

Changes of Endogenous Levels of ABA, IAA and GA-like Substances in Fruitlets of Parthenocarpic Persimmon

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Summary

The reproductive organ of parthenocarpic persimmon (*Diospyros kaki* Thunb. 'Hiratanenashi') was sampled from -5 to 122 days after flowering (DAF). The endogenous levels of abscisic acid (ABA) and indole-3-acetic acid (IAA) were analyzed by gas chromatograph-mass spectrometry (GC-MS), and those of gibberellin (GA)-like substances were determined by the dwarf rice bioassay. Fruitlet abscission occurred mainly from 13 to 62 DAF and peaked at 41 DAF. A relatively rapid increase of fresh weight of fruitlets occurred until 41 DAF; thereafter, the growth rate was moderate. The weight of the calyx increased until 13 DAF. The ABA concentrations in fruitlets was higher from flowering to 28 DAF than the remainder period. The calyx at 13 DAF had the maximum ABA concentration of all parts analyzed. IAA in the ovary/fruitlet increased about 18 times in content and 5 times in concentration from -5 to 0 DAF, and decreased to less than half in content by 13 DAF. The concentration of GA-like substances in fruitlets at flowering was also high, and increased at 41 and 122 DAF. At 5 days before flowering, the calyx had a higher concentration and content of three phytohormones than the ovary. These results suggest that the genetical increases of IAA and GA-like substances in the fruitlet of the parthenocarpic persimmon during flowering may result in continued growth, but the interaction between increased ABA and decreased IAA and GA-like substances in the fruitlet before fruitlet-drop may induce its abscission.

Key Words: abscisic acid, calyx, fruit set, indole-3-acetic acid, parthenocarpy.

Introduction

The production of seedless fruits is one of the most important horticultural objectives. Parthenocarpy, which grows seedless fruit, is believed to be regulated by several phytohormones (Schwabe and Mills, 1981), as is fruitlet abscission (Addicott, 1982). Thus in development of chemical control techniques of fruit thinning, growth, quality and harvesting, it is important to determine precisely the changes of endogenous phytohormone levels. Recently the endogenous phytohormones of fruits have been determined with GC-MS using internal standards in orange (Kojima, 1995), parthenocarpic mandarin (Kojima et al., 1996b) and pollinated citrus (Kojima, 1996).

In persimmon fruits, endogenous levels of phytohormones have been investigated by bioassays. Sobajima et al. (1969) assayed auxin levels in relation to the physiological abscission of fruitlets. Hirata et al. (1978) assayed the endogenous levels of main five phytohor-

mones. However, to our knowledge, no prior studies measured ABA and IAA levels of persimmon fruits with GC-MS.

There are several reports that calyx removal inhibited the growth of the persimmon fruitlet during the first half of growth stage (Sobajima et al., 1969; Yonemori et al., 1995). Thus, it seems that the calyx plays an important role on the fruitlet growth, but endogenous levels of its phytohormones have not been determined with GC-MS.

We quantified changes of ABA, IAA and GA-like substance levels in the fruitlet and calyx of parthenocarpic persimmon to investigate the roles of these phytohormones in fruit set and growth.

Materials and Methods

1. Plant Materials

Twenty 30-year-old trees of persimmon 'Hiratanenashi' (*Diospyros kaki* Thunb.), which shows a high degree of pseudo-parthenocarpy, growing in the experimental orchard of Kyoto Pref. Univ., were used in this study. Two thousand flower buds in the middle part of a shoot were covered with paper bags a few days before flowering to prevent pollination. Thus 'Hiratanenashi' showed only parthenocarpy in this report. Two hundred

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flower buds were tagged and observed for abscission weekly from May 30 to September 25. For hormonal analysis, at least 50 reproductive organs were collected on each sampling date, -5, 0, 13, 28, 41, 61, 90 and 122 DAF. As for 61, 90 and 122 DAF, each of the fruitlet sampled was immediately separated into flesh and peel (Fig. 2-A-I). From the separated flesh tissue, only central part which includes axial bundles was excised from the central axis region; all separation procedures were performed in a chilled box kept below 5 °C. The separated tissues were immediately weighed, frozen in liquid nitrogen and stored at -40 °C until analyzed.

2. Phytohormone Purification

Soluble polyvinylpyrrolidone and butylated hydroxytoluene were added to the sample, which was homogenized in 80% ethanol and filtered (Kojima, 1995). The filtrate was divided into two portions; equivalent to 4 g fresh weight (gFW) for ABA and IAA analysis, and equivalent to 20 gFW for GA-like activity analysis.

3. ABA and IAA Analysis

Both d_6 -ABA [purity 99%, purchased from Shoko Co., LTD, Tokyo, synthesized according to the method of Rivier et al. (1977)] and ^{13}C -IAA (Kojima, 1995) were added to the filtrate as the internal standards. The purification was performed according to the method of Kojima (1995, 1996). The filtrate was evaporated to the aqueous phase, which was acidified, filtered through membrane filter and partitioned against diethyl ether.

Dried ether extract was dissolved with 30% methanol and fractionated with an HPLC system equipped with a UV detector. The HPLC column was Developsil ODS-5 (150 × 6.0 mm I. D., Nomura Chem. Co., LTD, Aichi) maintained at 40 °C. The sample was eluted with 30% methanol solution (20 mM acetic acid) at a flow rate of 1.5 ml/min. The effluents corresponding to the retention time of ABA and IAA were collected separately.

The fractions of methylated ABA and IAA samples were injected into a GC-MS with selected ion monitoring (Kojima, 1995; 1996). IAA content was calculated by monitoring ion m/e 189 and 195. ABA content was calculated by the methods of Rivier et al. (1977) monitoring ion m/e 190 and 194.

4. GA-like Substances Analysis

Purification was performed according to the method of Kojima (1995, 1996): The filtrate equivalent to 20 gFW was evaporated to the aqueous phase, which was acidified, filtered through membrane filters and partitioned against ethyl acetate. The organic layer was partitioned against 0.5 M K_2HPO_4 . The acidified aqueous phase was partitioned against ethyl acetate (EtAc). The dried EtAc extracts were purified with Sephalyte DEA.

The dried extracts were dissolved in 30% methanol and fractionated with the above-mentioned HPLC sys-

tem. The sample was eluted with a gradient of methanol in 0.1% acetic acid at a flow rate of 1.35 ml min^{-1} (Ogata et al., 1996). The methanol concentration was increased linearly from 30 to 80% until 25 min, held at 80% until 45 min, increased linearly to 100% until 46 min, held at 100% until 60 min, decreased linearly to 30% until 61 min, and held at 30% until 80 min. The effluent was collected at 4-min interval until 48 min (12 fractions). The fraction was dried and dissolved in 50% acetone.

The bioassay procedure was similar to the 'modified micro-drop bioassay' (Nishijima and Katsura, 1989): Dwarf rice seeds were treated with GA synthesis inhibitor. The fraction was applied on 10 seedlings. After the calculation as GA_3 equivalent, 12 values from 12 fractions were summed up as the value of GA-like substances.

Results

1. Abscission and Growth

Figure 1-A shows a change of an abscission rate in the ovary/fruitlet of the parthenocarpic persimmon.

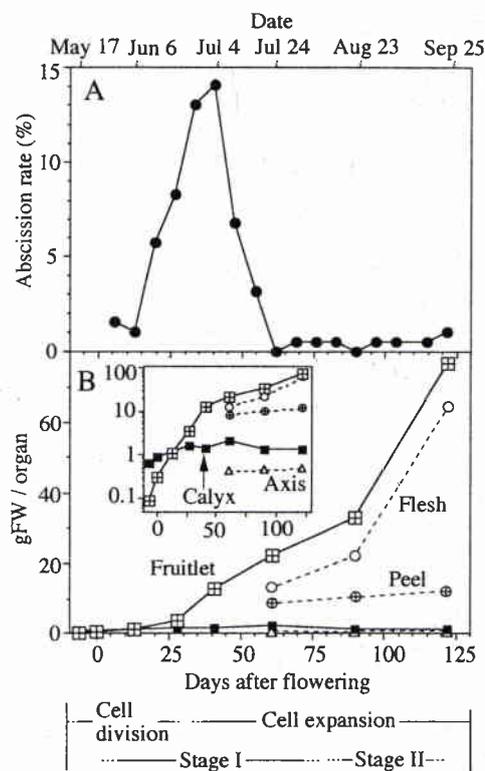


Fig. 1. Changes in abscission rate (A) and fresh weights (B) of the reproductive organs in parthenocarpic persimmon. Fresh weights of organs are also expressed on a logarithmic scale (insert in B). From 61 DAF a fruitlet was divided into an axis, flesh and peel. The upper horizontal axis shows sampling date for hormone analysis. Cell growth states and fruit growth stages described by Hirata and Hayashi (1978) are shown in a lower part of figure.

Fruitlet abscission occurred mostly from 13 to 62 DAF, peaking at 41 DAF.

Figure 1-B shows changes of fresh weights in the reproductive organs. The increase in the fruitlet weight from 61 to 122 DAF was mainly contributed by the increase in the flesh weight. The fresh weight of the ovary/fruitlet increased linearly as a steep slope on a logarithmic scale from -5 to 41 DAF, and thereafter increased as a gentle slope (insert in Fig. 1-B). The calyx weighted more than the ovary before flowering; its weight increased until 13 DAF.

2. Endogenous Phytohormones

Figure 2 shows changes of levels of ABA, IAA and GA-like substances in the developing reproductive organs. ABA concentration in the ovary/fruitlet was

higher from 0 to 28 DAF than the remainder period (Fig. 2-A-I). Within the fruitlet from 61 to 122 DAF, ABA concentrations decreased in the flesh and peel and increased in the axis. In the calyx, ABA concentration reached maximum at 13 DAF and then declined. ABA content in the ovary/fruitlet continued to increase from -5 to 61 DAF (Fig. 2-A-II). ABA content in the calyx from -5 to 13 DAF was higher than in the ovary/fruitlet.

IAA concentration of the ovary/fruitlet showed a sharp peak at flowering and increased again at 122 DAF (Fig. 2-B-I). IAA content of the ovary/fruitlet increased about 18 times from -5 to 0 DAF and decreased to less than half at 13 DAF (Fig. 2-B-II). IAA concentration increased in the peel at 90 DAF and substantially in the flesh at 122 DAF (Fig. 2-B-I). IAA concentration in the calyx was higher than that in the fruitlet

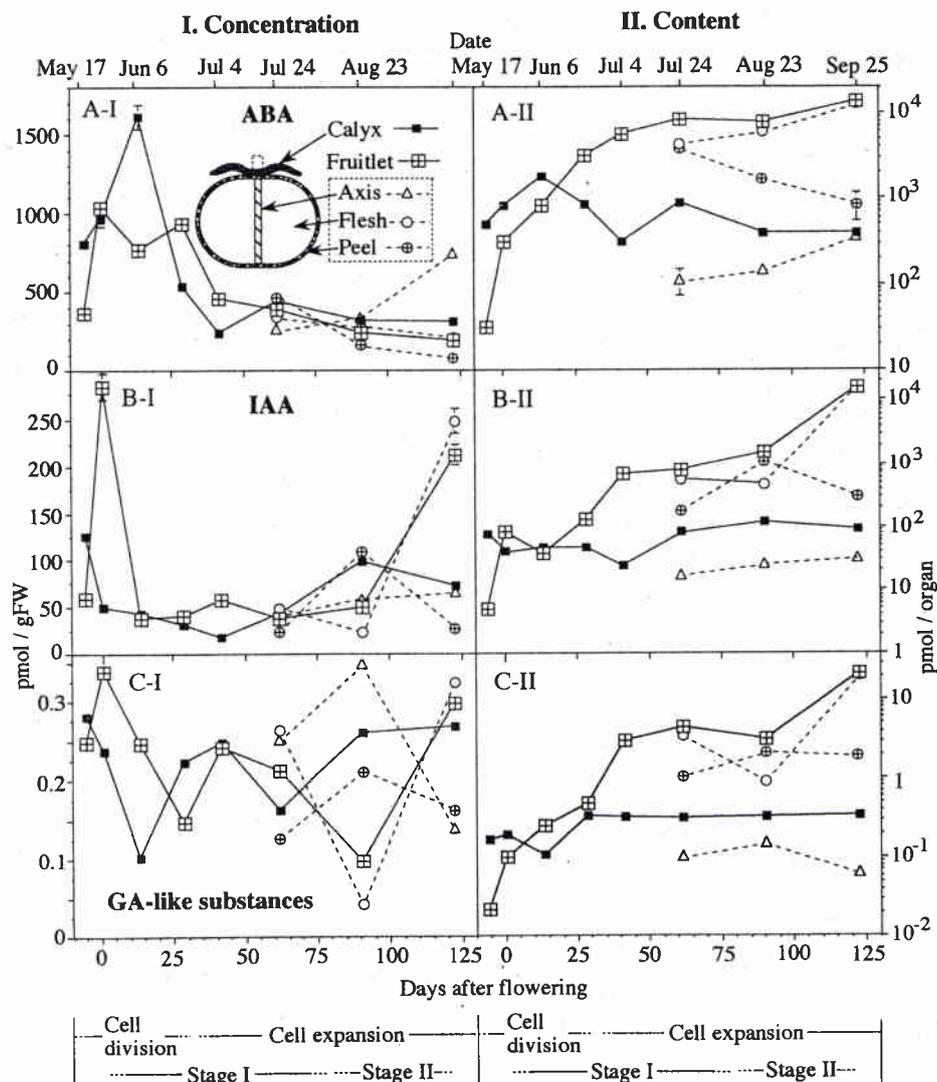


Fig. 2. Changes in concentrations (I) and contents (II) of ABA (A), IAA (B) and GA-like substances (C) of the reproductive organs. GA-like substances are expressed as GA_3 equivalents detected by the rice bioassay. For ABA and IAA, means of three determinations and their SE are shown ($n=3$), and where vertical bars are not shown the limits are within the dimensions of the symbols.

before flowering, tended to decrease to 41 DAF and thereafter increased.

The changing pattern of concentration of GA-like substances differed from those of ABA and IAA (Fig. 2-A, B, C-I). It should be considered that values of ABA and IAA were determined only as active forms, whereas values of GA-like substances included their precursors. The concentration and content of GA-like substances in the ovary/fruitlet increased rapidly at 0, 41 and 122 DAF (Fig. 2-C-I, II). In the fruitlet, the changing pattern of the axis and the peel differed from that of the flesh (Fig. 2-C-I). The calyx kept nearly constant content from 28 to 122 DAF (Fig. 2-C-II).

Discussion

The persimmon fruit has a double-sigmoid growth curve which is divided into three stages. Hirata and Hayashi (1978) determined the period of these stages in the persimmon 'Hiratanenashi': stage I, 0-81; stage II, 82-119; stage III, 120-146 DAF. The distinction between cell division and cell expansion is also important for the investigation of the mechanism of the fruitlet growth (Gillaspy et al., 1993). It was reported that in the 'Hiratanenashi' fruitlet cell division of the flesh stopped at 28 DAF (Hirata and Hayashi, 1978).

1. Fruitlet Growth and ABA

Our study showed that the calyx contained high concentrations of ABA at an early stage (Fig. 2-A-I). Yonemori et al. (1995) found that in the persimmon 'Hiratanenashi', calyx removal at the early stage inhibited fruitlet growth and decreased the ABA content of the fruitlet. The fruitlet had higher concentrations of ABA during one month after flowering than after 41 DAF (Fig. 2-A-I). The high concentrations of ABA of fruitlets just after flowering were also observed in tomato (Kojima et al., 1993), parthenocarpic mandarin (Kojima et al., 1996b) and pollinated citrus (Kojima, 1996). The positive effects of ABA on the fruit growth have been reported in citrus (Kojima et al., 1995) and strawberry (Ofosu-Anim et al., 1996). Thus, there is the possibility that ABA in the fruitlet and calyx may promote fruitlet growth at the early stage.

2. Fruitlet Growth, and IAA and GA-like Substances

In an early period of cell division, the fruitlet had the sharp peak of IAA concentration (Fig. 2-B-I), confirming the tendency examined by the auxin bioassay (Hirata et al., 1978). The level of GA-like substances of the fruitlet also increased at the same period (Fig. 2-C-I, II), which coincided with the previous report (Hirata et al., 1978). In the parthenocarpic fruitlet in this period, the increases of auxin level were reported in tomato (Mapelli et al., 1978) and citrus (Kojima, 1997), and the increases of GA-like substances level were reported in tomato (Mapelli et al., 1978) and grape (Wang et al., 1993). In the present study, pollination had been per-

fectly prevented. Thus the increases of these hormones in the persimmon in cell division stage can not be attributed to the influence of pollination but to a genetic program.

Crane (1965) proposed the hypothesis that the central requirement for the initiation of the fruitlet growth might be any of regulators for the triggering of an assimilate supply because parthenocarpy in figs was able to be induced by auxins, GAs or cytokinins. Furthermore, Nitsch (1970) suggested that a threshold concentration of phytohormones might exist for fruit-set of a parthenocarpic fruit. Thus, our results suggest that IAA and/or GA-like substances in the fruitlet of parthenocarpic persimmon may exceed their threshold concentration and thereby may assure the continuance of assimilate import.

In the early period of the cell expansion stage, the level of GA-like substances in the persimmon fruitlet increased (Fig. 2-C-I, II) as in tomato fruits (Mapelli et al., 1978). These results support the assumption that GA is necessary for cell expansion (Gillaspy et al., 1993).

From 0 to 13 DAF the concentration of IAA and GA-like substances decreased (Fig. 2-B, C-I). Whereas in the same period IAA content decreased to less than half (Fig. 2-B-II), GA-like substances content continued to increase (Fig. 2-C-II). The apparent decrease of the concentration of GA-like substances is attributed to dilution because of a large fresh weight increase. The decrease of IAA concentration is due to dilution as well as to its metabolism (catabolism and conjugation). This decrease indicates that as to IAA in the ovary/fruitlet from 0 to 13 DAF the rate of metabolism plus export is over the rate of biosynthesis plus import.

3. Abscission and Phytohormones

The diameter of abscising 'Hiratanenashi' fruit started to decrease from one week before its abscission (Suzuki et al., 1988), when physiological changes should have already occurred. The high concentrations of ABA and the steep decrease of IAA concentration in the fruitlet occurred before the abscission peak (Fig. 2-A, B-I) as reported for in citrus (Takahashi et al., 1975). ABA in the fruitlet may induce abscission via the induction of ethylene synthesis (Goren, 1993), but low endogenous auxin content in the fruits may, in part, lead to fruit abscission. The external application of synthetic auxin to the fruitlet of persimmon 'Hiratanenashi' at 10 DAF decreased fruitlet abscission (Maotani et al., 1989). Thus, the increase of ABA level and the decrease of IAA level in the persimmon fruitlet may induce its abscission cooperatively.

As to the above-mentioned role of ABA, apparently there are conflictive effects: the promotion of fruitlet growth and abscission. ABA may effect positively on the fruitlet growth (Kojima et al., 1995; Ofosu-Anim et al., 1996), and simultaneously fruitlet abscission through ethylene increase (Goren, 1993). It may be thought that

the promotion of fruitlet abscission by ABA is a harmful side effect which can not be avoided, or a positive effect for decrease of fruit loads.

The concentration of GA-like substances in the fruitlet decreased before the abscission peak (Fig. 2-C-I). The external application of GA₃ to the fruitlet of the persimmon 'Hiratanenashi' from full bloom to 20 days later inhibited fruitlet abscission (Hasegawa et al., 1991). Additionally, it was reported that the decrease of endogenous GAs level by the application of GA biosynthesis inhibitor stimulated markedly abscission of citrus fruitlets (Kojima et al., 1996a). Thus the decrease of GA-like substances level in the fruitlet may also induce its abscission.

In conclusion, it is suggested that IAA and GA-like substances in the fruitlet increased at flowering play a role in the continuance of its growth, and the increase of ABA and the decreases of IAA and GA-like substances in the fruitlet induce its abscission cooperatively.

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Literature Cited

- Addicott, F. T. 1982. *Abscission*. p. 97-152. Univ. of California Press, Berkeley and Los Angeles, Calif.
- Crane, J. C. 1965. The chemical induction of parthenocarp in the 'Calimyrna' fig and its physiological significance. *Plant Physiol.* 40: 601-610.
- Gillaspy, G., H. Ben-David and W. Gruissem. 1993. Fruits: a developmental perspective. *Plant Cell* 5: 1439-1451.
- Goren, R. 1993. Anatomical, physiological, and hormonal aspects of abscission in citrus. *Hort. Rev.* 15: 145-182.
- Hasegawa, K., N. Kuge, T. Mimura and Y. Nakajima. 1991. Effects of KT-30 and GA₃ on the fruit set and the fruit growth of persimmon cvs. Saijo and Hiratanenashi. *J. Japan. Soc. Hort. Sci.* 60: 19-29 (In Japanese with English summary).
- Hirata, N. and S. Hayashi. 1978. Physiological studies of developing and ripening fruit of the Japanese persimmon. IV. Morphological studies of the Japanese persimmon fruit in two cultivars (Fuyu, seeded variety and Hiratanenashi, seedless variety). *Bull. Fac. Agric. Tottori Univ.* 30: 14-25 (In Japanese with English summary).
- Hirata, N., S. Hayashi and K. Tanabe. 1978. Physiological studies of developing and ripening fruit of the Japanese persimmon. V. Relationship of fruit growth to changes in auxin, gibberellin, cytokinin, abscisic acid and ethylene contents in fruit of the Japanese persimmon. *Bull. Fac. Agric. Tottori Univ.* 30: 26-37 (In Japanese with English summary).
- Kojima, K., S. Kuraishi, N. Sakurai and K. Fusao. 1993. Distribution of abscisic acid in different parts of the reproductive organs of tomato. *Scientia Hort.* 56: 23-30.
- Kojima, K. 1995. Simultaneous measurement of ABA, IAA and GAs in citrus - role of ABA in relation to sink ability. *JARQ* 29: 179-185.
- Kojima, K., Y. Yamada and M. Yamamoto. 1995. Effects of abscisic acid injection on sugar and organic acid contents of citrus fruit. *J. Japan. Soc. Hort. Sci.* 64: 17-21.
- Kojima, K. 1996. Changes of abscisic acid, indole-3-acetic acid and gibberellin-like substances in the flowers and developing fruitlets of citrus cultivar 'Hyuganatsu'. *Scientia Hort.* 65: 263-272.
- Kojima, K., A. Goto and S. Nakashima. 1996a. Effects of uniconazole-P on abscission and endogenous ABA, IAA and GA-like substances levels of satsuma mandarin fruitlet. *Biosci. Biotech. Biochem.* 60: 901-902.
- Kojima, K., M. Yamamoto, A. Goto and R. Matsumoto. 1996b. Changes of ABA, IAA and GAs contents in reproductive organs of Satsuma mandarin. *J. Japan. Soc. Hort. Sci.* 65: 237-243.
- Kojima, K. 1997. Changes of ABA, IAA and GAs levels in reproductive organs of citrus. *JARQ* 31: 278-282.
- Maotani, T., A. Suzuki, T. Nishimura, O. Kumamoto, K. Oshima and Y. Yamanaka. 1989. Control of physiological fruit drop of Japanese persimmon 'Hiratanenashi'. *J. Japan. Soc. Hort. Sci.* 58: 557-562 (In Japanese with English summary).
- Mapelli, S., C. Frova, G. Torti and G. P. Soressi. 1978. Relationship between set, development and activities of growth regulators in tomato fruits. *Plant Cell Physiol.* 19: 1281-1288.
- Nishijima, T. and N. Katsura. 1989. A modified micro-drop bioassay using dwarf rice for detection of femtomol quantities of gibberellins. *Plant Cell Physiol.* 30: 623-627.
- Nitsch, J. P. 1970. Hormonal factors in growth and development. p. 427-472. In: A. C. Hulme (ed.). *The Biochemistry of Fruits and Their Products* Vol. 2, Academic Press, London.
- Ofosu-Anim, J., Y. Kanayama and S. Yamaki. 1996. Sugar uptake into strawberry fruit is stimulated by abscisic acid and indoleacetic acid. *Physiol. Plant.* 97: 169-174.
- Ogata, T., H. Hasukawa, S. Shiozaki, S. Horiuchi, K. Kawase, I. Iwagaki and H. Okuda. 1996. Seasonal changes in endogenous gibberellin contents in satsuma mandarin during flower differentiation and the influence of paclobutrazol on gibberellin synthesis. *J. Japan. Soc. Hort. Sci.* 65: 245-253 (In Japanese with English summary).
- Rivier, L., H. Milon and P. E. Pilet. 1977. Gas chromatography-mass spectrometric determinations of abscisic acid levels in the cap and the apex of maize roots. *Planta* 134: 23-27.
- Schwabe, W. W. and J. J. Mill. 1981. Hormones and parthenocarpic fruit set. *Hort. Abst.* 51: 661-698.

- Sobajima, Y., M. Ishida, K. Kiyokawa and M. Sakiyama. 1969. Investigations on the cause and control of physiological dropping in the Japanese persimmon fruit. II. Effects of pollination and gibberellin treatment on the physiological dropping of fruits and changes of auxin in the fruits. Sci. Rep. Kyoto Pref. Univ. Agri. 21: 12-23 (In Japanese with English summary).
- Suzuki, A., H. Iwanaga, Y. Murakami and T. Maotani. 1988. Relationship between changes in ethylene evolution and physiological drop of persimmon fruit. J. Japan. Soc. Hort. Sci. 57:167-172 (In Japanese with English summary).
- Takahashi, N., I. Yamaguchi, T. Kono, M. Igoshi, K. Hirose and K. Suzuki. 1975. Characterization of plant growth substances in *Citrus unshiu* and their changes in fruit development. Plant Cell Physiol. 16: 1101-1111.
- Wang, J., S. Horiuchi, T. Ogata and H. Matsui. 1993. Relationship between the formation of parthenocarpic berries and endogenous plant hormone contents in seedless grape cultivars. J. Japan. Soc. Hort. Sci. 62: 9-14 (In Japanese with English summary).
- Yonemori, K., K. Hirano and A. Sugiura. 1995. Growth inhibition of persimmon fruit caused by calyx lobe removal and possible involvement of endogenous hormones. Scientia Hort. 61: 37-45.

単為結実のカキの幼果における ABA, IAA および GA 様物質の内生量の変化

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摘 要

単為結実のカキ '平核無' の子房/幼果を開花前5日から開花後122日まで採取し、アブシジン酸(ABA)とインドール酢酸(IAA)はガスクロマトグラフィー質量分析計で、ジベレリン(GA)様物質はわい性イネの生物検定で各内生量を測定した。幼果は開花後41日をピークとして13~62日に集中的に落果した。幼果の相対的な生重量の増加は、開花後41日までは急激で、以後はゆるやかであった。がくの生重量の増加は開花後13日までにほぼ終わった。幼果のABA濃度は開花日から28日後まで高く、その後低下した。がくのABA濃度は開花後13日にすべての分析値中の最高値であった。子房中のIAAは、開花前5日と比べて開花日に含量が約18倍に増加して高濃度を示し、開花後13日には含量が半分以下に減少して

濃度も急低下した。幼果のGA様物質の濃度も、開花日に高い値を示し、その後41および122日に上昇した。開花前5日のつぼみではがくの3種類の植物ホルモンは濃度および含量とも、子房よりも高い値であった。以上の結果から、カキの単為結実の幼果では開花時のIAAおよびGA様物質の遺伝的な増加が幼果の生長を継続させること、ならびにその後の幼果中のABA濃度の上昇およびIAAとGA様物質の濃度の低下が共同的に落果を促進することが示唆された。

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