Physiological and Chemical Changes Associated with Watercore Development Induced by GA in Japanese Pear ‘Akibae’ and ‘Housui’

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Summary

We examined physiological- and chemical-characteristics of watercored fruits induced by gibberellin (GA) paste treatment in Japanese pear (*Pyrus pyrifolia* Nakai) ‘Akibae’ and ‘Housui’. ‘Akibae’ was more susceptible to the development of watercore induced by GA treatment during fruit maturation than was ‘Housui’. ‘Akibae’ fruit accumulated sucrose during its fruit maturation, reaching a level two times higher at maturity than did ‘Housui’. Analyses of watercore tissues in two cultivars showed marked drops of firmness and total lipid contents concomitant with increases in electrolyte leakage and sugar content in both cultivars. Ethanol insoluble solids (EIS) contents in watercore fruit consistently decreased with the extent of the disorder in ‘Akibae’ but they remained nearly constant in ‘Housui’. The amount of water- and *trans*-1,2-cyclohexanediamine-*N,N,N',N'*-tetra-acetic acid (CDTA)-soluble pectic polysaccharides increased as watercore extended in both cultivars. ‘Akibae’ had more hemicellulose and cellulose (FW basis) fractions than did ‘Housui’, their weights decreased continuously as the watercore zone expanded. Therefore, we conclude that the physiological and chemical changes, corresponding to watercore development, induced by GA treatment progressed faster in ‘Akibae’ than they did in ‘Housui’.

Key Words: GA, hemicellulose, pectin, sugars, watercore.

Introduction

The occurrence of watercore during fruit development lowers fruit quality and storage potential in some Japanese pear (*Pyrus pyrifolia* Nakai) cultivars. Generally, watercore is known to be induced by high leaf/fruit ratio and gibberellin (GA) treatment (Sakuma et al., 1995, 1998; Tamura et al., 2002). This physiological disorder is accompanied with chemical changes, i.e. altered sugar metabolism (Kajiura et al., 1976; Yamaki et al., 1976), and reduced pectin content (Yamaki et al., 1977). Moreover, Inomata et al. (1993) reported a change in membrane permeability, related to maturation and ripening indices, including ground color development in watercore afflicted fruit.

A new Japanese pear cultivar ‘Akibae’ was released in 1997 as a progeny between ‘Osa-Nijisseiki’ and ‘Kosui’ is characterized as being self-compatible and resistant to black spot disease (*Alternaria alternata* (Fr.) Keissler Japanese pear pathotype)(Tanabe et al., 2001). Despite the high productivity, high soluble solid content and good storage potential, this cultivar develops severe watercore early in its maturation, unlike other cultivars (Tanabe et al., 2001).

In our present work, we compared 1) the pattern of GA-induced watercore, sugar accumulation and the physiological changes during fruit maturation of ‘Akibae’ and ‘Housui’ fruit, and 2) the physiological and chemical characteristics, in the watercored tissues, including cell wall components, were investigated in relation to the severity of the problem.

Materials and Methods

Plant Materials

Japanese pear fruits of cultivars, ‘Akibae’ and ‘Housui’ growing in the orchard of Tottori University, were treated 40 days after full bloom (DAFB) with a GA paste (Kyowahakko Co., Japan) to induce watercore. Treated and control fruits were harvested on September 1, 2001, cut transversely and indexed for watercore. The index ranked watercore fruits into four groups: (0) no symptom of watercore, a sound portion of watercored fruit; (1) slight watercore, cross-sectional area showing less than 5% watercore; (3) moderate watercore, covering 10 to 30% of the area and (5) severe watercore, more than 50% of the area. To determine the ethanol insoluble solids (EIS), tissues were immediately processed into 80% ethanol slurries according to Chun and Huber (1997) and stored at –20 °C. The fruit flesh was sliced and diced, frozen immediately in liquid nitrogen and stored at –30 °C until used.

Received; October 10, 2002. Accepted; March 6, 2003.

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This research was supported by Japan Society for the Promotion of Science (No. P00331).

Determination of firmness, soluble solids, membrane leakage, sugar composition and total lipids

Firmness was measured with Rheometer (RT-3010D, Rheotech, Japan). The skin was removed from the equator of half-cut fruit and firmness was measured with a flat-tipped probe (0.8 cm diameter). The cross-head speed of Rheometer was $100 \text{ mm} \cdot \text{min}^{-1}$, and driving depths were 10 mm. Values are expressed as Newton (N). Soluble solid content was measured with a digital refractometer (Atago, Japan). Solute-leakage of excised tissues was measured by placing 10 discs (10 mm diameter $\times$ 3 mm thickness) in 100 ml of deionized water according to Simon (1977). Leakage was measured with a conductivity meter for 2 hr at 30 min intervals. The rate of leakage was expressed as percent of total electrolyte that leaked from discs that were boiled for 20 min. Total soluble sugars were extracted from 10 g of homogenated tissues of watercored fruit with 80% ethanol. All samples were filtered with $0.20 \mu\text{m}$ of Millex-LG (Millipore, USA). And the filtrate was concentrated to 5 ml using rotary evaporator. An aliquot was analyzed with a HPLC (Hitachi 655A-12) equipped with a Shodex NH2P-50 column that was eluted with 75% acetonitrile. Total lipid extraction was determined by the method of Folch et al. (1957). The

total lipid is evaporated under a stream of nitrogen, dried in an oven at 80°C for 12 hr and weighed.

Preparation of EIS and extraction of cell wall components

EIS were prepared by the method of Huber (1984) using 80% ethanol homogenates. Briefly, the frozen tissue was homogenized at full speed for 1 min using homogenizer (Ultra-Turrax T25, IKA-Labortechnik, Germany) in ethanol to a final concentration of 80%. The homogenate was boiled for 20 min to inactivate enzymes and it was filtered through Miracloth (Calbiochem, USA). The residue was sequentially washed with chloroform:methanol (1:1, v/v), 80% ethanol and acetone before being dried at 37°C .

Soluble pectin fractions and 4% and 24% KOH soluble hemicellulosic fractions were isolated as described by Cheng and Huber (1996) and Maclachlan and Brady (1994), respectively. Briefly, the EIS were sequentially extracted (200 mg/50 ml) once with deionized water, twice with 50 mM CDTA in 50 mM sodium acetate (pH 6.5) for 12 hr at room temperature, once with 50 mM Na_2CO_3 , containing 20 mM Na-borohydride for 6 hr at 1°C , and continuously stirred. At the end of each extraction, the mixtures were centrifuged ($18,000 \times g$, 10 min) and the supernatants filtered

Fig. 1. Changes of fruit quality parameters and watercore occurrence during fruit maturation in gibberellin-treated Japanese pear 'Akibae' and 'Housui'. Bars represent SE of 9 to 30 replications.

through glass microfibre filters (GF/C, Whatman, England). The Na_2CO_3 extracts were neutralized with acetic acid and pellets rinsed with 200 ml of 80% ethanol and acetone for hemicellulose extraction. Prior to hemicellulose extraction, starch was removed by incubating the EIS in 100 mM Hepes (pH 6.9) containing α -amylase (Wako, Japan) and 0.02% NaN_3 at 37 °C for 20 hr. Polysaccharides that precipitated by adding ethanol to a final concentration of 80% were kept at -20 °C for at least 4 hr, and collected on a GF/C filter. The pellets were rinsed with 200 ml of 80% ethanol and acetone, then extracted sequentially with 4% KOH and 24% KOH, containing 0.1% Na-borohydride, and continuously stirred for 24 hr at room temperature (MacLachlan and Brady, 1994). Cellulose was determined by the method of Tabuchi and Matsumoto (2001).

Total uronic acids in the EIS (5 mg) were hydrolyzed by sulfuric acid (Ahmed and Labavitch, 1980). Starch in the EIS was extracted according to Campbell et al. (1990) using DMSO. EIS was suspended in DMSO:water (9:1, v/v; 20 ml/50 mg EIS) and stirred at room temperature for 24 hr after which the slurry was centrifuged at $2,000 \times g$ for 5 min. The dissolved starch in the supernatant was precipitated with ethanol for 12 hr (Frankhauser et al., 1989), and then centrifuged ($12,000 \times g$, 20 min). The starch pellet was resuspended in 20 ml of 0.01 N NaOH. And its concentration determined as glucose equivalents using anthrone. Uronic

acids and total sugar contents in each fraction were detected by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) and phenol-sulfuric acid method (Dubois et al., 1956), respectively.

Results and Discussion

Watercore occurrence and fruit maturation in GA-treated Japanese pears

Changes in soluble solids content, fruit growth and flesh firmness of GA-treated fruits during their late development revealed that soluble solids content of 'Akibae' and 'Housui' during their fruit maturation period reached 16.3% and 13.1%, respectively (Fig. 1). In both 'Akibae' and 'Housui', the GA-treatment influenced their growth rates strongly, resulting in a 25 and 21% increase in fruit weight, respectively. Fruit firmness did not differ significantly between the two cultivars except that it was slightly high in 'Akibae' near maturity (Fig. 1). The susceptibility to the watercore disorder to GA was greater in 'Akibae' than in 'Housui', resulting in 100 to 67% watercored fruits at the full maturity, respectively (Fig. 1). Furthermore, susceptibility to watercore by control fruits was 50 and 0% in 'Akibae' and 'Housui', respectively at full maturity (data not shown). Thus it is assumed that 'Akibae' may be very sensitive to watercore. That the occurrence of watercore increased concurrently with fruit maturation

Fig. 2. Accumulation of soluble sugars during development of gibberellin-treated Japanese pear 'Akibae' and 'Housui'. Bars represent SE of 3 replications.

in both cultivars (Fig. 1) agrees with a previous report by Kajiura et al. (1976), and that GA application accelerated the occurrence of watercore (Sakuma et al., 1995).

Sugar accumulation pattern during fruit maturation in two Japanese pears

Fructose and glucose contents decreased gradually with fruit maturation in 'Akibae' but remained unchanged in 'Housui' (Fig. 2). Conversely, sucrose content increased dramatically in both cultivars, but that of 'Akibae' attained a level more than twice that of 'Housui' at full maturity (Fig. 2).

The concentration and composition of sugars in 'Housui' are quite similar to those reported by Moriguchi et al. (1992). However, 'Akibae' accumulated an abnormally high amount of sucrose, although this phenomenon is common in Japanese pear fruits during fruit maturation (Kajiura et al., 1979). Moreover, 'Akibae' accumulated sucrose dramatically during its early stage of maturation, about 2 weeks earlier than that of 'Housui' (Fig. 2).

Physiological changes of watercored tissues in two cultivars

Flesh firmness of fruits using excised tissue preparation revealed that at equal watercore index, 'Akibae' fruits were firmer than those of 'Housui' (Fig. 3), but that the reduction in firmness was evident in severely watercored tissue, especially for 'Akibae'. The reduction of the I-5 fruits was 67% of the sound tissue from normal I-0 fruits.

Electrolyte leakage in the incubation medium was most rapid in severely watercored I-5 tissues than the I-0 ones in 'Akibae' and 'Housui' (Fig. 3). Contrarily, total lipid contents in excised tissues were lower in watercore than sound tissues in both cultivars, but the magnitude of the loss was greater for 'Akibae' (Fig. 3). Inomata et al. (1993) reported that GA application on 'Housui' cultivar induced earlier membrane permeability than that in the control. Thus, it seems likely that increased membrane permeability or fruit ripening may be a primary cause for watercore disorder.

Fig. 3. Firmness, total lipids contents and electrolyte leakages in sound tissues (I-0) and watercored tissues (I-1, 3, 5) in gibberellin treated Japanese pear 'Akibae' and 'Housui'. Bars represent SE of 20 replications (Firmness) and 5 replications (total lipids and electrolyte leakages). I represents watercore index: 0 = sound tissue, 1 = slight watercore, 3 = moderate watercore, 5 = severe watercore; see text.

Fig. 4. Amounts of soluble sugars in sound tissues (I-0) and watercored tissues (I-1, 3, 5) in gibberellin treated Japanese pear 'Akibae' and 'Housui'. Bars represent the SE of 3 replications. I represents watercore index: 0 = sound tissue, 1 = slight watercore, 3 = moderate watercore, 5 = severe watercore; see text.

Table 1. Ethanol insoluble solids (EIS) and cell wall components in sound and watercored tissues in gibberellin-treated Japanese pear 'Akibae' and 'Housui'.

Index ^z	Cell wall parameters (mg · g ⁻¹ FW)								Starch
	EIS	Pectin			Hemicellulose			Cellulose	
		Total	Water	CDTA	Na ₂ CO ₃	4% KOH	24% KOH		
‘Akibae’									
0	15.5 ^y	2.33	0.001	0.209	0.482	0.957	1.30	4.24	3.09
1	14.3	2.39	0.003	0.200	0.468	0.992	1.22	4.23	2.72
3	13.2	2.25	0.017	0.222	0.449	0.928	1.02	3.96	2.15
5	12.1	2.33	0.020	0.271	0.422	0.814	0.917	3.82	1.37
‘Housui’									
0	7.70	2.03	0.001	0.121	0.605	0.235	0.788	2.00	0.425
1	7.54	1.98	0.002	0.120	0.581	0.198	0.799	1.93	0.508
3	7.34	1.90	0.002	0.137	0.573	0.143	0.832	2.15	0.527
5	7.44	1.91	0.002	0.151	0.561	0.114	0.687	2.14	0.652

^z Watercore index: 0 = sound tissue, 1 = slight watercore, 3 = moderate watercore, 5 = severe watercore; see text.

^y Data represent the mean of two analyses. See *materials and methods* for extraction details.

Soluble sugars of watercored tissues in two cultivars

Analyses of excised tissues of 'Akibae' and 'Housui' showed high accumulations of sugars in severe watercore tissue. The total soluble sugar contents paralleled the watercore indices in two cultivars, such that they increased 8 and 10% above than of sound tissues in 'Akibae' and 'Housui', respectively (Fig. 4). Although sucrose was the main sugar in 'Akibae', both sucrose and fructose were almost equally accumulated in 'Housui'. Yamaki et al. (1976) reported that sorbitol contents increased more in tissues of watercored 'Housui' fruits than they did in sound tissues. Our data reveal that sorbitol did not increase much in 'Akibae' (Fig. 4), so that the accumulation of sorbitol is unlikely to be the primary cause of watercore disorder in this cultivar.

Changes in cell wall component of watercored tissues in two cultivars

Analysis for EIS content in the excised tissues of 'Akibae' and 'Housui' showed that it decreased only in 'Akibae' with elevated watercore index (Table 1). Total pectin content extracted from EIS differed little between watercored and sound tissues in 'Akibae', but it progressively decreased in 'Housui' as reported by Yamaki et al. (1976). The contents of water- and CDTA-soluble pectic polysaccharides increased significantly in watercored tissues, but their levels were quite low compared with other crops (Chun et al., 1999; Rose et al., 1998). The level of Na₂CO₃-soluble wall-bound pectin, decreased with the severity of watercore in both cultivars. The amount of total soluble pectins including water-, CDTA- and Na₂CO₃-soluble fraction was nearly the same in the two cultivars (Table 1). The total soluble cell wall pectin concentration remained relatively con-

stant during fruit ripening, but their proportions changed (Kondo et al., 1999; Rose et al., 1998). Our results indicated that the substantial solubilization of Na_2CO_3 -soluble fraction coincides with an increase in water- and chelator-soluble fractions. Similar results were reported for tomato fruits (Carrington et al., 1993). The 4 and 24% KOH soluble hemicelluloses decreased continuously with the development of watercore in the two cultivars. The 4% KOH soluble fractions that was more readily extracted from the cell wall than 24% KOH soluble one, usually contains a high proportion of hemicellulosic polysaccharides (Rose et al., 1998). In 'Akibae', it was almost 4 times higher than that of 'Housui' (Table 1). The 24% KOH soluble fraction, that is tightly bound to the cell wall and to cellulose microfibrils (Rose et al., 1998), and the cellulose level in the EIS also decreased as the index value increased in 'Akibae', but not in 'Housui' (Table 1). Our results showed that the 24% KOH soluble fraction in the two cultivars amounted to 53% and 85% of the total hemicelluloses in the severely watercored tissues in 'Akibae' and 'Housui', respectively (Table 1). Hence, we assumed that the difference in the 4 and 24% KOH soluble hemicelluloses reflects the differences in compositional cell wall changes that accompany watercore development.

Consequently, our results showed changes in cell wall components accompanying watercore disorder to be more apparent in 'Akibae', compared with 'Housui', indicating that textural modifications could occur in the watercored tissues in Japanese pears (Yamaki et al., 1976). The degradation of cell wall component (Table 1) along with increased membrane leakage (Fig. 3) would elevate the chances of watercore occurrence by increasing the osmotic stress as suggested in apple fruit (Marlow and Loescher, 1984). However, it is unclear whether the changes of cell wall components in those tissues are induced by watercore directly or indirectly by other factors associated with maturation. In our experiment, we regarded the unaffected tissues of watercored fruits as sound tissue or the control instead of comparing each sound tissue with a separate watercore index as performed earlier (Yamaki et al., 1976).

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GA処理で誘導したニホンナシ '秋栄' および '豊水' 果実のみつ症に伴う生理・化学的变化

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摘 要

ニホンナシ '秋栄' および '豊水' において GA 処理はみつ症発生を加速させる。本研究では '秋栄' と '豊水' の両品種を使い、GA 処理によって誘導されたみつ症に伴う生理・化学的变化を調査した。GA 処理に伴うみつ症の発生率は、'豊水' に比べて '秋栄' で高かった。また、'秋栄' の成熟に伴う糖蓄積は独特の様相を示し、成熟期には '豊水' に比べ2倍のスクロースを蓄積した。両品種ともみつ症の進行にともない果肉硬度の低下、脂質含量の低下、電解質漏出の増加がみられた。みつ症が発生した '秋栄' の組織のエタノール不溶性物

質含量は、症状の進行にともない減少したのに対し、'豊水' では顕著な変化はなかった。水可溶性および CDTA 可溶性ペクチン含量は両品種でみつ症の進行にともない増加した。'秋栄' は '豊水' に比べ、2倍以上のヘミセルロースおよびセルロース含量を示し、みつ症の進行にともないそれらの含量は低下した。これらの結果から、'秋栄' のみつ症発症率が高かった原因として、スクロースの過剰な蓄積と、GA 処理で誘導したみつ症に伴う組織の生理・化学的变化、特に細胞壁関連物質の品種間差が関係する可能性が示唆された。