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Changes of ABA, IAA and GAs Levels in Reproductive Organs of Citrus

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Abstract

This review provides information about the levels of abscisic acid (ABA), indole-3-acetic acid (IAA) and gibberellins (GAs) in reproductive organs and the effects of uniconazole-P application on those hormones in citrus to analyze their possible relationships with fruit set and growth. Endogenous phytohormone levels in flowers and fruitlets were studied in 'Hyuganatsu' (Citrus tamurana [Hort.] Ex. Tanaka), which had no parthenocarpic ability, and Satsuma mandarin (Citrus unshiu Marc. cv. Ozaki). ABA concentration in the style of Satsuma increased 6-fold from the bud to the anthesis stage. Stamens in Hyuganatsu contained higher concentrations of IAA and GAs than in Satsuma. Immediately after pollination, Hyuganatsu showed peaks of ABA and IAA concentrations in fruitlets and parthenocarpic Satsuma also showed smaller peaks of ABA and IAA concentrations. Parthenocarpic fruitlets contained higher concentrations of GAs than pollinated fruitlets after fruit-set. 'Sepals' which included floral disks, sepals and receptacles in both setting types contained higher concentrations of ABA than in the nonsetting type, suggesting that ABA in 'sepals' plays a positive role. 'Sepals' in both setting types contained higher concentrations of IAA after fruit-set, suggesting that IAA from 'sepals' may hinder fruitlet abscission. Uniconazole-P, a GA biosynthesis inhibitor, markedly stimulated fruitlet abscission in Satsuma and increased 4-fold the concentration of endogenous ABA in fiuitlets.

Discipline: Horticulture
Additional key words: abscisic acid, gibberellin, indole-3-acetic acid, fruit drop, fruit-set

Introduction

The production of seedless fruits has been a major objective for a long time in the citrus industry. Thus, the analysis of the mechanism of parthenocarpy is essential. Fruit set and growth which are related to parthenocarpy, are drastic processes that are regulated by several phytohormones[41]. Since mechanical harvesting of citrus fruits is also important, fruitlet abscission which is regulated by several phytohormones has been studied[13]. Recently, El-Otmani et al.[7] have reviewed plant growth regulators in citrus, and provided a comprehensive overview of published information.

1) Exogenous hormone effects

In the case of fruit-set and growth, the effects of exogenous hormone on reproductive organs in citrus have been widely studied. Gibberellin (GA) application promoted fruit-set in mandarin[5] and enhanced the accumulation of the metabolite[39]. Auxin application also affected the growth of mandarin fruitlets[14]. Naylor[37] stated that whereas auxins are most effective in inducing parthenocarpy in multiseeded fruits, GAs are influential in fruits with a few ovules.

2) Endogenous hormones

Although it has been suggested that abscisic acid (ABA) plays an inhibitory role in growth[17,45,48], promotive effects of ABA were also reported in sink tissues[16,28,30,34,40]. Brenner and Cheikh[4] stated that sinks may regulate processes in the leaves by removing ABA which may result in stomatal closure and depression of photosynthesis.

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A style of a flower was assumed to act as a sense organ for wilting[11]. It was reported that the style showed the highest level of ABA in a citrus flower[12,15]. However, no direct causal relationship between the ABA level in the style and pollination has been demonstrated.

Takahashi et al.[42] reported the presence of peaks of ABA and indole-3-acetic acid (IAA) concentrations in fruitlets of Satsuma mandarin. Garcia-Papi and Garcia-Martinez[8] suggested that more than 1 hormone may be involved in the fruit-set based on the use of fruitlets of seeded and seedless 'Clementine' mandarin fruits. Talon et al.[43] analyzed GAs and free and conjugated ABA and IAA of 2 related species of seedless mandarins, suggesting that the parthenocarpic ability was mainly influenced by the hormonal status of the fruitlet. Talon et al.[44] suggested that the endogenous GA content in developing ovaries was the limiting factor controlling parthenocarpy.

3) Abscission

Addicott[1] stated that abscission is controlled directly by phytohormones. ABA was originally discovered as an abscission-promoting hormone. The promoting effect of exogenously applied ABA on abscission has been documented in several plants[1]. Goren[13] suggested that the abscission of citrus fruits may be induced by ABA by the following process: (1) ABA induced ethylene synthesis, (2) which increased the activity and production of hydrolytic enzymes. (3) The enhanced enzymes degraded the cell wall in the abscission zone, (4) which resulted in the fruit separation. On the other hand, auxins, which include IAA, delay abscission, generally[13]. Based on the relationships between endogenous phytohormone contents and the abscission pattern, Takahashi et al.[42] suggested that the sudden decrease of the auxin content from the maximum peak as well as the appearance of an ABA peak might be important in the abscission of citrus fruitlets. GA is not considered to be a primary hormone in the control of fruit abscission when applied to trees of various species in the field[13].

This review provides information regarding ABA, IAA and GAs levels in both pollinated and parthenocarpic fruitlets and uniconazole-P effects on those hormones in citrus to define possible relationships with the fruit set and growth.

Procedure for simultaneous measurement of ABA, IAA and GAs

1) Purification and fractionation

Hormone fractionation was performed according to the method of Kojima et al.[27,28]. The sample to which soluble polyvinylpyrrolidone, ^{3}H-ABA and ^{13}C-IAA had been added was homogenized in 80% ethanol and filtered. The aqueous filtrate after evaporation was adjusted to pH 2.8 and filtered through membrane filters (0.22 μm pore size). The aqueous filtrate was partitioned against diethyl ether. The dried extract was fractionated with an HPLC system using an ODS column equipped with an ultra-violet detector. The effluents corresponding to the retention times of ABA and IAA were collected. The remaining effluent was collected for GA analysis. The aqueous phase after diethyl ether extraction was partitioned against ethyl acetate. The ethyl acetate layer was partitioned against 0.5 M K_2HPO_4. The aqueous phase was adjusted to pH 2.5 and was partitioned against ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4 overnight. The dried ethyl acetate layer and the effluent except for the ABA and IAA fractions in the ether extract were combined and purified by Sepralyte DEA.

2) Determination of ABA, IAA and GAs levels

ABA content was determined by the methods of Kojima et al.[23,29]. The methylated ABA fraction was injected into a gas chromatography (GC) system using a capillary column equipped with a ^{63}Ni electron capture detector. A portion of methylated sample was injected into the HPLC system for collection of the methylated ABA. The radioactivity of the collected fraction was measured in a scintillation counter for the correction by recovery rates.

IAA content was determined by the methods of Cohen et al.[6]. The methylated IAA fraction was injected into GC using a capillary column equipped with a mass spectrometer based on the split-less technique.

The bioassay procedure for GAs was similar to the "modified micro-drop bioassay"[38]. The collected sample after drying was diluted successively 3 times with 50% acetone[27,28]. The highest of all values over detectable levels obtained from gradually diluted samples was used as respective GA values.

Distribution and changes in phytohormone levels within bud and flower[32,33]

1) Plant materials

Nine 20-year-old 'Hyuganatsu' trees (*Citrus tamurana* [Hort.] Ex. Tanaka) and three 20-year-old trees of Satsuma mandarin (*Citrus unshiu* Marc. cv. Ozaki) grafted on trifoliate orange (*Poncirus trifoliata* Raf.) growing in the same experimental orchard of the Kuchinotsu Branch in Nagasaki, Japan were used. Since Hyuganatsu is a self-incompatible cultivar, and has no potential for setting parthenocarpic fruits[46], emasculation is not necessary. Satsuma mandarin, male-sterile variety, shows natural parthenocarpy and a higher fruit-set. The day the stigma could be seen through a crack in petals was designated as "the day of anthesis". At least 60 buds and flowers each were sampled at random. Samples were immediately separated into parts and frozen in liquid nitrogen, which was performed in a chilled room (10°C), and stored at -75°C.

2) ABA level

Gillissen[11] suggested that the style is a sense organ that sends the information for wilting of all floral organs after successful pollination. The highest ABA levels were observed in styles in both varieties (Table 1). Goldschmidt[12] also observed the same tendency in Shamouti orange, and discussed the role of ABA in the styles. Harris and Dugger[15] determined ABA levels of the style in Washington navel orange, which was devoid of pollen and was not pollinated by the pollen of other trees, and stated that ABA levels of the style increased without pollination. In Satsuma, ABA concentration of the style increased 6-fold from the bud to anthesis stage, confirming the tendency as a parthenocarpic citrus (Table 1). Moreover, pollination led to a 3 time increase of the concentration of ABA in the styles of Hyuganatsu 8 DAA (pollination, 11,000; no pollination, 3,900 pmol/g fresh weight)[32]. Thus, the increase of the ABA level in the style by pollination may be the signal leading to wilting.

In both varieties, ABA concentrations in the stamens decreased upon anthesis, while those in petals increased (Table 1), confirming the results of Goldschmidt[12]. In Hyuganatsu, ABA concentration of the stamens decreased by less than one-fourth. In tomato, it has also been reported that ABA concentration in the stamens decreased upon anthesis[24]. These decreases of ABA concentrations in stamens may be related to the maturation of the pollen within an anther.

3) IAA level

Satsuma showed lower concentrations of IAA in the stamens, petals and 'sepals' than Hyuganatsu (Table 2). In the stamens, Satsuma did not show any change in IAA concentrations from the bud to the flower stage, while in Hyuganatsu which forms normal pollen grains, the concentration doubled.

Table 1. ABA levels in parts of buds, 5 days after anthesis (DAA) and of flowers, at anthesis in Hyuganatsu (H) and Satsuma mandarin (S)

Part	Cultivar	Bud (pmol / gFW)	Flower (pmol / gFW)
Style[a]	H	3,300	2,400
	S	470	3,100
Ovary	H	730	540
	S	780	900
Stamen	H	1,300	390
	S	910	700
Petal	H	130	450
	S	290	410
'Sepal'[b]	H	2,500	990
	S	1,200	790
Total	H	1,100	700
	S	600	720

a): Styles include stigmas.
b): 'Sepal' includes a floral disk, a sepal and a receptacle.

Table 2. IAA levels in parts of buds, 5 days after anthesis (DAA) and of flowers, at anthesis in Hyuganatsu (H) and Satsuma mandarin (S)

Part	Cultivar	Bud (pmol / gFW)	Flower (pmol / gFW)
Style[a]	H	50	67
	S	72	69
Ovary	H	49	91
	S	150	83
Stamen	H	120	240
	S	51	52
Petal	H	130	230
	S	55	38
'Sepal'[b]	H	170	110
	S	68	48
Total	H	130	190
	S	62	47

a): Styles include stigmas.
b): 'Sepal' includes a floral disk, a sepal and a receptacle.

In the tomato flower which forms normal pollen grains, it was reported that the IAA concentration of the stamens increased by more than 4 times during the same period[26]. The lack of change of the IAA level in the stamens in Satsuma may be due to the abortion of anthers, while the rapid increase of the IAA level in Hyuganatsu might be related to the maturation of the pollen grains within the anther.

4) GAs level

Hyuganatsu exhibited higher concentrations of GAs in the stamens than Satsuma (Table 3), which might be related to the presence of pollen within the anther. GAs levels in styles decreased considerably in both varieties, but the physiological significance is not understood. The GAs levels in ovaries and 'sepals' increased (see fruitlet chapter).

Changes of phytohormone levels in fruitlet[32,33,35]

1) Plant materials

The plant materials and sampling method described in the chapter of bud and flower were used. For sampling and observation of both cultivars, the flower buds, which bore leaves, were covered with paper bags. An appropriate number of Hyuganatsu materials were hand-pollinated. For hormonal analysis, at least 60 organs each were sampled at random. Fruitlets with attachments were immediately separated into fruitlets and 'sepals' which included floral disks, sepals and receptacles (Fig. 1 (Hw)).

Table 3. GAs levels in parts of buds, 5 days after anthesis (DAA) and of flowers, at anthesis in Hyuganatsu (H) and Satsuma mandarin (S)

Part	Cultivar	Bud (pmol / gFW)	Flower (pmol / gFW)
Style[a)]	H	1.4	0.0
	S	6.1	1.6
Ovary	H	1.6	4.0
	S	2.8	3.6
Stamen	H	4.5	3.5
	S	0.5	0.9
Petal	H	4.7	1.1
	S	1.3	0.9
'Sepal'[b)]	H	1.9	3.5
	S	0.7	3.9
Total	H	3.8	2.1
	S	1.4	1.5

a): Styles include stigmas.

b): 'Sepal' includes a floral disk, a sepal and a receptacle.

2) Fruitlet growth

In most of the fruits, early development can be divided into 3 phases[10]: phase I, period of ovary development, fertilization and fruit-set; phase II, period of cell division, seed formation and early embryo development, and phase III, period of cell expansion and embryo maturation. In citrus, cell division occurs in phases I and II[2].

Fig. 1 (Hn, Sn) shows the changes in the number of retained ovaries/fruitlets. The pollinated fruitlets of Hyuganatsu stopped falling after phase II, while non-pollinated fruitlets fell completely by 36 days after anthesis (DAA) (Fig. 1 (Hn)). Some of the growing pollen tubes penetrated into the ovaries of Hyuganatsu 5 days after pollination[46]. Thus in Hyuganatsu, phase I corresponded to the period from 0 to about 10 DAA. Phase II corresponds to the period from 10 to about 30 DAA, because Bain[2] reported that the period of cell division corresponds to about 1 month of early development in the orange fruit. On the other hand, Satsuma mandarin did not exhibit any peak of fruitlet abscission, because only inflorescences with leaves were used for the experiment (Fig. 1 (Sn)).

The fresh weight of the pollinated fruitlets increased linearly on a logarithmic scale after anthesis, while that of non-pollinated fruitlets increased at a lower rate (Fig. 1 (Hw)). 'Sepals' after pollination showed slightly heavier fresh weights than those without pollination. In parthenocarpic mandarin, the fresh weight of ovaries/fruitlets continued to increase before anthesis, whereas that of 'sepals' increased slightly (Fig. 1 (Sw)).

3) ABA level

Pollinated fruitlets of Hyuganatsu showed a peak of ABA concentration in phase I (Fig. 2 (Hc)), and the parthenocarpic fruitlet also showed a peak (Fig. 2 (Sc)). Although it has been suggested that ABA plays an inhibitory role in growth[17,45,48], promotive effects of ABA in sink tissues have also been reported[16,25,30,40]. Brenner et al.[3] suggested that ABA functions as a promoter of sink activity. Thus if this hypothesis is valid, ABA in fruitlets may promote sink activity in phase I to ensure fruit-set in both types of citrus.

Pollinated fruitlets showed a lesser extent of fruitlet abscission than non-pollinated fruitlets (Fig. 1 (Hn)). However, pollinated fruitlets contained a

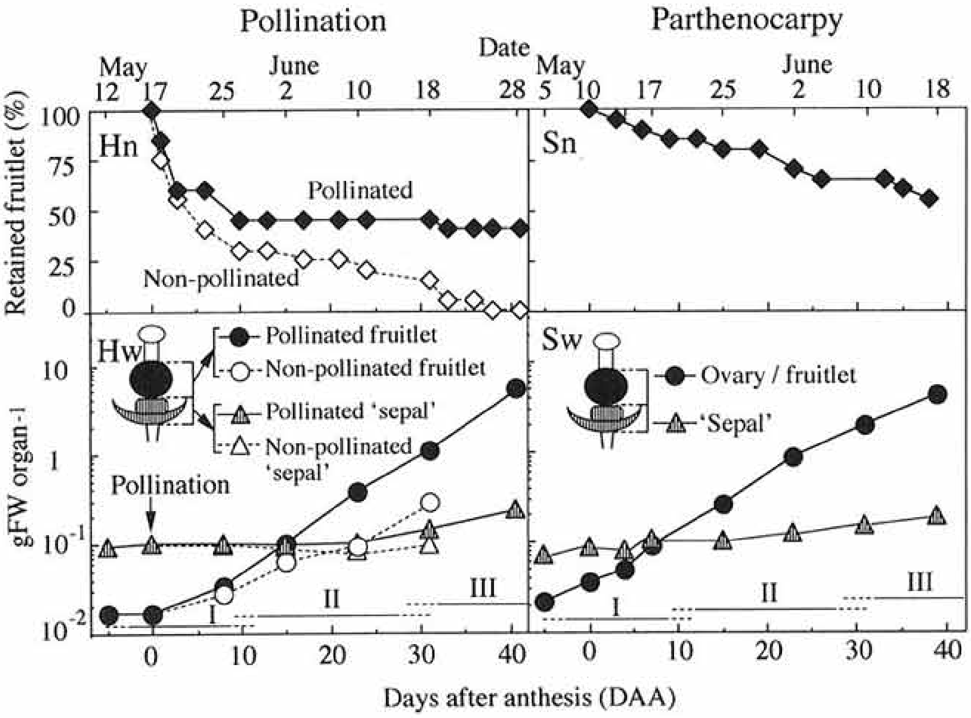

Fig. 1. Changes in the number of retained reproductive organs and fresh weight of reproductive organs and 'sepals' in Hyuganatsu (Hn, Hw) and Satsuma mandarin (Sn, Sw) 'Sepals' includes a floral disk, a sepal and a receptacle.

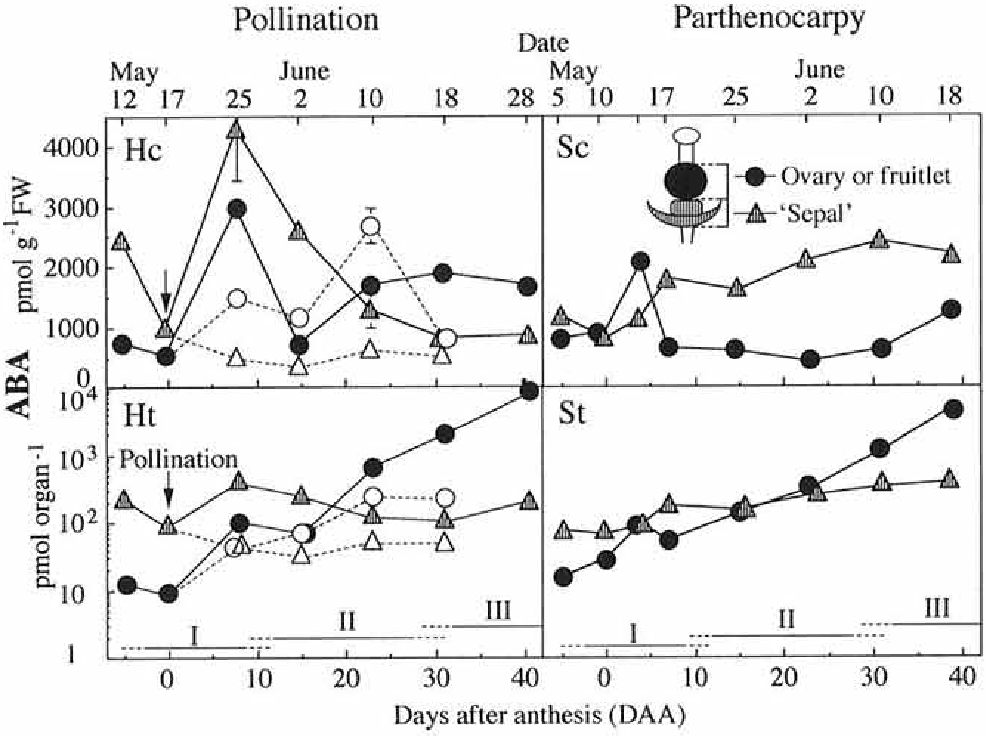

Fig. 2. Changes in concentrations and contents of ABA in the developing ovaries/fruitlets and 'sepals' of Hyuganatsu (Hc, Ht) and Satsuma mandarin (Sc, St)
The downward arrow shows the date of pollination. Means of 3 determinations and their SE are indicated (n = 3), and where vertical bars are not shown the limits are within the dimensions of the symbols. The upper horizontal axis shows the sampling date for hormone analysis.

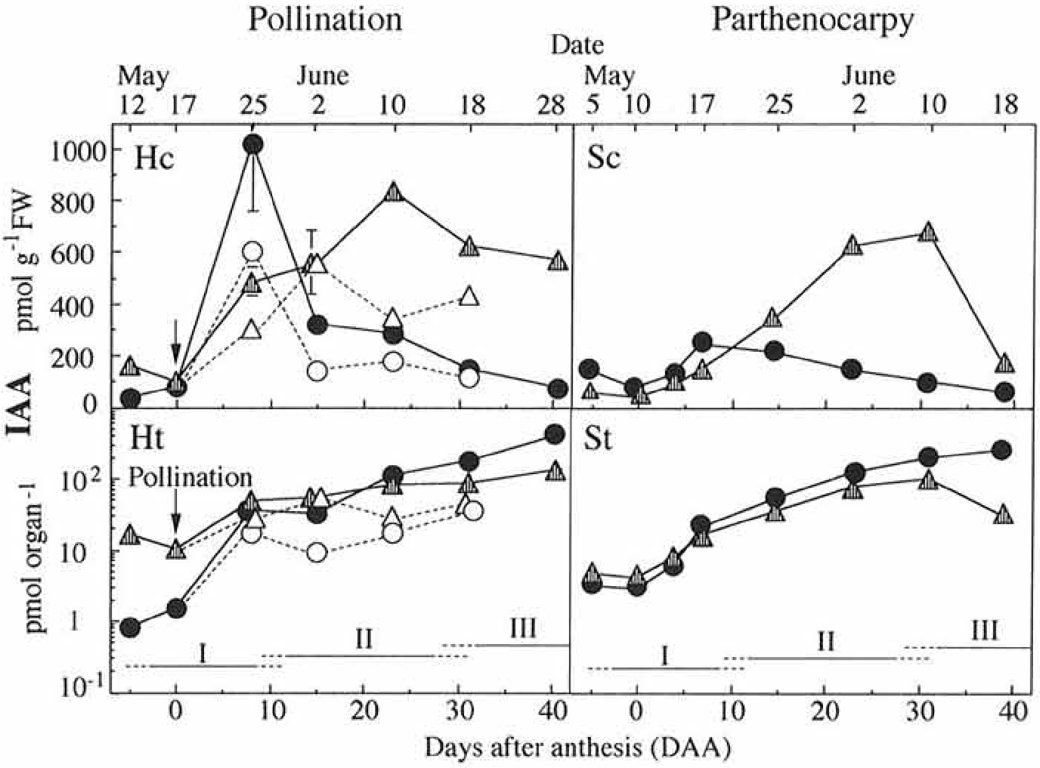

Fig. 3. Changes in concentrations and contents of IAA in the developing ovaries/fruitlets and 'sepals' of Hyuganatsu (Hc, Ht) and Satsuma Mandarin (Sc, St)
Means of 3 determinations and their SE are indicated (n = 3), and where vertical bars are not shown the limits are within the dimensions of the symbols.

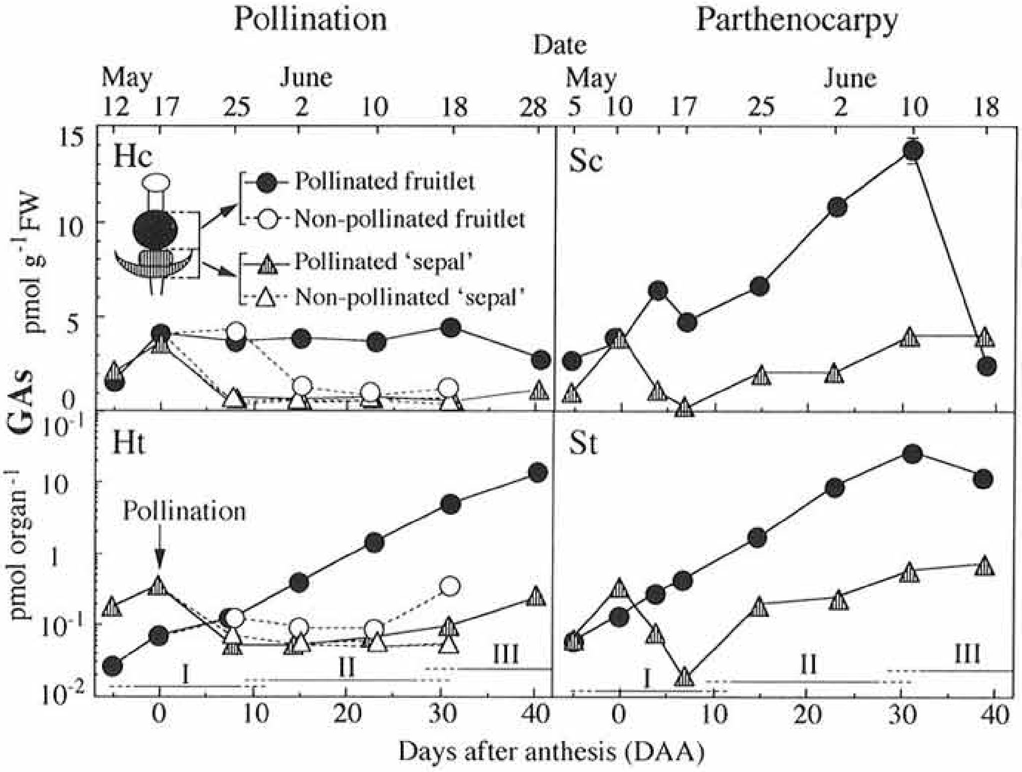

Fig. 4. Changes in concentrations and contents of GAs in the developing ovaries/fruitlets and 'sepals' of Hyuganatsu (Hc, Ht) and Satsuma mandarin (Sc, St)
GAs are expressed as GA_3 equivalents detected by the dwarf rice bioassay.

higher level of ABA than non-pollinated fruitlets in phase I (Fig. 2 (Hc, Ht)). Additionally, 'sepals' of pollinated fruitlets contained much higher levels of ABA in phases I and II. These results do not support the hypothesis that endogenous ABA directly promotes abscission immediately after fruit-set. However, it is possible that the promotive effect of abscission by ABA was masked by the inhibitory effect of other phytohormones.

In 'sepals', changes in the patterns of ABA concentrations differed among pollinated, non-pollinated and parthenocarpic fruitlets (Fig. 2 (Hc, Sc)). 'Sepals' after pollination showed a peak of ABA concentration in phases I and II, and 'sepals' after parthenocarpy contained higher levels than 'sepals' in the absence of pollination in phases II and III. Thus the fact that 'sepals' in both setting types contained higher levels of ABA concentration suggests that ABA in 'sepals' plays a beneficial role in the fruitlet growth and/or retention.

4) IAA level

'Sepals' after both pollination and parthenocarpy contained higher concentrations of IAA than fruitlets around phase II (Fig. 3 (Hc, Sc)), although I had assumed that 'sepals' would exhibit lower concentrations of IAA in all phases. Auxin inhibited the rise in polygalacturonase and cellulase activity in the abscission zones and delayed fruit abscission[18]. Iwahori et al.[20] reported that IAA applied via the peduncle markedly retarded the abscission between the fruitlet and 'sepal' of citrus explants. Thus IAA from 'sepals' may inhibit the hydrolase activity and hinder fruitlet abscission.

The pollinated fruitlets exhibited the highest peak of IAA concentration in phase I throughout the experimental period (Fig. 3 (Hc)) and the IAA content increased by more than 10 times compared with that at anthesis (Fig. 3 (Ht)). Non-pollinated fruitlets also showed a peak of IAA concentration (Fig. 3 (Hc)). Parthenocarpic fruitlets showed an increase of IAA concentration in phase I (Fig. 3 (Sc)), confirming the earlier report based on *Avena* curvature test[42]. Guardiola and Lazaro[14] observed that the application of synthetic auxins had a direct stimulatory effect on the fruitlet growth of mandarin. Thus, the IAA increase in phase I is likely to promote the growth of ovary/fruitlets.

5) GAs

Parthenocarpic fruitlets contained higher concentrations of GAs than pollinated fruitlets (Fig. 4 (Hc, Sc)), confirming the results reported in grapes[19] and pears[9]. Talon et al.[43] observed that the reproductive organ of the parthenocarpic mutant of citrus contained slightly higher levels of GA_1, GA_{20} and GA_4 than the pollinated cultivar. Talon et al.[44] suggested that the endogenous GA level in the developing ovaries in the parthenocarpic cultivars was the limiting factor controlling the fruit-set.

In both pollinated and parthenocarpic fruitlets GAs contents continued to increase dramatically in phases I and II, but in non-pollinated fruitlets the contents did not increase in phase II (Fig. 4 (Ht, St)). On a fresh weight basis, fruitlets of both setting types showed higher levels of GAs than non-setting fruitlets around phase II (Fig. 4 (Hc, Sc)). The localized GA application on pistils of citrus flowers promoted the mobilization of assimilates into young ovaries[39]. Thus the higher levels of endogenous GAs recorded around phase II may play a major role in the assimilate accumulation to ensure the fruit-set.

Pollinated fruitlets which contained developing seeds showed higher concentrations and contents of GAs than non-pollinated fruitlets after phase II (Fig. 4 (Hc, Ht)). Leopold and Kriedemann[36] stated that developing seeds were substantial sources of GAs. Thus increased contents of GAs in pollinated fruitlets may be provided by the developing seeds.

On the other hand, in 'sepals', there was no difference in the GAs concentration between pollination and the absence of pollination (Fig. 4 (Hc)). Parthenocarpic 'sepals' contained higher concentrations of GAs than non-parthenocarpic 'sepals' after phase II (Fig. 4 (Hc, Sc)). The higher levels of GAs in 'sepals' may be involved in the growth of the parthenocarpic fruitlet after phase II. In both setting types, 'sepals' showed a small peak of GAs concentration at anthesis (Fig. 4 (Hc, Sc)). The increase of the GAs concentration in 'sepals' may promote ovary development before fruit-set.

Uniconazole-P effects on abscission and endogenous hormones levels in mandarin fruitlet[31]

The alteration of the level of endogenous phytohormones by a biosynthesis inhibitor is a useful approach for clarifying their roles. Uniconazole-P, a triazole-type growth retardant, acts as an inhibitor of GAs biosynthesis[21]. Fig. 5A depicts the changes in the number of retained fruitlets of Satsuma mandarin after the uniconazole-P treatment. Uniconazole-

Table 4. Concentrations of ABA, IAA, and GAs in mandarin fruitlets 7 and 21 days after treatment (DAT) with uniconazole-P[a)]

Treatment	ABA	IAA	GAs
	(pmol / gFW)		(pmol GA$_3$ eq./gFW)
[7 DAT]			
Control	640 ± 20	180 ± 1	5.5
Uniconazole-P	2,300 ± 32	110 ± 7	0.3
[21 DAT]			
Control	1,500 ± 37	51 ± 2	2.6
Uniconazole-P	5,900 ± 96	78 ± 2	1.8

All the values of ABA and IAA concentrations (3 measurements) are mean ± SE.
a): An inhihbitor of GAs biosynthesis.

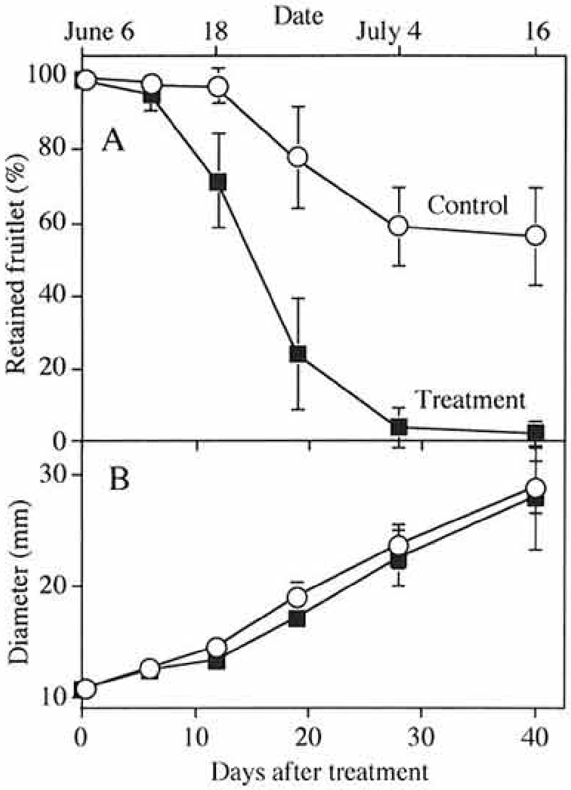

Fig. 5. Changes in the number of retained fruitlets (A) and equatorial diameter (B) of mandarin fruitlets treated with uniconazole-P

P accelerated fruitlet abscission markedly. Paclobutrazol, an inhibitor of GAs biosynthesis, also increased citrus fruit abscission[44)]. On the other hand, uniconazole-P decreased the growth of mandarin fruitlets slightly (Fig. 5B).

Table 4 shows the concentrations of endogenous ABA, IAA, and total GAs in mandarin fruitlets after uniconazole-P treatment. Uniconazole-P decreased the total level of GAs by less than one-tenth 7 days after treatment (DAT), confirming that uniconazole-P acts as an inhibitor of GA biosynthesis. Talon et al.[44)] suggested that GA may control the fruit development of parthenocarpic citrus and implied the occurrence of a threshold for GAs level. Thus, fruitlet abscission may be mainly due to the reduction of the total GAs level in fruitlets.

ABA levels in fruitlets treated with uniconazole-P were about 4-fold higher than those in the control both 7 and 21 DAT (Table 4). The treatment with paclobutrazol also induced a 3-fold increase of ABA in Satsuma fruits[47)]. However, in rice shoots, uniconazole-P did not affect the endogenous ABA level[22)]. The effects of uniconazole-P may be different between the fruitlet and the shoot. Endogenous ABA in the fruitlet which was increased by uniconazole-P may also promote the abscission of mandarin fruitlets in the sequential mechanism suggested by Goren[13)].

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