

Effects of Soil Application of the Naturally Occurring Form of Abscisic acid (S-ABA) on Development and Quality of Satsuma Mandarin Fruit

By

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Summary : In 2009 and 2010, we conducted experiments to investigate the effects of soil application of the naturally occurring form of abscisic acid (S-ABA), a type of plant hormone, at four concentrations (0, 0.1, 1, 10 ppm) on the development and quality of satsuma mandarin fruit. In both years, S-ABA was applied to the soil twice during the fruit development stage. In both years, S-ABA application promoted fruit development, indicated by increases in fruit fresh weight, as well as transverse and longitudinal diameters of fruit, on S-ABA-treated trees. At the same time, fruit coloration was delayed in fruits from S-ABA-treated trees. Sugar contents of fruit were reduced by S-ABA treatment, and the lowest sugar content was recorded with 10 ppm S-ABA treatment. No significant differences in acidity were observed between fruit from S-ABA-treated and control trees. In addition, the potassium contents of fruit juice from S-ABA-treated trees were elevated when compared with levels in control trees. The above results demonstrate that treatment of roots with S-ABA via soil application promotes fruit development and inhibits fruit maturation.

Key words : abscisic acid, fruit color, fruit development, S-ABA, soil application

Introduction

The plant hormone abscisic acid (ABA) is known to affect seed maturation and stomatal closure, as well as plant responses to environmental stresses such as drying and low temperatures. However, when ABA was first discovered, it was primarily thought of as a plant growth inhibitor. For this reason, ABA is less frequently used as a plant growth regulator (PGR) in horticultural contexts than other plant hormones. ABA exists in its naturally occurring form, S-(+)-ABA (S-ABA), and its non-naturally occurring enantiomer, R(-)-ABA (R-ABA). The non-naturally occurring form is reported to exhibit little or no biological activity when applied exogenously¹⁾. Composite ABA is a racemic mixture of S-ABA and R-ABA ; thus, the practical use of composite ABA must be carefully evaluated. Although expensive, the development of a method to mass produce the S-ABA isomer has facilitated the practical application of S-ABA in

horticultural production.

Based on the observation that, over the course of fruit development, endogenous ABA peaks during the immature stage and tends to increase in the latter and final stages of development, it is believed that the peak during the immature stage is related to physiological immature fruit abscission and that the increase in the latter and final stages of development is related to fruit maturation and accelerated senescence²⁾. Furthermore, the application of exogenous S-ABA has been reported to promote ripening in grapes³⁾, affect coloration in grapes and sweet cherries⁴⁻⁶⁾, and affect sugar accumulation in peaches, apples, sweet cherries and iyo (*Citrus iyo*)⁶⁻¹⁰⁾. It is anticipated that further research on the physiological effects of S-ABA on fruit development will lead to expanded use of S-ABA as a chemical control method for promoting fruit development and for increasing productivity.

In Japan, in terms of production volume and area

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cultivated, satsuma mandarin production exceeds that of each of the other fruit tree species cultivated, and is one of the country's most important tree fruit species. Although various PGRs are used in satsuma mandarin production to regulate flowering and fructification, promote ripening and coloration, and to reduce rind puffing, ABA is not registered as an agricultural chemical for this purpose. The exogenous application of ABA to fruit of satsuma mandarin has been shown to promote fruit development and sugar accumulation, although the ABA used for application was racemic type and multiple applications of ABA at high concentrations were necessary¹¹⁾. Meanwhile, KOJIMA *et al.* (1995)¹⁰⁾ directly injected S-ABA into *C. iyo* and observed increased sugar accumulation at low S-ABA injection rates.

In previous research, exogenous ABA has typically been applied directly to the tissue or organ of interest, namely fruit²⁾. We need to investigate the impact of exogenous ABA on the fruits in which the ABA was applied to the other organs or tissues. Thus, in the present study, we investigated the impact on fruit development of S-ABA applied to satsuma mandarin roots. In addition to reporting experimental results, we discuss the various physiological effects associated with S-ABA application.

Materials and Methods

1. Effects of soil application of ABA on fruit

Our study sample comprised satsuma mandarin 'Okitsuwase' trees (grafted onto trifoliolate orange (*Poncirus trifoliata*) grown in 60 L pots in a cultivated field on the grounds of the Tokyo University of Agriculture Atsugi campus. The experiment was conducted twice, first in 2009 and second in 2010, using 23-year-old and 6-year-old trees, respectively.

The following four treatments using naturally occurring ABA (S-(+)-ABA ; BAL Planning Co., Ltd.) were performed; soil application of water not containing S-ABA ; soil application of a 0.1 ppm S-ABA aqueous solution ; soil application of a 1 ppm S-ABA aqueous solution; and soil application of a 10 ppm S-ABA aqueous solution. Each S-ABA solution was applied to the soil twice during the fruit development stage. In both years, the first and second S-ABA applications to the soil (3 L per tree) occurred on July 29 and September 7, respectively. In 2009, fruit was initially thinned on July 16 and again on August 12 to a number of leaves per fruit of 25. In 2010, fruit was initially thinned on July 15 and again on August 12 to a number of leaves per fruit of 27. Pots were watered as needed, when the soil surface of the entire pot appeared to be dry. Fruits were harvested on October 17 in 2009 and on October 18 in 2010, and fruit

quality was assessed thereafter. In addition to measuring the transverse and longitudinal diameters of fruit, pericarp color was also assessed. L^* , a^* and b^* values of pericarp color were evaluated using a colorimeter (ZE 2000 ; Nippon Denshoku Industries Co., Ltd.). Next, fruit juice was obtained by squeezing and was passed through a No. 2 filter (Toyo Roshi Kaisha, Ltd.). Fruit juice was then analyzed for soluble solids content (Brix) using a digital sugar refractometer (PR-101 ; Atago Co., Ltd.). Acidity of fruit juice was also assessed by neutralization titration using 0.1N NaOH and expressed as equivalent citric acid content.

In addition, inorganic composition (potassium, magnesium, and calcium) of the fruit juice was analyzed by atomic absorption spectrophotometry, while sugar (glucose, fructose, and sucrose) and organic acid (citric acid and malic acid) contents were analyzed by HPLC.

2. Sugar and Organic Acid Analysis

Fruit juice samples were passed through 0.45 μm membrane filters (DISMIC-13cp ; Toyo Roshi Kaisha, Ltd.) and were analyzed for sugar and organic acid content by HPLC under the following conditions.

HPLC analysis of sugar content was performed using 2 \times 5 mm UK-Amino (Imtakt Corporation) and 46 \times 250 mm Union UK-Amino (Imtakt Corporation) columns as guard and primary columns, respectively, with 3 : 1 acetonitrile-pure water mixture as the mobile phase, delivered at a flow rate of 1 mL \cdot min⁻¹. Eluent was analyzed using a refractive index detector (RID-6A ; Shimadzu Corporation).

In 2009, HPLC analysis of organic acid content was performed using a Shim-pack SCR-101H column (Shimadzu Corporation) with 0.095% p-toluenesulfonic acid as the mobile phase, delivered at a flow rate of 1 mL \cdot min⁻¹. Eluent was analyzed using a conductivity detector (CDD-6A ; Shimadzu Corporation). In 2010, HPLC analysis was performed using a SUPELCOGEL H column (Supelco Inc.) with 0.1% phosphoric acid as the mobile phase, delivered at a flow rate of 0.8 mL \cdot min⁻¹. Eluent was analyzed using an ultraviolet spectrophotometer (SPD-6A ; Shimadzu Corporation).

3. Statistical Analysis

Analysis of variance (ANOVA) was performed using the Excel software package. Separation of means was evaluated using Tukey's multiple range test at the 5% level.

Table 1 Effects of ABA soil application on fruit weight, fruit size, SSC, and acidity.

Year	Treatment	Fruit weight (g)	Fruit diameter (mm)		SSC ^y (°Brix)	Acidity (%)
			transverse	longitudinal		
2009	ABA 0 ppm	78.0c ^z	55.6b	44.3b	11.3a	1.42ab
	ABA 0.1 ppm	80.4bc	55.3b	44.6ab	11.2a	1.34b
	ABA 1 ppm	88.2ab	57.3ab	44.3b	10.9ab	1.60a
	ABA 10 ppm	96.7a	58.9a	48.2a	10.2b	1.26b
2010	ABA 0 ppm	95.7b	59.5b	48.4b	10.5a	0.99a
	ABA 0.1 ppm	108.0a	62.0a	50.7a	9.8b	0.97a
	ABA 1 ppm	108.9a	62.4a	50.0a	9.4c	1.02a
	ABA 10 ppm	102.8ab	60.8ab	49.4ab	9.5bc	0.99a

^zDifferent letters in the column show significant differences by Tukey's multiple range test (P=0.05).

^ySoluble solids content.

Results

1. Effects of ABA on fruit size, fruit juice sugar content and acidity

In both 2009 and 2010, increased fruit weight was observed for trees receiving soil application of S-ABA. In 2009, fruit weight was found to increase with increasing S-ABA concentration, with the highest fruit weight (96.7 g) being recorded for the 10 ppm S-ABA treatment. In 2010, highest fruit weight (108.9 g) was recorded for the 1 ppm S-ABA treatment (Table 1). Similarly, increased fruit size in terms of both transverse and longitudinal diameter was observed in fruit from ABA-treated trees. In 2009, greatest fruit size was recorded for the 10 ppm S-ABA treatment, demonstrating that soil application of S-ABA promotes fruit development. In both years, highest soluble solids content (Brix) was observed under control treatment, and soluble solids content decreased with increasing concentration of S-ABA applied to the soil. No significant differences in acidity were observed between fruit from ABA-treated and fruit from control trees. In both years, highest value was recorded for the 1 ppm S-ABA treatment.

2. Effects on pericarp color

The results of pericarp color evaluation are presented in Table 2. L^* , a^* and b^* values were highest for the control and lower under S-ABA treatments. In both years, the lowest L^* , a^* and b^* values were recorded for the 10 ppm S-ABA treatment, representing the highest S-ABA concentration. The lower a^* and b^* values observed in fruit from S-ABA-treated trees indicates a delay in the disappearance of green color and in the appearance of yellow color of the pericarp, respectively. The delay in pericarp coloration was also observed in fruits on the trees.

Table 2 Effect of ABA soil application on fruit pericarp color.

Year	Treatment	L^*	a^*	b^*
2009	ABA 0 ppm	67.0a ^z	-4.31a	61.1a
	ABA 0.1 ppm	63.7ab	-8.25b	56.5b
	ABA 1 ppm	66.4a	-6.83b	58.7ab
	ABA 10 ppm	60.8b	-10.7c	51.1c
2010	ABA 0 ppm	68.4a	2.83a	67.4a
	ABA 0.1 ppm	67.2a	-4.51b	62.0b
	ABA 1 ppm	64.2b	-5.95b	60.7b
	ABA 10 ppm	61.4c	-6.50b	55.8b

^zDifferent letters in the column show significant differences by Tukey's multiple range test (P=0.05).

3. Effects on sugar and organic acid content and inorganic composition of fruit juice

In 2009, no differences were observed in the glucose and fructose contents of fruit juice between treatments. However, lower sucrose contents were observed in fruit from S-ABA-treated trees relative to fruit from non-treated control trees, with sucrose content tending to decrease with an increase in the concentration of applied S-ABA (Fig. 1). Although no treatment differences were observed for sucrose in 2010, glucose and fructose contents were lower in fruit from trees receiving 1 ppm and 10 ppm S-ABA than in fruit from control trees. In terms of the organic acid contents of fruit juice, in 2009, citric acid and malic acid contents of fruit from trees receiving 10 ppm S-ABA were higher than in fruit from control trees. The opposite results were observed in 2010, with the content of both acids being lower in fruit from trees receiving 10 ppm than fruit from control trees (Fig. 2).

In terms of the inorganic composition of fruit juice, in both years, potassium contents were observed to increase with S-ABA concentration applied to the soil, with the highest values being recorded for 10 ppm S-ABA treatment (Fig. 3). In both years, the highest magnesium content was recorded for the 10 ppm S-ABA treatment, but no correlations between magnesium

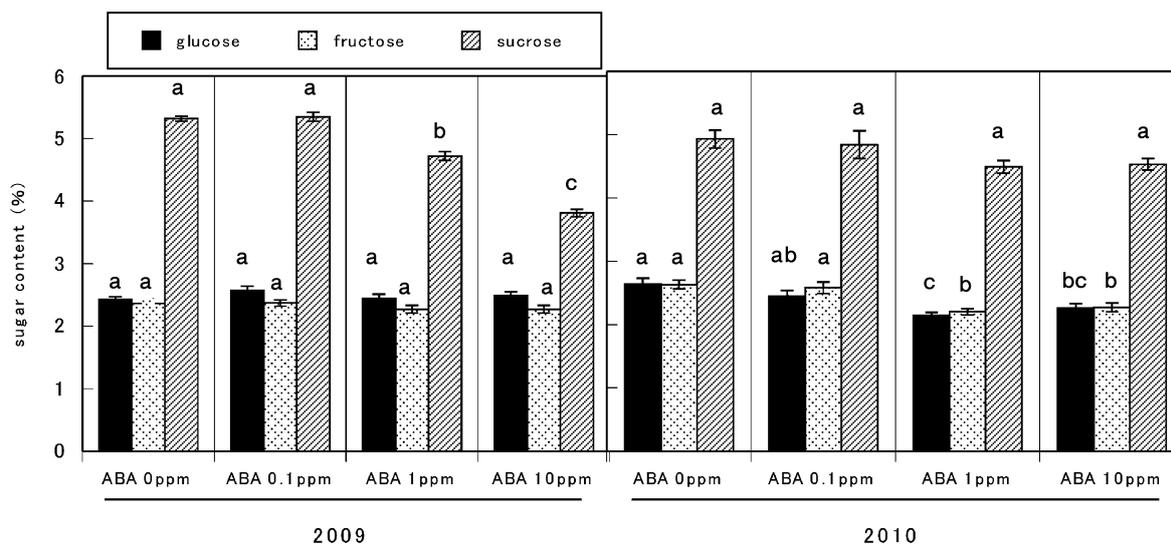


Fig. 1 Effects of soil application of ABA on glucose, fructose and sucrose contents in the fruit juice. Left expresses results in the year 2009 and right in the year 2010. The vertical lines show standard error. Different letters in each population show significant differences by Tukey's multiple range test ($P = 0.05$).

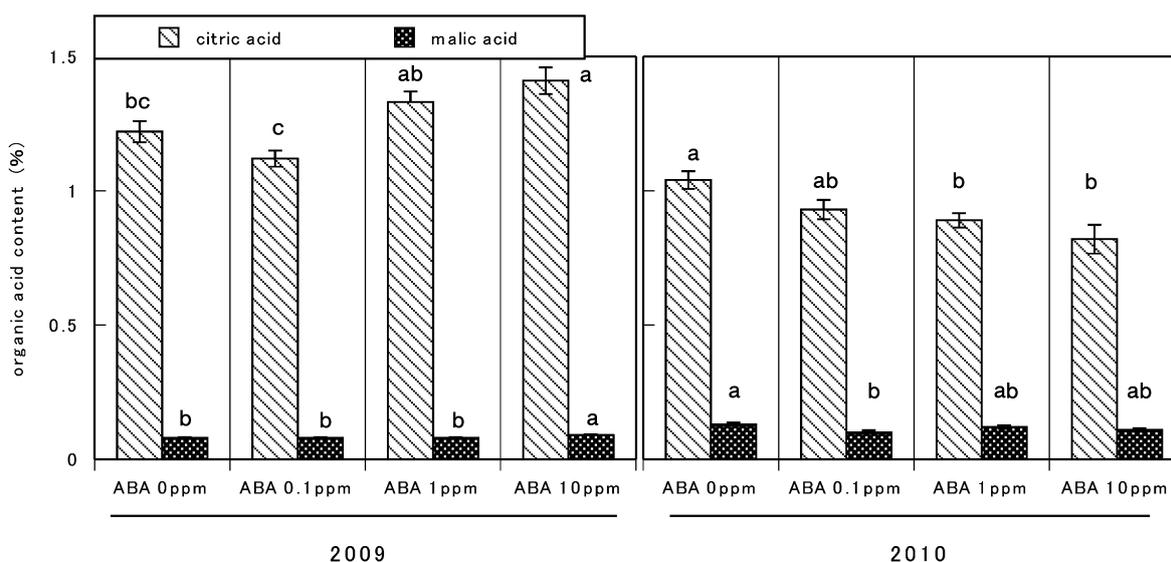


Fig. 2 Effects of soil application of ABA on citric acid and malic acid contents in the fruit juice. Left expresses results in the year 2009 and right in the year 2010. The vertical lines show standard error. Different letters in each population show significant differences by Tukey's multiple range test ($P = 0.05$).

content and S-ABA concentration applied to the soil were observed. In both years, calcium contents were found to be lower in fruit from S-ABA-treated trees than in fruit from control trees.

Discussion

The application of S-ABA to the root increased fruit weight and fruit enlargement in this experiment. The increase of fruit development by racemic type ABA applied to fruits has also been reported in satsuma mandarin¹¹. Exogenous S-ABA has higher physiological

activity than racemic type ABA and in grape, the berries treated with S-ABA showed two times more anthocyanin content than ones with racemic type ABA¹². The promotion of fruit development in satsuma mandarin may be due to translocation, to the fruits, of S-ABA that has been taken up or synthesized by the roots. While S-ABA is synthesized by the roots and is translocated to different organs and tissues¹³, it has not been verified in the case of satsuma mandarin that S-ABA applied to the roots enhances S-ABA synthesis and it is translocated to fruits¹⁴. Meanwhile, ABA controls and mediates plant

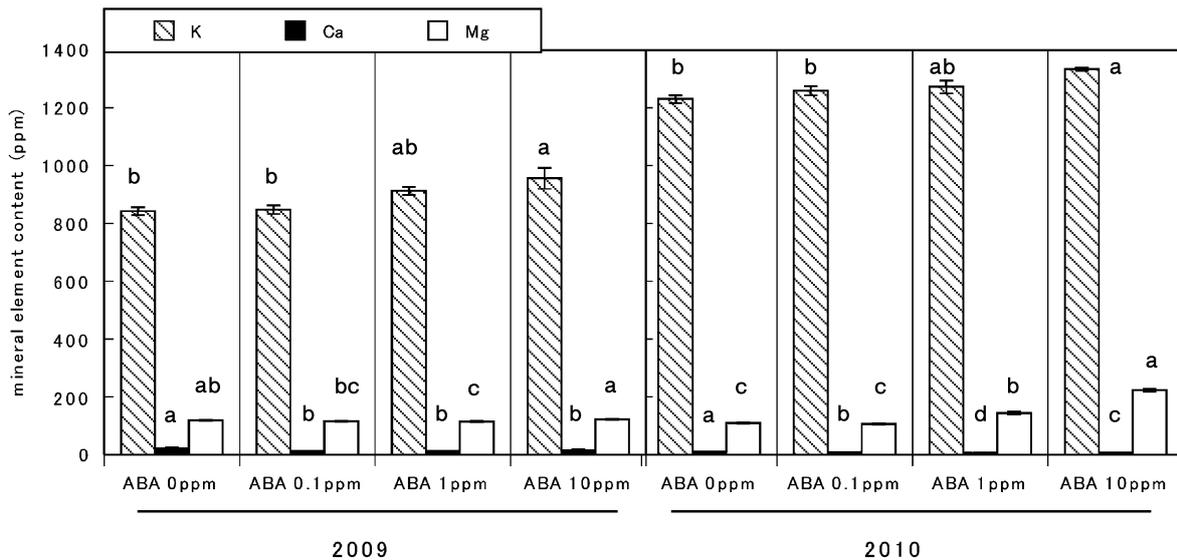


Fig. 3 Effects of soil application of ABA on potassium, calcium and magnesium contents in the fruit juice. Left expresses results in the year 2009 and right in the year 2010. The vertical lines show standard error. Different letters in each population show significant differences by Tukey's multiple range test ($P = 0.05$).

responses to environmental stresses and is involved in water-stress tolerance, salt tolerance and high temperature tolerance¹⁵. When satsuma mandarin is cultivated under the high temperatures of summer, they also experience a certain degree of water stress, which suppresses fruit development¹⁶. When conducting ABA treatment in summer, it is possible that S-ABA applied to soil promotes fruit development by alleviating this stress. As no fruit drop in S-ABA-applied trees was observed, S-ABA did not affect fruit development by diminishing fruit bearing.

DU and TACHIBANA (1995)¹⁷ reported that application of S-ABA to the roots of cucumber cultivated under high temperature conditions resulted in an increase in root S-ABA content and increased uptake of inorganic nutrients including potassium, calcium and magnesium. ZHENG *et al.* (1998)¹⁸ found that combined treatment of gibberellin A₃ and S-ABA to *Cymbidium* resulted in an increase in plant potassium content proportional to the concentration of S-ABA application. We found that potassium contents of fruit receiving soil treatments of S-ABA were greater than those in fruit receiving no S-ABA, and that potassium contents also increased in proportion to the concentration of S-ABA. We assume that S-ABA application stimulated root activity, which promoted the uptake and translocation of potassium and other inorganic nutrients. Although we found that, among the inorganic components of fruit, only calcium contents were lower under S-ABA treatment when compared with controls, the calcium contents of leaves were higher under S-ABA treatment (data not shown).

Thus, the effects of S-ABA on uptake and translocation of calcium may be different than for other inorganic nutrients.

In contrast with controls, soil application of S-ABA was observed to suppress coloration in satsuma mandarin. S-ABA applied to fruits promoted coloration in grape, sweet cherry and strawberry^{4,6,19} and suppressed the coloration in blueberry²⁰. When applied directly to grape or cherry fruit at the onset of fruit coloration, S-ABA has been reported to promote fruit coloration; similarly, coloration of satsuma mandarin fruit is promoted in fruit with high ABA content during the maturation stage^{4,6,21}. In the present study, however, application of S-ABA to roots during the fruit development stage (two times of application) appeared to have the opposite effect, inhibiting rather than promoting coloration. The effect of S-ABA on the fruit coloration may be different in the application to root during the fruit development from the application to fruit. Meanwhile, although it has been reported that direct application of ABA to fruits increases the sugar contents of peach, apple, *C. iyo*, and satsuma mandarin^{7,9,10,11}, in this experiment the application of S-ABA to roots resulted in decreased sugar content (Brix) in fruit at harvest. It has been reported that in the ripening process of satsuma mandarin fruit, glucose and fructose contents increase only slightly, while those of sucrose increase markedly, even beyond the harvest period²². Since in both 2009 and 2010, sucrose contents of fruit juice were not higher for fruits receiving S-ABA soil treatment in contrast with controls, S-ABA soil treatment appears to delay the

maturation. From the pericarp coloration and sugar content results, the application of S-ABA to roots during the fruit development stage may inhibit the physiological transition of fruit to the maturation stage, i.e., delayed onset of the maturation stage.

In addition to the inhibitory effects on fruit coloration and sugar accumulation, while organic acid contents generally tend to decrease over the course of fruit maturation, we observed that the organic acid content of fruit juice in S-ABA treatment increased in 2009 and decreased in 2010. As such, it remains unclear whether and to what degree ABA is involved in organic acid metabolism during the fruit development or maturation stage. This opposite result may have arisen from tree vigor, since we used old 23-year-old trees in 2009 and young 6-year-old trees in 2010. In the present study, S-ABA was applied twice, once in July and once in September, and we observed both the promotion of fruit development and the inhibition of maturation. Further investigation is necessary regarding the influence of application timing, and application number on fruit quality in S-ABA-applied trees.

This study showed that the application of S-ABA to the roots of satsuma mandarin can influence the development of distant organs such as fruit. Although the underlying mechanisms remain unclear, our results have confirmed useful information on moving towards the practical application of S-ABA.

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天然型アブシジン酸の土壤散布がウンシュウミカンの 果実肥大と品質に及ぼす影響

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要約：植物ホルモンの天然型アブシジン酸 (S-ABA) を土壤散布処理し、ウンシュウミカンの果実の肥大と品質に及ぼす影響を 2009 年と 2010 年に調べた。両年とも S-ABA 土壤散布 (0, 0.1, 1, 10 ppm) を果実肥大期の 7 月と 9 月に 2 回行ったところ、果実の肥大が促進され、S-ABA 区で果重、果実の横径、縦径が大きくなった。一方、果皮色は、S-ABA 処理区で着色が両年とも抑制された。糖含量は S-ABA 処理区で低くなり S-ABA 10 ppm 区が最も低かった。酸度は S-ABA 処理区と対照区間に有意な差はみられなかった。果汁中のカリウム含量が対照区に比較して S-ABA 処理区で高く、その処理濃度が高くなるにつれてカリウム含量が増加した。これらの結果より、根への S-ABA 処理は果実の肥大と成熟に作用することが示された。

キーワード：アブシジン酸, 果皮色, 果実肥大, S-ABA, 土壤散布

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