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Effects of Abscisic Acid Injection on Sugar and Organic Acid Contents of Citrus Fruit

Kiyohide Kojima*, Yoshio Yamada and Masashi Yamamoto
Kuchinotsu Branch, Fruit Tree Research Station, Kuchinotsu, Nagasaki 859-25

Summary

The effect of exogenous abscisic acid (ABA) injections into citrus fruits in autumn on fruit drop, ABA catabolism, and sugar and organic acid contents were evaluated. Fruit drop increased after ABA injection proportional to the ABA concentration. Ninety-two percent of citrus fruits were retained 16 days after injection with 0.2 μmol ABA per fruit. Injection of ^{14}C-ABA revealed that exogenously applied ABA was catabolized to a level of about 10% in the fruits within four days. Injection of ABA caused an increase in the glucose and fructose concentrations, but had no effect on the organic acid in the juice. ABA appears to play a role in increasing sugar content of fruit juice.

Introduction

Although various cultural methods have been tried to produce sweeter citrus fruits, e.g. growing trees in a container and in the greenhouse, limiting root growth covering of ground with waterproof sheet, etc., attempts using plant growth substances have not been successful. Essentially these growing methods caused water deficiency in plants. It is a well known fact that generally water deficiency remarkably increases abscisic acid (ABA) content in plants (Zeevaart and Creelman, 1988).

ABA has been considered to play a negative role in plant development (Walton, 1980; Zeevaart and Creelman, 1988). Recently, promotive effects of ABA on the accumulation of assimilates in the sink tissue have been reported (Beruter, 1983; Hein et al., 1984; Kojima et al., 1993b, 1994; Schussler et al., 1984). Based on these reports, the relationship between ABA and sugar level in fruit juice needs to be established. ABA, when sprayed on plants, is readily converted to trans-abscisic acid (t-ABA) and/or gradually broken down by sunlight (Marumo et al., 1990).

In this experiment, we determined the maximum amount of ABA which would not cause fruit abscission, the duration of ABA catabolism, and whether ABA increased sugar level in citrus juice or not.

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*Present address: Faculty of Agriculture, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606.

Materials and Methods

Plant and chemical materials

Citrus (*Citrus iyo* Hort. ex Tanaka cv. Miyauchi Iyokan) trees growing in the Kuchinotsu Branch orchard were utilized for this study. During the experiment, citrus trees were watered sufficiently every days to minimize formation of ABA from water deficiency. Randomly selected fruits were injected with ABA (Toray Industries Co., Tokyo, Japan) containing an emulsifying agent (Phosphoglycerate mutase: Tween 20 type; 16 : 1, wt/wt, Hokkou Kagaku Co., Kanagawa, Japan) except fruits on the control which were injected with the emulsifying agent only.

Experiment 1 (Fruit drop)

On 10 September 100 fruits, weighing about 130 g, were co-injected with a total of 0.2, 0.6 or 1.2 μmol ABA and the above emulsifying agent (0.1 ml, 0.2% vol/vol). The syringe neelde (0.50×

25 mm) was fully inserted into the stylar scar along the axis and slowly emptied to prevent overflowing. The number of retained fruits were counted during the following 16-day period.

Experiment 2 (Catabolism of ABA in fruit)

Twenty fruits weighing about 150 g with about 20 cm of stem and leaves attached were sampled. Basal ends of these stems were re-cut and immersed in water. They were incubated at 25°C under cool white fluorescent lamps (25 mmol·m^{-2}·S^{-1}) set for a 12-hr photoperiod.

A mixture of ABA (0.2μmol) and 122000 dpm [2-^{14}C] ABA (5.5mCi·$mmol^{-1}$, Amersham, Buckinghamshire, U.K.) in emulsifying agent (0.1 ml, 0.2% vol/vol) was injected into these fruits. Fruits were sampled at 24 hr intervals for four days and were stored at −70°C until analysis. The ABA was extracted and purified according to the modified method of Kojima et al. (1993a) as follows; plant tissue was homogenized in 80% ethanol and the homogenate filtered through a glass filter. The filtrate, designated at Step 1, was evaporated to the aqueous phase which was adjusted to pH 2.8 and re-filtered through a 0.22 μm pore membrane. The aqueous filtrate was partitioned three times against CH_2Cl_2 and the combined organic layer evaporated to dryness; the residue was re-dissolved in 25% CH_3CN solution, Step 2. A mixture of ABA was fractionated through an ODS column attached to an HPLC system equipped with an UV spectrophotometer. The eluate corresponding to the retention time of ABA was collected and designated as Step 3. Aliquots of each step were mixed with scintillation cocktail, and the radioactivity was measured in a scintillation counter. All procedures were performed under diffused light conditions to avoid the conversion of ABA to t-ABA.

Experiment 3a (ABA injections)

From 3 September, 10 fruits each on five 10-year-old trees were injected 3 times as a 4-day interval with a total of 0.2 μmol ABA and 0.1 mol of 0.2% (v/v) emulsifying agent, whereas for control comparable fruits on the same trees were injected with emulsifying agent alone. Treated and control fruits were selected at random from each tree. The experiment was terminated after 11 days. On sampling, fruits in each tree were subdivided into two groups.

Experiment 3b

From 27 October, 15 fruits each on six 15-year-old trees were injected 3 times at a 3-day intervals with a total of 0.2 μmol ABA and 0.5 ml of 0.2% (v/v) emulsifying agent, whereas for control comparable fruits on the same trees injected with emulsifying agent alone. When injected fruits were sampled after 9 days, fruits in each tree were subdivided into three groups.

Sugar and organic acid analysis

Juice of sampled fruits was squeezed and mixed per each group for sugar and organic acid analysis. The fruit juice was diluted 50 times with distilled water. Sugar components were measured by HPLC system (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a refractive index detector (RID-6A). The sample was eluted through gel permeation column (SCR 101N, Shimadzu) maintained at 40°C with distilled water at a flow rate of 1 ml·min^{-1}. The individual sugar concentrations were calculated from known peak areas of standard sugars. Organic acid was analyzed by acilyzer (Model 3, Fujihira Industry Co., Tokyo, Japan).

Results and Discussion

Experiment 1

The central axis region of citrus fruit consists of spongy parenchyma cells (Schneider, 1968), which adsorb injected liquid readily. Thus the injection method was used to investigate the effect of ABA. The advantages of the injection method are; a definite amount of ABA can be applied to fruit flesh through the tough skin; isomerization into trans-ABA and breakdown by sun light can be avoided. There is always the possibility of needle wound reaction, but no needle scar was visible to the naked eye even 2 weeks after injection. Additionally, it is possible to offset the effects of the wound reaction by a comparison with the control.

The highest level of applied ABA which does not cause abscission of fruits for about two weeks was determined by injecting three amounts of ABA into each of 100 fruits. Figure 1 shows changes in the number of retained fruits during 16 days after ABA injection. Fruit commenced to abscise 3 days after injection. Eighty-one of 100 fruits were retained 16 days after injection of 1.2 μmol ABA, while 92 were retained after an injection of 0.2 μmol ABA. As the amount of ABA injected increased, fruit drop increased. Sagee et al.

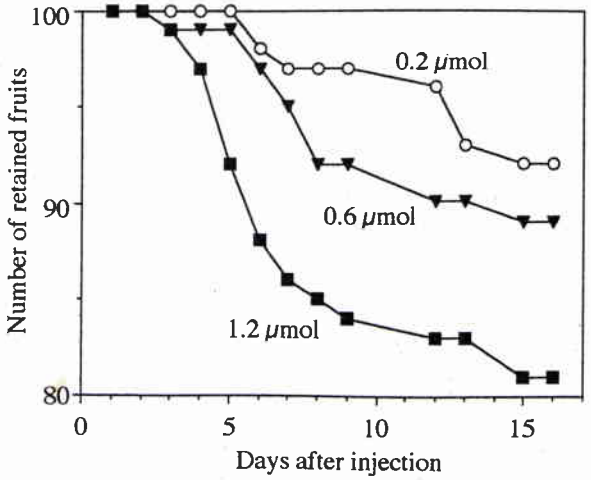

Fig. 1. Changes in the retained fruit number for 16 days after ABA injection. Each amount of ABA was injected into 100 fruits.

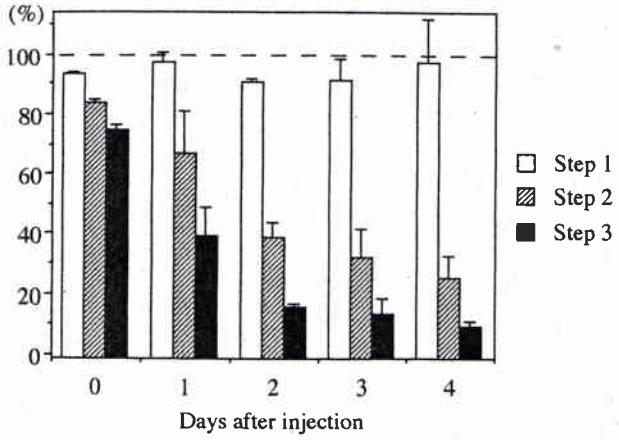

Fig. 2. Changes in the radioactivity in citrus fruits injected with ^{14}C-ABA. The experiment lasted for 4 days and the extracts were subjected to three purification steps. Data are expressed as percentage of the total radioactivity injected on day zero. Step 1 is the crude extract; Step 2 is the organic pahse after solvent partitioning; Step 3 is the fraction corresponding to the retention time of ABA on HPLC.

(1980) reported that ABA induced cellulase and polygalacturonase activities, which lead to abscission in citrus leaf explants. ABA may also induce the abscission by these same sequential mechanisms in the citrus fruit.

Experiment 2

The period of injected ABA catabolism was determined by co-injecting ABA (0.2 μmol) and radiolabelled ABA into fruits. As the fruits began to abscise 4 days after ABA injection, the ABA catabolism was investigated for this duration. Radioactivity in Step 1, which corresponds to the crude extract after filtration, remained at an almost constant level (Fig. 2). However, radioactivity in Step 2, which corresponds to the organic solvent extract, gradually decreased. In Step 3, which corresponds to the fraction after HPLC, radioactivity decreased more rapidly to 10% 4 days after injection. Thus, it is demonstrated that repeated injections of exogenous ABA are needed to induce the hormonal effect.

ABA is converted to ABA-β-D-glucocyl ester (ABA-GE), which is widespread in plants (Zeevaart and Creelman, 1988), especially in the central axis region of citrus fruits where ABA-conjugate concentration was the highest of all tissues analyzed and was about 14 times higher than ABA concentration (Harris and Dugger, 1986). In our trial, ABA can exist both in Step 1 which is the crude extract and Step 2 which is the organic phase after solvent partitioning; but ABA-GE exist only in Step 1. Radioactivity in Step 1 remained at an almost constant level, while that in Step 2 decreased gradually. Hence, there is a possibility that catabolized ABA-GE accounts for the difference in radioactivity between Steps 1 and 2 and that its increase reflects the rate of glucosylation; the central axis region of citrus fruits might have a high ability of glucosylation.

Experiment 3

The fruit drop and ABA catabolism studies indicated that the injection of 0.2 μmol ABA per fruit at 3- to 4-day intervals resulted in minimal fruit abscision. Table 1 shows the effects of ABA on sugar component and organic acid of the fruit juice. ABA had no effect on fruit weight and orgnaic acid in both Exp. 3a and b. In Exp. 3a, however, ABA significantly increased glucose and fructose concentrations but not that of sucrose; whereas in Exp. 3b, all three sugars in the treated fruits increased. Percentages of sucrose, glucose and fructose after ABA injection increased to almost the same level in both Exp. 3a and b.

Three possibilities for the cause of increase in sugar level by ABA can be considered: (a) ABA reduces the juice content which concentrates the sugars. This seems unlikely, because there is no

Table 1. Effects of injected ABA on sugar component and organic acid of citrus fruit juice.

Treatment[y]	Weight ($g \cdot fruit^{-1}$)	Sugar (%)[z]			Organic acid (%citric acid equiv.)
		Sucrose	Glucose	Fructose	
Exp. 3a					
Control	151±5	2.14 ± 0.07 (100)	1.12 ± 0.04 (110)	1.19 ± 0.04 (100)	2.33±0.07
ABA	152±5	2.37 ± 0.11 (111)	1.30 ± 0.05 * (116)	1.37 ± 0.06 * (115)	2.23±0.06
Exp. 3b					
Control	304±7	3.36 ± 0.19 (100)	1.36 ± 0.08 (100)	1.48 ± 0.09 (100)	1.73±0.03
ABA	315±6	4.14 ± 0.14 * (123)	1.68 ± 0.08 * (126)	1.83 ± 0.09 * (124)	1.70±0.07

* Significant difference from the controls at $P \leqq 0.05$.
[z] Values in parentheses are percentages of controls.
[y] Exp. 3a; n=10, Exp.3b; n=18.

difference in fruit weight between the control and ABA injected fruits. (b) ABA release sugars from stored carbohydrates and/or cell materials in segment membrane and fruit peel. (c) ABA causes the import of sugars from leaves and/or other fruits via the phloem. Further research is necessary to investigate changes in the carbohydrate components of the segment membrane and fruit peel, and in the phloem transport after ABA treatment. In short, our results demonstrate that ABA increases the concentration of soluble sugars in citrus juice.

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Literature Cited

Beruter, J. 1983. Effects of abscisic acid on sorbitol uptake in growing apple fruits. J. Exp. Bot. 34 : 737-743.

Harris, M. J. and W. M. Dugger. 1986. The occurrence of abscisic acid and abscisyl-β-D-glucopyranoside in developing and mature citrus fruit as determined by enzyme immunoassay. Plant Physiol. 82 : 339-345.

Hein, M. B., M. L. Brenner and W. A. Brun. 1984. Concentration of abscisic acid and indole-3-acetic acid in soybean seeds during development. Plant Physiol. 76 : 951-954.

Kojima, K., S. Kuraishi, N. Sakurai, T. Itou and K. Tsurusaki. 1993a. Spatial distribution of abscisic acid and 2-trans-abscisic acid in spears, buds, rhizomes and roots of asparagus (*Asparagus officinalis* L.). Scientia Hortic. 54 : 177-189.

Kojima, K., S. Kuraishi, N. Sakurai and K. Fusao. 1993b. Distribution of abscisic acid in different parts of the reproductive organs of tomato. Scientia Hortic. 56 : 23-30.

Kojima, K., Y. Yamada and M. Yamamoto. 1994. Distribution of ABA and IAA within a developing Valencia orange fruit and its parts. J. Japan. Soc. Hort. Sci. 63 : 335-339.

Marumo, S., S. K. Lee and Y. Kamuro. 1990. Microbial production of optically active abscisic acid by *Botrytis cinerea* and its plant hormonal activity. Plant Cell Technol. 2 : 503-513.

Sagee, O., R. Goren and J. Riov. 1980. Abscission of citrus leaf explants. Plant Physiol. 66 : 750-753.

Schneider, H. 1968. The anatomy of citrus. p. 19. In: W. Reuther, H. J. Webber and L. D. Batchelor (eds.). The citrus industry Vol. II. Univ. Calif., Berkeley.

Schussler, J. R., M. L. Brenner and W. A. Brun. 1984. Abscisic acid and its relationship to seed filling in soybeans. Plant Physiol. 76 : 301-306.

Walton, D. C. 1980. Biochemistry and physiology of abscisic acid. Ann. Rev. Plant Physiol. 31 : 453-489.

Zeevaart, J. A. D. and R. A. Creelman. 1988. Metabolism and physiology of abscisic acid. Ann. Rev. Plant Physiol. 39 : 439-473.

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カンキツ果実へのアブシジン酸注射が糖および有機酸濃度に及ぼす影響

児島清秀・山田彬雄・山本雅史

果樹試験場口之津支場　859-25　長崎県南高来郡口之津町

摘　要

秋季に'宮内伊予柑'を供試し，注射針で果実内にABAを注入し，ABAが果肉の糖と有機酸濃度に及ぼす影響を調べた．

1．9月上旬に，ABAの0.2，0.6，1.2 μmolを，それぞれ100個の果実に注射した．ABAの注入量が多いほど落果が多くみられた．しかし，0.2 μmolのABAでは，16日後でも92%は落果しなかった．

2．果実内でのABAの代謝速度を調べるために，9月上旬に，枝付きの果実を採取後，微量の ^{14}C-ABAと0.2 μmolのABAを果実に注入し，水さしで，25℃の室内においた．注入後，果実を毎日，4日連続して採取し，抽出精製後，HPLCでABAの画分を分取し，放射能を測定した．4日目には放射能が始めに与えた量の10%にまで減少した．

3．1と2の結果から適切な条件として，注入するABA量は0.2 μmol，注入する間隔は3または4日が得られた．

4．そこで，この条件を用い，9月中旬に5本の10年生の樹を供試し，1樹内にABA処理果と対照果をそれぞれ10果設定した．ABA処理果には0.2 μmolのABA（展着剤水溶液を含む）と対照果には展着剤水溶液を注射した．11月上旬にも6本の15年生の樹を供試し，1樹内にそれぞれ15果設定し，同様に注射した．処理時期と果実の大きさの異なる上記2回の実験ともに，注射したABAは，有機酸には影響しなかったが，果汁中のブドウ糖，および果糖濃度を有意に増加させた．ABA処理果と対照果の果実重はほぼ同じであったので，ABAの果肉の糖量を上昇させる作用が認められた．