruited, chilling-tolerant.

Fig. 4. Free ABA content in field-picked MG and ripe tomato fruit, and in nonchilled MG fruit ripened in a 20C chamber (1984). NY = 'New Yorker', large-fruited, chilling-sensitive; EC = 'Early Cherry', small-fruited, chilling-sensitive; 79 = line 79-546, large-fruited, chilling-tolerant; 281 = line 281, cherry, chilling-tolerant.

Table 1. Differences in ABA content and their significance between control and chilled tomato fruit, both chilling-sensitive and tolerant.

Year	Lines ²	Difference (ng ABA/mg DW)	t value	Pr > t
1984	NY	2.04 ± 0.62	3.29	0.0110
1984	79	2.18 ± 0.62	3.51	0.0080
1984	EC	2.33 ± 0.62	3.76	0.0056
1984	281	2.66 ± 0.62	4.28	0.0027
1986	NY	2.33 ± 0.46	5.05	0.0010
1986	79	2.56 ± 0.46	5.57	0.0005

²NY = 'New Yorker', large-fruited, chilling-sensitive; EC = 'Early Cherry', small-fruited, chilling-sensitive; 79 = line 79-546, large-fruited, chilling-tolerant; 281 = line 281, cherry, chilling-tolerant.

The extracts were millipore-filtered (1.2 μ m pore size), rinsed, and vacuum-evaporated at 28C to the aqueous phase. The pH was adjusted to 3.5, and the sample was partitioned three times against an equal volume of chloroform that was then evaporated.

The HPLC method for ABA isolation (Horgan, 1981) was based on that of Ciha et al. (1977). An Altex 110A HPLC system (Altex, Fullerton, Calif.) had a preparative column (250 $\times$ 10 mm, Altex Ultrasil-ODS, particle size 10 μ m) and an analytical col-

umn (250 $\times$ 4.6 mm, Altex Ultrasphere-ODS, particle size 5 μ m), both protected by a guard column (30 $\times$ 4.6 mm cartridge, aquapore RP 300, particle size 10 μ m; Brownlee Labs, Santa Clara, Calif.). Millipore-filtered samples of 3 ml (0.3 ml 100% methanol and 2.7 ml water) were injected into a 2.0-ml injector loop of the preparative column, and a gradient ran from 0% methanol (5 mM ammonium acetate) to 100% methanol for 32 min with a flow rate of 4.5 ml·min⁻¹. ABA was detected by UV absor-

bance at 254 nm and recorded by integrator. The ABA peak (predetermined by standards, Sigma, St. Louis) was collected, partitioned at pH 3.5, and dried as described above. A sample of 40 μ l (25% methanol) was injected into a 20- μ l injector loop of the analytical column, the mobile phase being 30% methanol for 5 min, followed by a gradient to 60% for 20 min with a flow rate of 0.7 ml·min⁻¹. The ABA peak was collected, partitioned, and dried as with the preparative column.

To the sample in 25 μ l 100% methanol was added an internal standard of 2(2-chlorophenyl)-2(4-chloro-phenyl)-1, 1, 1-trichloro-ethane(O, P'-DDT) as 0.5 ng/ μ l, along with 0.1 ml of methylated ether. Methylation involved diazomethane by the use of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald), as described by Schlenk and Gellerman (1960). After vortexing and a wait of 10 min, the samples were dried under N₂ and dissolved in 25 μ l ethyl acetate. Injections of 2 μ l were made onto a 3-m glass column (6 mm o.d., 2 mm i.d., packed with OV-17; Supelco, Bellefonte, Pa.) of the gas chromatography (Hewlett-Packard 5730A). The injector was at 300C, the oven had a gradient from 220 to 280C at 2C/min, and the carrier gas was 5% methane and 95% argon. Detection was by electron capture at 300C and recording was by an integrator.

Recovery of ABA was 72% (a range of 68% to 78%), estimated by running 40-mg samples through the procedure with and without the addition of 200 ng of ABA. The difference between regular and spiked samples was calculated as percentage recovery. Verification of ABA was made using mass spectrometry (GC-MS) in representative samples [Finnigan (San Jose, Calif.) model 1015 GC-MS and System Industries Data System, operated by the Dept. of Chemistry, Cornell Univ.].

The method used here for ABA isolation has proved to be reproducible, using the same weight of tissue for extraction. Comparative extraction times demonstrated that 1 hr was sufficient (0.79 $\pm$ 0.03 and 0.73 $\pm$ 0.03 ng ABA/mg DW after 1 and 24 hr duration with stirring, respectively). Details of the method and the DW of lyophilized tissue extracted vary among kinds of plant, so that slight modifications are needed. For potato tubers, squash fruit, and cabbage heads, a polyvinylpyrrolidone (PVPP) treatment is necessary to remove phenols and clean the extract before injecting onto HPLC columns.

In both years, the effect of 12 days chilling at 2C was a 2- to 3-fold increase in free ABA (P = 0.0001). This was the case in both 1984 (Fig. 2) and 1986 (Fig. 3), whether the line was sensitive or tolerant (Table 1). The results of the 1984 experiment could have been influenced by water loss. However, similar results from the more controlled humidity conditions of the 1986 experiment suggest that chilling was primarily responsible. Thus, chilling of MG tomato fruit resulted in an increase in free ABA content in tomato fruit pericarp, regardless of cultivar, chilling-sensitivity, or fruit type (large vs.

Fig. 5. Free ABA content in field-harvested MG, turning, pink, and ripe tomato fruit, and in nonchilled MG fruit ripened in a 20C chamber (1986). NY = 'New Yorker', large-fruited, chilling-sensitive; 79 = 79-546, large-fruited, chilling-tolerant.

cherry). Exogenous ABA pretreatment gave some protection against chilling injury in cotton (Rikin et al., 1979) and reduced symptoms of damage in cucumber seedlings (Sasson and Bramlage, 1981).

When chilled tomatoes were ripened at 20C, there was a significant decrease in ABA content of the large-fruited NY and 79 (Figs. 2 and 3) in 1984 ($P = 0.0019$) and 1986 ($P = 0.0001$), in contrast to cherry fruit (EC and 281), where ABA remained near the high value for chilled fruit (Fig. 2). The drop in ABA content from the high value in the chilled MG fruit to that in the ripened large fruit was noticeable even at the turning stage (Fig. 3; $P = 0.0001$). There were nonsignificant differences between chilling-sensitive and tolerant lines. The chilled and ripened chilling-sensitive NY had a tendency for higher ABA content than the similarly treated tolerant line 79 in 1984 and 1986 (Figs. 2 and 3), and a chilled and ripened chilling-sensitive EC tended to have a higher ABA content than tolerant 281 (Fig. 2), suggesting that the tolerant lines can ripen after chilling

without high ABA levels. Chilling sensitivity or tolerance did not appear to affect the ABA content of the chilled fruit, before or after ripening. Type of fruit had more of an effect, with ripened cherry fruit having a higher ABA content per milligram DW than the large-fruited types. Other differences have been found between large and cherry-type fruit in response to ripening after chilling, as in electrolyte leakage experiments (King and Ludford, 1983) and electron transport experiments (Crooks, 1985).

When control MG fruit were ripened at 20C in darkness with no chilling involved, there was a different pattern in ABA content from fruit ripened on the plant. This was seen in 1984 over all four cultivars with $P = 0.0001$ (Fig. 4), and also in 1986 in NY and 79 with $P = 0.0001$ (Fig. 5), particularly at the pink and red stages. With unchilled tomato, ABA peaks after the MG stage and drops again before ripening (McGlasson, 1978). Chamber-ripened nonchilled fruit continued to increase in ABA content instead of reaching a peak and falling

off, as with field-ripened fruit (Fig. 5), possibly due to the time involved after harvest (≈ 12 to 14 days in the ripening room). This was the case in 1984 and 1986 at both humidities.

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