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Effect of water stress on flower-bud formation and plant hormone content of satsuma mandarin (*Citrus unshiu* Marc.)

Yoshiko Koshita^{a,*}, Toshio Takahara^b

^a Department of Grape and Persimmon Research, National Institute of Fruit Tree Science, Akitsu, Hiroshima 729-2494, Japan

^b Department of Citrus Research, National Institute of Fruit Tree Science, Kuchinotsu, Nagasaki 859-2501, Japan

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Abstract

The effect of water stress on plant hormones (GAs, IAA and ABA) level in the leaves and flower-bud formation of the satsuma mandarin (*Citrus unshiu* Marc.) trees was investigated to determine the relationship between flower-bud induction and the level of endogenous plant hormones as a result of water stress. Severe water stress (−1.5 to −2.0 MPa) in autumn, which causes heavy leaf fall, reduced the percentage of flowering nodes by one third of the moderately water-stressed ones (−0.5 to −1.0 MPa). The quantity of GA_{1/3} from the middle of October through early December was significantly higher in the leaves of the trees under severe water stress than in the leaves of the trees enduring moderate water stress. The content of IAA in the leaves of the trees under moderate water stress was higher in late February. These findings indicate that the levels of GA_{1/3} are enhanced by severe water stress, higher in the leaves from the branches that produce fewer flowers during flower-bud induction periods. The levels of IAA were higher in the leaves from the branches that produced more flowers during the season when flower-buds develop.

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1. Introduction

Development of the flower controlling mechanism in citrus cultivation is very important because of alternate-year bearing. Because the prices of fruit fluctuate according to yield,

Abbreviations: GA, gibberellic acid; IAA, indole acetic acid; ABA, abscisic acid; MeCN, acetonitrile

* Corresponding author. Tel.: +81-846-45-4754; fax: +81-846-45-5370.

this phenomenon is undesirable for growers. Therefore, control of the flowering process and understanding the physiological mechanism of the induction of flower-buds in citrus is indispensable to the industry.

Flower-bud induction of the satsuma mandarin in Japan occurs from September to December (Osaki and Saso, 1942) and the physiological development season is late December to early March (Iwasaki et al., 1959).

Citrus flowering is influenced by various growing conditions or treatments. For example, a 'Tahiti' lime tree that is severely water-stressed will produce more flowers than one that is moderately water-stressed (Southwick and Davenport, 1986). Inoue (1989) reported that a low-temperature treatment increased the rate of leafless inflorescence. The ringing treatment in the middle of October enhanced the number of the flower-buds in the next spring (Koshita et al., 1999).

Plant growth regulators have been applied exogenously to elucidate the roles of plant hormones in flower-bud induction of citrus. The conclusion was that exogenously applied GA reduces the number of flowers in the following spring (Hirose, 1968). On the other hand, some research has been done to elucidate the relationship between the GA content during flower-bud induction and the flowering response (Koshita et al., 1999; Ogata, 1997; Southwick and Davenport, 1987). We suggested in an earlier study of the satsuma mandarin (1999) that endogenous $GA_{1/3}$ and IAA were involved in flower-bud formation. Contrary to this, Ogata (1997) concluded that the GA_1 contents in the leaves caused by differences in fruit load of the branch might not be a key factor of the flower-bud formation. Southwick and Davenport (1987) also concluded that endogenous GAs and ABA might not play a key role in flower-bud formation that was controlled by drought and chilling treatments. To find the answer to this inconsistency and understanding the relationship between GAs and other uninvestigated plant hormones, we quantified $GA_{1/3}$, $GA_{4/7}$, IAA and ABA in the leaves of the satsuma mandarin simultaneously under conditions of flower suppression or induction, severe and moderate drought, during the flower-bud inductive and developmental period.

2. Materials and methods

2.1. Plant material

Six 3-year-old satsuma mandarin trees grafted onto *Poncirus trifoliata* rootstocks and grown in pots were used for this experiment. On September 9, 1996, some fruits were removed to adjust the leaf fruit ratio to 40. The pots were kept in the orchard of the Department of Citrus Research, National Institute of Fruit Tree Science, Kuchinotsu, Nagasaki. They were kept on the open ground but covered when rainy weather arrived.

2.2. Drought treatment of the trees

Different conditions of water stress (severe and moderate) were imposed from middle September 1996 to the harvest date, November 21, 1996. The trees under severe water stress were irrigated approximately once every 7–10 days. The trees under moderate water stress were irrigated once every 3 days. Six trees showing uniform growth were selected from 12 trees and three trees were used for each set of conditions.

2.3. Measurement of the water potentials of the trees

The water potential was measured before dawn with the pressure chamber method of Scholander et al. (1965). Two leaves from each tree were picked and used immediately to determine the water potential. Water potential was measured before irrigation of the severely water-stressed trees.

2.4. Sampling for plant hormone analysis

On October 21 and December 4, 1996 and February 24, 1997, the leaves of the vegetative shoots were picked, frozen immediately in liquid N₂, and stored at –80 °C until plant hormone extraction.

2.5. Plant hormone analysis

The endogenous plant hormones (GA, IAA and ABA) in the leaves were extracted according to Koshita et al. (1999). Briefly, 10 g of the samples were homogenized and extracted in 80% acetone containing 100 mg l⁻¹ butylhydroxytoluene (BHT) and subjected to solvent partitioning. The extracts were further purified by high-performance liquid chromatography (HPLC). Conditions of HPLC were as follows; column ODS (PEGASIL ODS, Senshu Pak, 150 mm × 6 mm i.d.); temperature 40 °C; flow rate 1.5 ml min⁻¹; solvent A; 5% MeCN with 0.5% CH₃COOH; solvent B 80% MeCN with 0.5% CH₃COOH; gradient profile 0–5 min 0% of B, 5–50 min 0–33% of B, 50–70 min 33–100% of B. Under these conditions, the retention times of the plant hormones were IAA 34 min, ABA 44 min, GA_{1/3} 29 min, and GA_{4/7} 59 min. ¹³C₆IAA and d6ABA were used as internal standards for the IAA and ABA analysis, respectively, and quantified by GC/MS-SIM. The contents of GA_{1/3} and GA_{4/7} were quantified by ELISA using an anti-GA₄ antibody (Nakajima et al., 1991). The GA_{1/3} and GA_{4/7} were tentatively identified based on the retention time of the HPLC.

2.6. Assessment of floral, foliar formation, and leaf fall ratio

Floral and foliar formations of the next spring were assessed on April 23, 1997. All shoots generated from vegetative branches were assessed, and buds were classified into three types: vegetative type, leafless inflorescence, and leafy inflorescence. The leaf fall ratio was determined by counting the number of leaves on the trees soon after adjustment of the leaf fruit ratio on September 9, 1996, and on April 9, 1997.

3. Results

3.1. Effect of water stress on the satsuma mandarin trees

The water potentials of the severely and moderately water-stressed trees fluctuated, as shown in Fig. 1. Several leaves from each treatment were dropped during drought treatment,

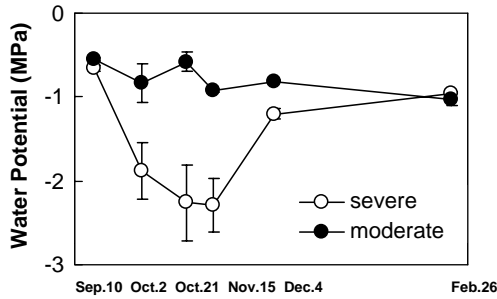


Fig. 1. Water potentials of the trees observed from September 10 to February 26. Vertical bars represent S.E. of the mean ($n = 3$) and were not shown when smaller than the data symbol.

middle September to November 21, 1996, and the leaf fall ratio was higher in the severely water-stressed trees than in the moderately water-stressed trees (Table 1).

3.2. Effects of water stress on vegetative- and floral-shoots production

The severely water-stressed trees produced fewer flower-buds than the moderately water-stressed ones, and the ratio of the leafy inflorescence was 3-fold higher than that of the leafless inflorescence. On the other hand, almost all the nodes of the moderately water-stressed trees produced flowers and the ratio of the leafless inflorescence was 1.7-fold higher than that of the leafy inflorescence (Table 1).

3.3. $GA_{1/3}$ and $GA_{4/7}$ contents in the leaves

The $GA_{1/3}$ content on October 21, during the drought treatment and on December 4 was significantly higher in the leaves of the severely water-stressed trees (Fig. 2A). On the other hand, there were no significant differences in the contents of $GA_{4/7}$ in the leaves (Fig. 2B).

3.4. IAA and ABA contents in the leaves

Though the IAA content on October 21 and December 4 was not different in either treatment, there was a 2-fold higher content in the moderately water-stressed trees than in

Table 1
The effect of drought treatment on flowering characteristics and sprouting in the following spring for vegetative branches and leaf fall ratio of satsuma mandarin

Drought treatment	Percentage of flowering node per total node	Percentage of vegetative node per total node	Percentage of leafless inflorescence per total node	Percentage of leafy inflorescence per total node	Percentage of sprouted node per total node	Leaf fall ratio
Severe	75.6 ± 10.2 ^a	24.4 ± 10.2	18.4 ± 1.1	57.2 ± 9.4	96.5 ± 4.2	43.3 ± 8.4
Moderate	98.7 ± 1.0	1.26 ± 1.0	62.5 ± 9.2	36.3 ± 8.2	99.9 ± 0.2	13.9 ± 6.5

^a Mean ± S.E., $n = 3$.

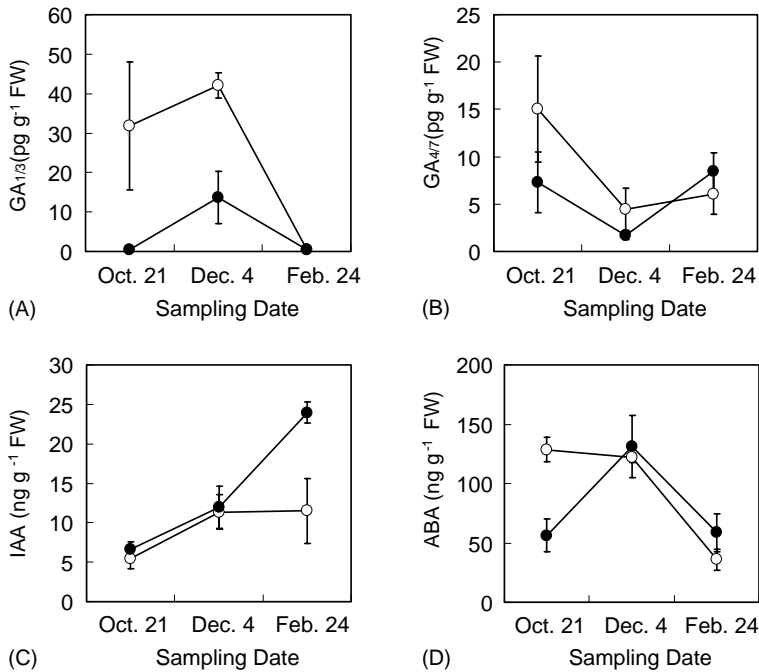


Fig. 2. Differences in the content of GA_{1/3} (A), GA_{4/7} (B), IAA (C) and ABA (D) contents in the leaves of trees under severe (○) and moderate (●) water stress. Vertical bars represent S.E. of the mean ($n = 3$).

the severely water-stressed trees on February 24 (Fig. 2C). During the drought treatment, there was no difference in the content of IAA in the severely and moderately water-stressed trees.

The ABA content of the severely water-stressed trees was 2-fold higher than that of the moderately water-stressed trees on October 21. There was no difference in the ABA content on December 4 and February 24 (Fig. 2D).

4. Discussion

There have been some reports that soil drought affects flower-bud formation in citrus. Southwick and Davenport (1986) reported that both continuous and cyclical water-stress treatment induces flowering. On the other hand, less flowering was often observed in satsuma mandarin cultivation under conditions of severe water stress (Katayama et al., 1989). Because the different rootstock cultivars showed different drought tolerance (Rao et al., 1987), the variety of the rootstock might be one of the factors to effect drought tolerance of the citrus trees.

The defoliation in September was reported to reduce the number of flower-buds (Osaki and Saso, 1942). In our study, more leaves fell from the severely water-stressed trees than from the moderately water-stressed trees (Table 1). These results suggest a leaf fall

induced by very severe water stress might have an effect on the formation of flower-buds.

It is not clear whether endogenous GAs have an effect on the fate of citrus buds. The chilling treatment, which is an inductive condition of citrus flowering, reduced the gibberellins in the leaves to undetectable levels but rose to slightly above pre-stress levels after an additional 2 weeks. On the other hand, another flower-inducing condition, drought, showed that the GA content of the leaves induced a 4-fold increase in the gibberellin level 1 week after the onset of water stress, followed by a gradual decrease over the following 3 weeks until the trees were watered again (Davenport, 1990). Thus, the sampling timing is one of the important factors when water stress is imposed on citrus. In our report, higher levels of GA_{1/3} were detected in the severely water-stressed trees during and after drought treatment, which resulted in poorer flowering in the following spring (Fig. 2A, Table 1). Our previous report also showed higher GA_{1/3} levels in the leaves of the fruit-bearing branches than in the vegetative branches (Koshita et al., 1999), however, there is a possibility that these observed different GA levels in our previous and present studies might be caused by different factors. Ogata (1997) reported that there was no correlation between GA₁ and flowering, but GA₂₀, which is identified as a precursor of the GA₁, was detected with higher activity in the leaves of late-harvested trees, which produce fewer flowers than those harvested earlier. The involvement of the GAs to citrus flowering is also demonstrated by application of paclobutrazol, which is known as an inhibitor of the GA biosynthesis, because more flower-buds were differentiated in the following season (Yamashita et al., 1997). Our study demonstrated the possibility of involvement of the endogenous GAs in flower-bud formation.

The relationship between the IAA content and flowering in citrus was not clearly demonstrated. In this study, a higher IAA content was only detected in the leaves of the moderately water-stressed trees on February 24, 1997, and there was no difference during the drought treatment (Fig. 2C). Therefore, endogenous IAA might not have been influenced by the drought treatment. In our previous report, a high IAA content in the leaves of the vegetative shoots was detected in late February; however, no relationship was found between the flower-bud formation and the IAA content in early December (Koshita et al., 1999). Because these two different treatments to induce the enhancement of flowering were accompanied by a higher IAA level in late February, it is possible that endogenous IAA acts on flower-bud development and not on induction.

The ABA content in the leaves was only different in October, when the conditions of water stress were different. In our previous report, higher levels of ABA were also detected in the leaves of the vegetative shoots rather than the bearing shoots (Koshita et al., 1999). Together with these two different flower-inducing or suppressing conditions and different ABA levels, there is a possibility that endogenous ABA might be one of the key factors of flower-bud formation, because September to December was reported to be the flower-bud-inducing season in Japan (Osaki and Saso, 1942).

In conclusion, the GA_{1/3} level in the leaves was higher in the less flower producing severely water-stressed trees during the flower-bud inducing period, and involvement of endogenous GAs in the leaves in flower-bud formation of the satsuma mandarin is suggested. Moreover, the higher levels of IAA in the leaves of the branches that produced more flowers in late February suggested the involvement of IAA in flower-bud development. Although

there was no correlation between the ABA contents and intensity of flowering on branches, there is the possibility that endogenous ABA might be one of the key factors, because different levels were observed during flower bud inducing period.

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