Stimulative Effect of Jasmonic Acid on Callus Formation in Apple Fruit

Satoru Kondo *, Naoko Tsukada and Yoshiyuki Niimi

School of Bioresources, Hiroshima Prefectural University, Shobara, Hiroshima 727–0023

Summary

The role of jasmonic acid (JA) in callus formation was investigated on discs taken from the pulp of apple (*Malus pumila* Mill. var. *domestica* Schneid.), sampled on 15, 25, and 35 days after full bloom (DAFB), and cultured on B5 medium, containing combinations of naphthalene acetic acid (NAA), benzyladenine (BA), and JA. On the 15 DAFB sample, 0.45 μM , 1.0 μM , and 4.5 μM JA in the presence of 10.7 μM NAA induced greater callus formation than did the sample without JA. However, this effect of JA at 15 DAFB was not observed except when it was combined with only NAA; 4.5 μM JA inhibited the callus formation. The presence of JA inhibited callus formation on discs taken 25 and 35 DAFB. Endogenous JA concentration of apple pulp at 15 DAFB was lower than that at 25 and 35 DAFB. These results indicate that the low endogenous JA concentration early in fruit development promotes callus formation; whereas, increasing JA concentration inhibits it.

Key Words: callus formation, jasmonic acid, *Malus pumila* var. *domestica*.

Introduction

The physiological roles of jasmonates have been investigated in many plants. In most cases, jasmonates are inhibitory to plant growth, e.g. jasmonic acid (JA) and methyl jasmonate (MeJA) inhibited cell division and enlargement in soybean (*Glycine max* Merrill) and radish (*Raphanus sativus* L.) (Ueda and Kato, 1982). We found that in apple (*Malus pumila* var. *domestica*) the concentrations of JA were high in the early growth stages of pulp development (Kondo et al., 2000), indicating that jasmonates may influence cell division or cell enlargement. However, there are no reports on the effect of JA on cell division and enlargement at the beginning of apple fruit development. The objective of this study is to ascertain the action of jasmonates in callus formation of young apple fruit.

Materials and Methods

'Tsugaru' apple fruit were obtained from fifteen randomly selected 11-year-old trees grafted onto Malling 26 (M. 26) rootstocks, growing in an open field at Hiroshima Prefectural University in 1999.

Samples were collected 15, 25, and 35 days after full bloom (DAFB). Discs, 4 mm in diameter and 3 mm in thickness were excised with a cork borer from apple pulp. The average weight of 30 discs was 14.5 ± 0.43 (SE) mg. To determine the effects on the induction of callus weight gain, 16 groups of six discs were placed on 40 ml B5 medium (Nomura and Masuda, 1998), containing 0.08 M sucrose. In addition, 0.89 μM BA with

10.7 μM NAA, 0.89 μM BA alone, or 10.7 μM NAA alone were included on 4 media; the last 4 groups were hormone-free. These discs were cultured in petri dishes with different concentrations of JA (0, 0.45, 1.0 and 4.5 μM) in a 4×4 factorial design. This entire treatment was replicated 5 times (80 total cultures). The medium was adjusted to pH 5.8 with 1 M KOH before adding 0.3% (w/v) gellan gum, and autoclaved at $1.2 \text{ kg} \cdot \text{cm}^{-2}$ for 20 min. JA in methanol was added after the temperature fell to 45°C. Three randomly selected discs per apple were placed in one petri dish; the plates were sealed with parafilm and incubated for 30 days at 25°C in the dark.

JA concentration in apple pulp was measured, according to Kondo et al. (2000). Fruit samples (15 g fresh weight; five replications of 50 fruit) were homogenized in 20 ml saturated NaCl solution, 1 ml 1 mol citric acid, and 50 ml diethyl ether that contained 11.3 μmol butylated hydroxytoluene as an antioxidant with 1 μg ($\pm$)-2-(2,3- $^2\text{H}_2$) JA as the internal standard. After JA in the sample was derivatized by pentafluorobenzyl (PFB) bromide, the level of the derivatives was determined by gas chromatography-mass spectrometry (GC-MS) [QP 5000; Shimadzu Scientific Instruments, Inc., Kyoto; column = CP-Sil 5CB(Chrompack, Inc., Mid-delburg, Netherlands; 0.25 mm $\times$ 25 m); linear He flow at $50.2 \text{ cm} \cdot \text{sec}^{-1}$, column temperature had a step gradient of 60°C for 2 min, 60 to 270°C at $10^\circ\text{C} \cdot \text{min}^{-1}$, and 270°C for 35 min; electron potential, 70 eV]. The analyses were performed using selected ion monitoring mode. Retention times of JA derivatives were *trans*-JA, 19.47 min; $^2\text{H}_2$ -JA, 19.47 min, and *cis*-JA, 19.75 min. Ions of *m/z* 392, 390, 211, and 209 were monitored for *trans*-JA, *cis*-JA and $^2\text{H}_2$ -JA. JA concentration was

Received; July 17, 2000. Accepted; August 30, 2000.

*Corresponding author: e-mail: s-kondo@bio.hiroshima-pu.ac.jp

measured from the ratio of peak areas for m/z 209 ($^2\text{H}_0$)/211 ($^2\text{H}_2$). The total of *trans*-JA and *cis*-JA is expressed as JA.

Standard errors and significant differences between treatments were performed using the Newmen-Keuls test (SPSS Inc., Chicago, Ill.)

Results and Discussion

The combination of BA plus NAA promoted greater increases in callus weight than did media with only BA, NAA or that free of hormones (Fig.1). JA at 4.5 μM was the least effective at promoting callus growth in a medium containing BA and NAA. Jasmonates had little or no effect on callus weight gain in discs incubated without hormonal additives or with BA, whereas some gained weight when JA was added to NAA-incubated

Fig. 1. Effect of JA concentration on callus weight gain of 'Tsugaru' apple discs from (A) 15, (B) 25, and (C) 35 DAFB. The discs on B5 medium with or without 0.89 μM BA plus 10.7 μM NAA, 0.89 μM BA, or 10.7 μM NAA were incubated with 0, 0.45, 1, and 4.5 μM JA for 30 days at 25°C in the dark. Data represent means $\pm$ SE of five replications. Data points within each treatment (BA+NAA, hormone-free, BA, and NAA) with different letters show significant differences as calculated by Newmen-Keuls test, $P \leq 0.05$.

Table 1. JA concentrations (nmol $\cdot$ kg FW $^{-1}$) in the pulp of apples.

Days after full bloom	JA concentration
15	92.1 $\pm$ 7.9
25	132.5 $\pm$ 8.3
35	213.2 $\pm$ 17.1

Data are means $\pm$ SE of five replications.

callus. JA at 0.45 μM , 1.0 μM and 4.5 μM at 15 DAFB promoted significantly more callus formation, compared to the sample without JA; but JA inhibited callus induction even when it combined with NAA at 25 and 35 DAFB.

Auxin induces cell expansion in plants, and cytokinin, which plays a role in cell division, becomes more active in the presence of auxin (Masuda et al., 1971). The promotive effect of JA in the presence of NAA 15 DAFB indicates that JA may stimulate cell division as cytokinin does. Although most studies have reported inhibitory action of JA on cell division and enlargement (Ravnikar and Gogala, 1990; Ravnikar et al., 1990; Ueda and Kato, 1982), others have shown that JA in lower concentrations stimulate them (Ravnikar et al., 1992; Yoshihara et al., 1989). There are some possible explanations for the difference on the effect of JA on the callus formation in apple pulp between 15 DAFB and 25 and 35 DAFB. One is that endogenous JA concentration in apple pulp more than doubled between 15 and 35 DAFB (Table 1). In the BA plus NAA treatment where callus formation increased greatly when compared to the other treatments, 4.5 μM JA inhibited callus formation only at 15 DAFB. However, at 25 and 35 DAFB all JA concentrations from 0.45 μM JA to 4.5 μM JA inhibited callus formation; the degree of growth inhibition increased with time. Therefore, JA at any concentration applied exogenously might inhibit callus formation at a later stage of fruit development because endogenous JA concentrations increase with time.

Another factor is that the cells of apple fruit generally divide for 30 days after fertilization and then gradually begins to enlarge (Ryugo, 1988). Therefore, in experiment, ovarian tissue 15 DAFB was still in the cell division stage. Hence, the promotion of callus formation by JA at 15 DAFB demonstrates that low JA concentrations may promote the cell division but inhibit cell enlargement. The threshold for response to JA may differ for each fruit growth stage and by interactions with other endogenous hormones.

Literature Cited

- Kondo, S., A. Tomiyama and H. Seto. 2000. Changes of endogenous jasmonic acid and methyl jasmonate in apples and sweet cherries during fruit development. J. Amer. Soc. Hort. Sci. 125: 282–287.
- Masuda, Y., M. Katsumi and H. Imaseki. 1971. Plant

- hormone. p. 193-250. Asakura Press, Tokyo.
- Nomura, K. and K. Masuda. 1998. Medium. p. 17-39. In: A. Komamine and K. Nomura (eds.). Experimental method of biological chemistry 41: Introduction of plant cell technology. Japan Scientific Societies Press, Tokyo.
- Ravnikar, M. and N. Gogala. 1990. Regulation of potato meristem development by jasmonic acid in vitro. J. Plant Growth Regulat. 9: 233-236.
- Ravnikar, M., J. Rode, N. Gogala and D. Benedicic. 1990. Regulation of organogenesis with jasmonic acid. Acta Hort. 280: 169-172.
- 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.
- Ryugo, K. 1988. Fruit growth and development. p.107-168. In: K. Ryugo (ed.). Fruit culture-Its science and art. Wiley, New York.
- 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. Bol. Chem. 53: 2835-2837.

リンゴ果実のカルス形成に及ぼすジャスモン酸の促進効果

近藤 悟・塚田直子・新美善行

広島県立大学生物資源学部 727-0023 庄原市七塚町 562

摘 要

満開後15日, 25日および35日のリンゴ果肉ディスクからのカルス形成に及ぼすジャスモン酸(JA)の影響を検討した。果肉ディスクをナフタレン酢酸(NAA), ベンジルアデニン(BA)およびJAを組み合わせた試験液を含むB5培地に置床した。満開後15日の果肉ディスクのカルス形成は, NAAと0.45 μ M, 1.0 μ Mおよび4.5 μ M各々のJA濃度の組合せで促進された。一方NAAのみとの組合せ以外ではJAの促進効果は観察されず, 4.5 μ M JAはカルス形成を抑制した。満開後25日および35日の果肉ディスクのカルス形成は, JAの存在によって抑制された。内生JA濃度は満開後25日および35日に比べ, 満開後15日で低かった。以上より, 果実発育のごく初期には内生JA濃度が低く, その時期に限りJAのカルス形成促進効果が認められること, また高濃度のJAは逆に阻害することが示唆された。