Changes of Endogenous Jasmonic Acid and Methyl Jasmonate in Apples and Sweet Cherries during Fruit Development

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ABSTRACT. *Trans*-jasmonic acid (JA), *cis*-JA, and *trans*-methyl jasmonate (MeJA) were quantified in pulp and seeds of ‘Tsugaru’ apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] and ‘Satohnishiki’ sweet cherry (*Prunus avium* L.). *Trans*-JA and *cis*-JA showed similar changes during development in both types of fruit. JA concentration was high in the early growth stages of apple pulp development, decreased with days after full bloom (DAFB), and then increased again during maturation. There was an initial decrease in concentration of MeJA in apple pulp, followed by a general increase towards harvest. Concentrations of JA and MeJA in the pulp of sweet cherry were high during early growth stages, then decreased towards harvest. PDJ treatment at 104 DAFB (preclimacteric stage) increased endogenous abscisic acid concentration and anthocyanin concentration at 122 and 131 DAFB (maturation stages) in apple. JA concentration in apple seeds was also high in the early growth stages, then decreased, and finally peaked at harvest. MeJA concentration in apple seeds increased towards harvest. In the seeds of sweet cherry, JA and MeJA concentrations generally increased until harvest. In both types of fruit, concentrations of JA and MeJA in the seeds were higher than those of pulp. On a dry weight basis, changes in concentration in the seeds preceded those in the pulp. These results demonstrate that relatively high amounts of JA and MeJA are associated with young developing fruit. These substances may have a role in regulation of fruit growth at early growth stages, though this has not been demonstrated. Chemical name used: n-propyl dihydrojasmonate (PDJ).

Jasmonates have been shown to be physiologically active in plants. For example, methyl jasmonate (MeJA) inhibits growth of rice seedlings (*Oryza sativa* L.) (Tsai et al., 1997), induces stomatal closure in olive leaves (*Olea europaea* L.) (Sanz et al., 1993), and increases anthocyanin accumulation in stems and leaves of tulips (*Tulipa gesneriana* L.) (Saniewski et al., 1998).

Exogenous application of jasmonic acid (JA) to table grape (*Vitis vinifera* L.) flower clusters inhibits pollen tube growth, resulting in induced seedlessness (Shiozaki et al., 1998). Jasmonates also influence ethylene metabolism in fruit. Saniewski et al. (1987a) reported that *trans*-methyl jasmonate (MeJA) increased or decreased 1-aminocyclopropane-1-carboxylic acid (ACC) concentration in tomato fruit (*Lycopersicon esculentum* Mill.) depending on the maturation stage. Similarly, in apple (*Malus sylvestris* var. *domestica*), MeJA differentially affects ethylene production in the fruit, depending on the fruit growth stage (Fan et al., 1997; Saniewski et al., 1987b, 1988). The effect of exogenous jasmonates on fruit differs depending on the concentration (Fan et al., 1998b), and these differences may be associated with differences in endogenous jasmonate concentrations. However, there have been few reports of endogenous jasmonate changes in developing fruit. Additionally, since seeds are often the site of synthesis for other plant hormones (Luckwill, 1969), it is possible that seeds may be an important site of jasmonate synthesis; however, changes in jasmonate concentration in seeds have not been demonstrated.

The effects of plant hormones on fruit maturation differ for climacteric and nonclimacteric fruit. Abscisic acid (ABA), rather than ethylene, plays a role in the onset of fruit maturation in nonclimacteric fruit (Kondo and Inoue, 1997). The influence of jasmonates on fruit maturation may also differ between climacteric and nonclimacteric fruit although this has not been investigated.

The JA analog, PDJ, influences growth of radish (*Raphanus sativus* L.) and rice in the field, and alters sugar and tartaric acid concentration in grape berries compared to controls (Fujisawa et al., 1996, 1997). ABA is synthesized from carotenoids through lipoxygenase action (Parry and Horgan, 1991a) and it has been reported that lipoxygenase activity increased in MeJA-treated apple fruit (Olias et al., 1992). PDJ application may influence ABA metabolism, and therefore fruit maturation. However, the effects of jasmonate on ABA in the fruit have not been examined.

In the present study, changes in endogenous concentrations of JA and its methyl ester, MeJA, in pulp and seeds of apple (climacteric) and sweet cherry (*Prunus avium*) (nonclimacteric) fruit were examined during fruit development. Additionally, endogenous ABA concentrations in fruit treated with PDJ were compared to that of control (nontreated) fruit.

Materials and Methods

PLANT MATERIAL. Fifteen randomly selected 10-year-old ‘Tsugaru’ apple trees, grafted onto Malling 26 (M. 26) rootstocks, and 15 randomly selected 10-year-old ‘Satohnishiki’ sweet cherry trees, grafted onto ‘Colt’ rootstocks growing in an open field at Hiroshima Prefectural University, were used in this study. Similar experiments were performed in 1997 and 1998. The yield of sweet cherry fruit in 1997 was too small and the fruit were too irregular to provide an accurate test, so only the 1998 results were used for this report.

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Fifty or more apples were collected from 15 trees at 10 to 20 d intervals from 20 to 131 d after full bloom (DAFB) for analysis of JA and MeJA and for the measurement of firmness and anthocyanin. Samples of no less than 50 fruit were collected from 15 sweet cherry trees at intervals of 4 to 7 d from 15 to 56 DAFB for the same analysis as performed on the apple samples. The firmness of individual fruit was determined by a Rheometer (NRM-2002J; Fudo Ind. Co., Tokyo; needle diameter = 1 mm) after harvest. Measurements were taken at two places on the equator of 10 fruit selected randomly from 15 trees at each sampling date. After fruit firmness was measured, anthocyanin concentration was determined by extracting from fruit skins of fixed weight in 1% HCl-methanol and determining absorbance at 530 nm by a spectrophotometer (U-2001; Hitachi Ltd., Tokyo). Anthocyanin is expressed as cyanidin 3-galactoside equivalents in apple and cyanidin 3-glucoside equivalents in sweet cherry (Mazza and Miniati, 1993). Pulp, seeds, and skin samples were separated carefully with a knife, frozen immediately in liquid nitrogen, and then stored at -30°C until analysis.

PDJ TREATMENT. Experiments were randomized complete block designs with three replications of each treatment. A 0.39 M PDJ (Nippon Zeon Co., Ltd., Tokyo) solution in distilled water was applied to apple fruit and spur leaves using a low pressure hand sprayer at 104 DAFB (immediately before coloring). The rest of the tree was shielded by a vinyl sheet. A distilled water solution was sprayed as the control treatment. Fifteen fruit per treatment (three, one-tree replications of five fruit in each treatment per tree) were collected for ABA and anthocyanin analysis of the fruit at 122 and 131 DAFB. In sweet cherry, 0.39 M PDJ was sprayed onto three trees, three branches per tree using a low pressure hand sprayer at 36 DAFB (immediately before coloring). For analysis of ABA and anthocyanin, 60 fruit for each treatment (three, one-tree replications of 20 fruit per tree) were collected at 42, 49, and 56 DAFB. For each sampling time, fruit from a different branch were used (three treated branches, one per collection date).

JASMONATE ANALYSIS. JA and MeJA analyses in both species were quantified with a modification of the method of Mueller et al. (1993). Fruit samples [20 g pulp fresh weight (FW); five replications of 10 to 30 fruit selected randomly from 15 trees] and seeds (10 g FW; five replications) were homogenized with 1000 ng ($\pm$)-2-(2,3- $^2\text{H}_2$) JA and ($\pm$)-2-(2,3- $^2\text{H}_2$) MeJA as the internal standards in a 20 mL saturated NaCl solution, 1 mL 1 M citric acid, and 50 mL diethyl ether containing 11.3 μM butylated hydroxytoluene as an antioxidant. Internal standards were prepared from 2-(2-pentyl)-2-cyclopentenone through catalytic semi-deuteriogenation of acetylenic intermediates with deuterium gas in pyridine (Seto et al., 1996). After centrifugation (10 min at

Fig. 1. Changes of endogenous *trans*- and *cis*-JA concentrations in the pulp of apple [(A) fresh weight (FW) basis and (B) total of *trans*- and *cis*-JA concentrations on dry weight (DW) basis] and sweet cherry [(C) FW basis and (D) total of *trans*- and *cis*-JA concentrations on DW basis]. Data are means $\pm$ SE of five replications.

2000 g_n), the ether phase was removed and the aqueous layer was extracted a second time with 50 mL diethyl ether containing 11.3 μM butylated hydroxytoluene. The pooled ether extracts were dried under N_2 . The residue was dissolved in 200 μL chloroform/diisopropylethylamine, 1:1 (v/v), and derivatized 60 min at 50°C with 10 μL pentafluorobenzyl (PFB) bromide. The derivatization mixture was then dried under N_2 .

The residue was dissolved in 5 mL *n*-hexane and added onto a column of silica gel (5 mm i.d. $\times$ 14 cm) [250 mg of silica gel 60 (Merck KGaA, Darmstadt, Germany)]. The sample was eluted with 7 mL *n*-hexane/ether, 2:1 (v/v), dried under N_2 , redissolved in 50 μL *n*-hexane/ether, 2:1 (v/v), and 1 μL analyzed using gas chromatography-mass spectrometry (GC-MS) [QP 5050; Shimadzu Scientific Instruments, Inc., Kyoto, Japan; DB-1 column (J & W Scientific, Inc., Folsom, Calif.; 0.32 mm $\times$ 30 m, 0.25 μm film thickness); linear He flow at 53.1 cm^3s^{-1} , column temperature step gradient, 100°C for 0.5 min, 100 to 180°C at $20^{\circ}\text{C}\cdot\text{min}^{-1}$, 180 to 250°C at $30^{\circ}\text{C}\cdot\text{min}^{-1}$, and 250°C for 30 min; electron potential, 70 eV]. The quantitative analyses were carried out in the selected ion monitoring mode.

Retention times of the PFB derivatives are as follows: *trans*-JA, 7.62 min; *cis*-JA, 7.78 min; $^2\text{H}_2$ -JA, 7.62 min, *trans*-MeJA, 5.30 min; *cis*-MeJA, 5.45 min; $^2\text{H}_2$ -MeJA, 5.30 min. Ions were monitored for JA/[$^2\text{H}_2$]($\pm$)-JA as follows: m/z 392, 390, 211, and 209. The concentration of JA in the original extract was determined from the ratio of peak areas for m/z 209 ($^2\text{H}_0$)/211 ($^2\text{H}_2$). For MeJA/[$^2\text{H}_2$]($\pm$)-MeJA, ions were monitored as follows: m/z 226, 224, 195, 193, 153, and 151. MeJA concentration was measured from the ratio of peak areas for m/z 224 ($^2\text{H}_0$)/226 ($^2\text{H}_2$). To identify PFB-jasmonates in the samples, the fragmentation patterns were compared with those of the chemical standards.

ABA ANALYSIS. The *cis*-ABA concentration was measured with a modification of the method described previously (Kondo and Tomiyama, 1998). Ten gram FW pulp samples (five replications

of 10 to 30 fruit selected randomly from 15 trees) were homogenized in 80% methanol, containing 925 Bq [^3H]-ABA. The extract was filtered and reduced to the aqueous phase in vacuo. The pH was adjusted to 2.5 with 0.1 M phosphoric acid and extracted with dichloromethane. Solvent was removed in vacuo and the residue was redissolved in 4.8 M acetonitrile. High-performance liquid chromatography (HPLC) (Gilson Medical Elec. Inc., Middleton, Wis.) analysis was conducted as follows; column = μ Bondapak C_{18} (Waters Millipore Co., Milford, Mass.; 8 mm i.d. $\times$ 10 cm); mobile phase of acetonitrile with 20 mM acetic acid (4.8 M to 9.6 M acetonitrile over 8 min and then held at 9.6 M for 7 min); flow rate of 2.0 mL $\cdot$ min $^{-1}$; detector = UV 254 nm. The fraction corresponding to the retention time of *cis*-ABA standard was collected, dried in vacuo, and methylated with diazomethane. Half of the ABA-methyl ester was redissolved with methanol and quantified by gas chromatography using an electron capture detector (G-6800; Yanaco Co., Kyoto, Japan) using a Neutrabond-5 column (GL Sciences, Inc., Tokyo; 0.53 mm $\times$ 30 m, 2 μ m film thickness) with column temperature 150 to 210 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}\cdot$ min $^{-1}$. Recovery of ABA was estimated by measuring its radioactivity using a liquid scintillation system (LSC-3600; Aloka Co., Tokyo). *Cis*-ABA in the samples was identified through comparison to the chemical standard using GC-MS (QP 5050; Shimadzu Scientific Instruments, Inc., Kyoto).

STATISTICAL ANALYSIS. Data were presented as means $\pm$ SE and student's *t* test was used to determine the significance of PDJ treatment (SAS Inst., Inc., Cary, N.C.).

Results and Discussion

Trans-JA, *cis*-JA, and *trans*-MeJA were detected in pulp and seeds of apple and sweet cherry. The mass spectrum of *trans*-PFB-JA and *cis*-PFB-JA showed the same fragment pattern. The relative ion abundances of the samples were almost the same as standard JA and MeJA.

Trans-JA concentration in apple pulp increased from 89 to 158 nmol $\cdot$ kg $^{-1}$ FW between 20 and 41 DAFB (Fig. 1A). This was followed by a sharp decrease that leveled off by 83 DAFB. *Cis*-JA concentration also reached its maximum of 6 nmol $\cdot$ kg $^{-1}$ FW at 41 DAFB, decreased through 83 DAFB, and then increased gradually towards harvest (131 DAFB). The concentration of total JA on a dry weight (DW) basis followed a similar pattern (Fig. 1B).

Trans-JA concentration in the pulp of sweet cherry increased to 48 nmol $\cdot$ kg $^{-1}$ FW by 26 DAFB, decreased rapidly to 11 nmol $\cdot$ kg $^{-1}$ FW by 36 DAFB, then decreased gradually towards harvest (56 DAFB) (Fig. 1C). The concentration of *cis*-JA increased gradually from 15 to 26 DAFB, then increased rapidly to 10 nmol $\cdot$ kg $^{-1}$ FW by 30 DAFB, followed by a decrease until harvest.

In apple pulp, the percentage of *cis*-JA was 3% to 5% of total JA from 20 to 83 DAFB, then rose to around 10% from 83 DAFB until harvest (Fig. 1A). In sweet cherry pulp, the percentage of *cis*-JA was $\approx$ 5% of total JA from 15 to 26 DAFB, then rose to $\approx$ 25% from 26 DAFB to harvest (Fig. 1C). Fruit firmness decreased dramatically from 3.9 N $\cdot$ mm $^{-2}$ on 30 DAFB to 0.7 N $\cdot$ mm $^{-2}$ on 36 DAFB (Fig. 2B). This suggests that the pulp might switch to the maturation stage between 30 and 36 DAFB. The sharp increase in *cis*-JA concentration from 26 to 30 DAFB in sweet cherry may indicate a role in the decrease in fruit firmness. In tomato fruit, for example, the percentage of *cis*-JA increased during early fruit maturation (Fan et al., 1998b). These observations suggest a

relationship between the increase in percentage of *cis*-JA and fruit maturation.

Trans-MeJA concentration in apple pulp decreased from 0.63 nmol $\cdot$ kg $^{-1}$ FW to 0.36 nmol $\cdot$ kg $^{-1}$ FW between 20 and 111 DAFB, then increased to 0.54 nmol $\cdot$ kg $^{-1}$ FW by harvest (Fig. 3A). In sweet cherry pulp, the concentration decreased gradually from 0.67 nmol $\cdot$ kg $^{-1}$ FW to 0.40 nmol $\cdot$ kg $^{-1}$ FW between 15 DAFB and harvest (Fig. 3C). Gansser et al. (1997) have also reported that high concentrations of MeJA were found in immature strawberry fruit (*Fragaria $\times$ ananassa* Duchesne) at $\approx$ 10 d after anthesis, and decreased steadily during fruit development. The concentration of *trans*-MeJA in the pulp of both species on a DW basis followed a similar pattern (Fig. 3B and D).

Results of our study indicate that endogenous JA concentration is highest during the early stages of apple and cherry fruit development. The possible role of JA during this time is unknown. However, there are several reports of JA effects on cell division. JA and MeJA have been reported to retard cell division in soybean callus [*Glycine max* (L.) Merr.] (Ueda and Kato, 1982). In contrast, tuberonic acid and its glycoside, which are JA metabolites, induce potato tuberization (*Solanum tuberosum* L.) via cell division (Koda et al., 1988; Yoshihara et al., 1989). Ravnkar et al. (1992) reported that exogenous JA concentrations of 0.01 to 1 μM stimulated cell division in potato leaves.

Fig. 2. Firmness and anthocyanin concentration in (A) apple and (B) sweet cherry fruit. Data are means $\pm$ SE of 10 fruit.

Fig. 3. Changes of endogenous *trans*-MeJA concentrations in the pulp of apple [(A) fresh weight (FW) basis and (B) dry weight (DW) basis] and sweet cherry [(C) FW basis and (D) DW basis]. Data are means $\pm$ SE of five replications.

Anthocyanin concentration in 'Tsugaru' apple fruit increased after 111 DAFB and fruit firmness decreased (Fig. 2A). Kondo et al. (1991) reported that the internal ethylene production and ABA concentration of 'Tsugaru' apple fruit increased rapidly after 109 DAFB. Together, these observations suggest that the fruit reached the climacteric stage at around 110 DAFB. During this same time, endogenous *trans*-JA, *cis*-JA and *trans*-MeJA concentrations also increased (Figs. 1A and 3A). Treatment of preclimacteric apple fruit with JA and MeJA is reported to stimulate ACC oxidase activity and increase ethylene production (Miszczak et al., 1995; Saniewski et al., 1986). Additionally, JA and MeJA treatments promote degreening of apple fruit (Fan et al., 1998a). Thus, the increase of both endogenous JA and MeJA at 110 DAFB, as observed in the present study, may play a role in the initiation of the maturation process in apple fruit.

In sweet cherry, the anthocyanin concentration increased dramatically after 42 DAFB, which corresponds with the maturation time (Fig. 2B); however, increases in JA and MeJA concentration in sweet cherry pulp were not observed during this period (Figs. 1C and 3C).

PDJ, whose activity is stronger than native JA isomers, has been found to promote fruit maturation (accumulation of sugar and anthocyanin) of peach [*Prunus persica* (L.) Batsch (Peach Group)] (Takeuchi and Kamuro, 1997). Endogenous ABA concentration and anthocyanin concentration in apple pulp increased significantly in PDJ compared with control treatments (Fig. 4A and B). Color formation is an important

indicator that identifies the state of maturation of apple fruit (Mazza and Miniati, 1993). Ethylene and ABA may act as triggers for fruit maturation since both ethylene evolution and ABA concentration in apple fruit increase prior to coloring and sugar accumulation (Kondo et al., 1991). PDJ treatment occurred at 104 DAFB corresponding to the preclimacteric period, and the increase in ABA and anthocyanin concentrations in response to the treatment suggests that exogenous PDJ application at preclimacteric stages may promote apple fruit maturation.

PDJ treatment failed to influence endogenous ABA concentration and antho-

cyanin accumulation in sweet cherry (data not presented). In grape berries, which are nonclimacteric fruit, PDJ application at veraison promoted sugar increase and anthocyanin accumulation in the fruit, although the influence on ABA was not investigated (Fujisawa et al., 1997). ABA concentration in grape berries increases dramatically after veraison, decreasing from the maturation stage until harvest (Kondo and Kawai, 1998). ABA concentration in 'Satohnishiki' sweet cherry fruit reached a maximum around 35 DAFB, then decreased towards harvest (Kondo and Tomiyama, 1998). The PDJ treatment by Fujisawa et al. (1997) might have been performed during the increase of endogenous ABA in grape berries, while in the present study with sweet cherry, PDJ was applied during the decrease of ABA. Since PDJ treatment at the maturation stage did not influence fruit maturation (the accumulation of sugar and anthocyanin) of grape berries (Fujisawa et al., 1997), the influence of PDJ application during

Fig. 4. Effect of PDJ application on endogenous *cis*-ABA concentration in the (A) pulp and anthocyanin concentration in the (B) peel of apple. Data are means $\pm$ SE of three replications. **Significant at $P \leq 0.05$ or 0.01, respectively.

Fig. 5. Changes of endogenous *trans*- and *cis*-JA concentrations in the seeds of apple [(A) fresh weight (FW) basis and (B) total of *trans*- and *cis*-JA concentrations on dry weight (DW) basis] and sweet cherry [(C) FW basis and (D) total of *trans*- and *cis*-JA concentrations on DW basis]. Data are means $\pm$ SE of five replications.

the increase of ABA, at which time lipoxygenase activity is high (Parry and Horgan, 1991b), should be investigated in sweet cherry.

Fig. 6. Changes of endogenous *trans*-MeJA concentrations in the seeds of apple [(A) fresh weight (FW) basis and (B) dry weight (DW) basis] and sweet cherry [(C) FW basis and (D) DW basis]. Data are means $\pm$ SE of five replications.

6A and C). The seed plays an important role in fruit growth. For example, seed abortion in apple fruit prevents continued fruit growth (Kondo and Mizuno, 1989). However, peach fruit treated with gibberellic acid₃ (GA₃) after seed removal maintained fruit growth (Nakagawa et al., 1973). In grape berries, both ABA concentration and sugar concentration in the skin are higher for seeded fruit than for GA₃-treated seedless fruit (Kondo and Kawai, 1998). This implies that hormones produced in the seed may influence hormone levels in the pulp and contribute to fruit growth. In the present study, JA and MeJA concentrations in the seeds of both apple and cherry were generally higher than those in the pulp. Furthermore, when JA concentrations in the seeds and pulp of both

Trans-JA in apple seeds averaged 162 nmol $\cdot$ kg $^{-1}$ FW at 20 DAFB, decreased until 62 DAFB, and then increased through harvest, where the concentration averaged 363 nmol $\cdot$ kg $^{-1}$ FW (Fig. 5A). The concentration of *cis*-JA showed similar changes. *Trans*-JA concentration in the seed of sweet cherry increased from 26 DAFB until harvest and reached a peak of 237 nmol $\cdot$ kg $^{-1}$ FW at 49 DAFB (Fig. 5C). Changes in *cis*-JA concentration were again similar to those of *trans*-JA. There was a general increase in concentration of *trans*-MeJA in the seeds of apple and sweet cherry towards harvest (Fig.

types of fruit were compared on a DW basis, the changes were similar, with the changes in the seeds preceding those of the pulp (Figs. 1B and D, 5B and D). Also, the changes in MeJA on a DW basis in the seed and pulp were similar to those of JA (Figs. 3B and D, 6B and D).

In summary, results show an association between JA and MeJA in young developing apple and sweet cherry fruit. Seed concentrations of JA and MeJA were higher than those of the pulp, but changes on a DW basis were similar in both seeds and pulp. Increases of JA and MeJA concentration in apple pulp on and after climacteric and of anthocyanin by PDJ treatment at pre-climacteric stages suggest that jasmonate may be related to fruit maturation in apple.

Literature Cited

- Fan, X., J.P. Mattheis, and J.K. Fellman. 1998a. Responses of apples to postharvest jasmonate treatments. *J. Amer. Soc. Hort. Sci.* 123:421–425.
- Fan, X., J.P. Mattheis, and J.K. Fellman. 1998b. A role for jasmonates in climacteric fruit ripening. *Planta* 204:444–449.
- Fan, X., J.P. Mattheis, J.K. Fellman, and M.E. Patterson. 1997. Effect of methyl jasmonate on ethylene and volatile production by 'Summerred' apples depends on fruit developmental stage. *J. Agr. Food Chem.* 45:208–211.
- Fujisawa, H., M. Koshiyama, H. Seto, S. Yoshida, and Y. Kamuro. 1997. Effects of jasmonic acid compounds on fruit setting, fruit growth, ripening and cold-resistance. *Acta Hort.* 463:261–266.
- Fujisawa, H., H. Seto, S. Yoshida, and Y. Kamuro. 1996. Promoting effects of jasmonic acid analog, n-propyl dihydrojasmonate (PDJ), on plant growth. *Proc. 23rd Annu. Mtg. Plant Growth Regulat. Soc. Amer.* p. 111–116.
- Gansser, D., S. Latza, and R.G. Berger. 1997. Methyl jasmonates in developing strawberry fruit (*Fragaria ananassa* Duch. cv. Kent). *J. Agr. Food Chem.* 45:2477–2480.
- Koda, Y., E.S.A. Omer, T. Yoshihara, H. Shibata, S. Sakamura, and Y. Okazawa. 1988. Isolation of a specific potato tuber-inducing substance from potato leaves. *Plant Cell Physiol.* 29:1047–1051.
- Kondo, S. and K. Inoue. 1997. ABA and ACC content during growth of 'Satohnishiki' cherry fruit, and effect of ABA and ethephon application on fruit quality. *J. Hort. Sci.* 72:221–227.
- Kondo, S. and M. Kawai. 1998. Relationship between free and conjugated ABA levels in seeded and gibberellin-treated seedless, maturing 'Pione' grape berries. *J. Amer. Soc. Hort. Sci.* 123:750–754.
- Kondo, S. and N. Mizuno. 1989. Relation between early drop of apple fruit and endogenous growth regulators, and effects of MCPB, GA₃ plus GA₄ and BA sprays on fruit abscission. *J. Jpn. Soc. Hort. Sci.* 58:9–16.
- Kondo, S. and A. Tomiyama. 1998. Changes of free and conjugated ABA in the fruit of 'Satohnishiki' sweet cherry and the ABA metabolism after application of (s)-(+)-ABA. *J. Hort. Sci. Biotech.* 73:467–472.
- Kondo, S., J. Uthabutra, and H. Gemma. 1991. Comparison of 1-aminocyclopropane-1-carboxylic acid, abscisic acid and anthocyanin content of some apple cultivars during fruit growth and maturation. *J. Jpn. Soc. Hort. Sci.* 60:505–511.
- Luckwill, L.C., P. Weaver, and J. MacMillan. 1969. Gibberellins and other growth hormones in apple seeds. *J. Hort. Sci.* 44:413–424.
- Mazza, G. and E. Miniati. 1993. Anthocyanins in fruits, vegetables, and grains. CRC Press, Boca Raton, Fla.
- Miszczak, A., E. Lange, M. Saniewski, and J. Czapski. 1995. The effect of methyl jasmonate on ethylene production and CO₂ evolution in 'Jonagold' apples. *Acta Agrobotanica* 48:121–128.
- Mueller, M.J., W. Brodschelm, E. Spannagl, and M.H. Zenk. 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. *Proc. Natl. Acad. Sci. USA* 90:7490–7494.
- Nakagawa, S., I. Kiyokawa, H. Matsui, and H. Kurooka. 1973. Fruit development of peach and Japanese pear as affected by destruction of the embryo and application of gibberellins. *J. Jpn. Soc. Hort. Sci.* 42:104–112.
- Olias, J.M., L.C. Sanz, J.J. Rios, and A.G. Perez. 1992. Inhibitory effect of methyl jasmonate on the volatile ester forming enzyme system. *J. Agr. Food Chem.* 40:266–270.
- Parry, A.D. and R. Horgan. 1991a. Carotenoid metabolism and the biosynthesis of abscisic acid. *Phytochem.* 30:815–821.
- Parry, A.D. and R. Horgan. 1991b. Carotenoids and abscisic acid (ABA) biosynthesis in higher plants. *Physiol. Plant.* 82:320–326.
- Ravnikar, M., B. Vilhar, and N. Gogala. 1992. Stimulatory effects of jasmonic acid on potato stem node and protoplast culture. *J. Plant Growth Regulat.* 11:29–33.
- Saniewski, M., J. Czapski, and J. Nowacki. 1987a. Relationship between stimulatory effect of methyl jasmonate on ethylene production and 1-aminocyclopropane-1-carboxylic acid content in tomatoes. *Biol. Plant.* 29:17–21.
- Saniewski, M., J. Czapski, J. Nowacki, and E. Lange. 1987b. The effect of methyl jasmonate on ethylene and 1-aminocyclopropane-1-carboxylic acid production in apple fruits. *Biol. Plant.* 29:199–203.
- Saniewski, M., A. Miszczak, L. Kawa-Miszczak, E. Wegrzynowicz-Lesiak, K. Miyamoto, and J. Ueda. 1998. Effects of methyl jasmonate on anthocyanin accumulation, ethylene production, and CO₂ evolution in uncooled and cooled tulip bulbs. *J. Plant Growth Regulat.* 17:33–37.
- Saniewski, M., J. Nowacki, E. Lange, and J. Czapski. 1986. The effect of methyl jasmonate on ethylene and 1-aminocyclopropane-1-carboxylic acid production in preclimacteric and postclimacteric 'Jonathan' apples. *Fruit Sci. Rpt.* 13:193–200.
- Saniewski, M., J. Nowacki, E. Lange, and J. Czapski. 1988. The effect of methyl jasmonate on anthocyanin accumulation, ethylene production and ethylene-forming enzyme activity in apples. *Fruit Sci. Rpt.* 15:97–102.
- Sanz, L.C., J.C. F-Maculet, E. Gomez, B. Vioque, and J.M. Olias. 1993. Effect of methyl jasmonate on ethylene biosynthesis and stomatal closure in olive leaves. *Phytochem.* 33:285–289.
- Seto, H., S. Fujioka, H. Fujisawa, K. Goto, H. Nojiri, H. Yamane, and S. Yoshida. 1996. Preparation of (±)-2-(2,3-³H₂) jasmonic acid and its methyl ester, methyl (±)-2-(2,3-³H₂) jasmonate. *Biosci. Biotech. Biochem.* 60:1709–1711.
- Shiozaki, S., M. Pan, T. Ogata, S. Horiuchi, and K. Kawase. 1998. Jasmonic acid effects on gibberellic acid-induced seedlessness in 'Neo Muscat' table grapes. *J. Hort. Sci. Biotech.* 73:768–773.
- Takeuchi, Y. and Y. Kamuro. 1997. Physiology and present situation for practical use of brassinosteroid and jasmonoid compounds. *Chem. Regulat. Plants* 32:74–86.
- Tsai, F.Y., C.C. Lin, and C.H. Kao. 1997. A comparative study of the effects of abscisic acid and methyl jasmonate on seedling growth of rice. *Plant Growth Regulat.* 21:37–42.
- Ueda, J. and J. Kato. 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. *Physiol. Plant.* 54:249–252.
- Yoshihara, T., E.S.A. Omer, H. Koshino, S. Sakamura, Y. Kikuta, and Y. Koda. 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). *Agr. Biol. Chem.* 53:2835–2837.