Effects of S-ABA on Seedling Growth of Papaya (*Carica papaya* L.)

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Key words: Plant growth regulator, Abscisic acid, Anatomy

Summary

S-abscisic acid (S-ABA) impact on plant growth was studied in papaya (*Carica papaya* L. cv. Tainung No. 2.). Young seedlings were examined with various concentration of S-ABA aqueous solutions, using foliar spray and medium supply methods. Two weeks after treatment, foliar spray ones showed stem diameter, leaf number and fresh weight could be increased by applying S-ABA. Besides, this plant growth regulator could be used to improve root number, but could not affect average root length. However, foliar spray of S-ABA with high concentration in 1 ppm had no effect on growth of papaya seedlings. For ones with medium supply, papaya seedlings had positive effects on introducing various concentration of S-ABA solutions, especially on fresh weight of root. Seedling anatomy results showed that S-ABA could be used to reduce diameters of palisade cells in leaf, hence to increase number and length of the palisade cells. Furthermore, cell layer number of cortex in stem and root were also increased by applying S-ABA treatments. Consequently, the foliar application of S-ABA had positive effect on leaf growth, thereafter indirectly improved stem and root growth. Moreover, the introducing of medium supply method directly enhanced root growth.

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Introduction

Abscisic acid (ABA) is ubiquitous in plants, and its biosynthetic and catabolic pathways have been elucidated. Previously, ABA was believed to be involved in abscission. It plays an important role in plant responses to environmental stress and plant pathogens (Rock, 2000). ABA can be divided into two forms by structures. The first one is S-ABA which is naturally occurring enantiomer in plant, and the other is unnatural enantiomer called R-ABA (Murphy, 1984; Vaughan and Milborrow 1984; Windsor *et al*., 1992; Kamuro *et al*., 2001). The plant hormone S-abscisic acid (S-ABA) is important in higher plants. It involved the control of a wide plant of physiological and developmental processes (Cutler *et al*., 1997). Only the natural form (S-ABA) can effect on rapid responses such as stomatal closure (Finkelstein and Somerville, 1990). Although ABA generally plays mostly inhibitory roles, but some research has been reported it can promote plant growth. Natural type ABA shows opposite physiological effects compared with racemic ABA, which is chemically synthesized. For example, S-ABA can be used to promote plant growth in a low dosage and inhibit growth in a high dosage (Kamuro, *et. al*., 1997; Nozawa-Gloria, 2003). Moreover, low concentration of S-ABA appeared to promote elongation of coleoptile segments in oats and wheat when the coleoptile tip was not excised, although it was not significantly promoted when the tip was removed (McWha and Jackson, 1976). In addition, normal levels of endogenous ABA are required to maintain shoot development, particularly of effects on plant water balance (Sharp *et al*., 2000). Most of S-ABA researches were demonstrated in temperate fruit tree and cereal crops. However, S-ABA functions in plants were still obscure, and no research was found in tropical fruit tree field. Therefore a purpose of this research aimed to clarify effects and roles of S-ABA on growth of seedlings in papaya is desired.

Materials and Methods

Papaya seeds cv. Tainung No. 2 were obtained from Known You Seed Co., Ltd., Kaohsiung, Taiwan. The seeds were soaked in distilled water for 24 hours, followed by sown in 128 cells of seedling trays contained a medium of peat-moss, sand, and vermiculite (1:1:1, v/v). Two months after sowing, 4-5 true leaves of seedlings were expressed. Uniform seedlings were selected and transplanted into plastic pots (10 cm in diameter). Two experiments were carried out as below.

Experiment I. Effects of S-ABA on growth of papaya seedlings

1. Foliar spray of S-ABA

Seedlings were treated by foliar spray with various concentration of S-ABA (0.01, 0.1, and 1 ppm) solutions and sprayed with distilled water as a control once a week. Hoagland's solution (80 ml/plant) was applied weekly and kept moisture interval by water (80 ml/plant). Two weeks after treatment, eight seedlings were taken from each treatment to measure leaf number, stem diameter, plant height, fresh weight, root number, and root length. This experiment was conducted in greenhouse at Department of Horticulture, National Chung Hsing University, Taichung, Taiwan during 1 and 14 of March, 2011. Average temperature was 30 ± 2 °C in daytime and 18 ± 2 °C in nighttime. S-ABA (90%) using in this experiment was produced by BAL Planing Co., Ltd., Japan.

2. Medium supply of S-ABA

Various concentration of S-ABA (0.01, 0.1, and 1 ppm) solutions (30 ml/plant) were supplied into medium and supplemented with distilled water as control once a week. Subsequent protocols were based on the method described above, and these two experiments were conducted simultaneously.

Experiment II. Effects of S-ABA on anatomical characteristics of papaya seedlings

The concentration of S-ABA in this experiment was selected from experiment I that had more positive effect on seedling growth. Seedlings were treated by distilled water (control), S-ABA (0.1 ppm) by foliar spray or by medium supply weekly. Five seedlings in each treatment were randomly chosen to investigate anatomy of leaf, stem, and root by free hand section method. Plant materials were used as follows; the 3rd leaf (from top), internodes between the 6th and 7th node (from basal), and section of main root at 3 cm from basal of stem. Four sections in each part were selected for staining by safranin solution and immediately investigated under microscope. This experiment was conducted in greenhouse at Department of Horticulture, National Chung Hsing University, Taichung, Taiwan during 12 and 26 of May, 2012. Average temperature was 32 ± 2 °C in daytime and 22 ± 2 °C in nighttime.

Results

Experiment I. Effects of S-ABA on growth of papaya seedlings

1. Foliar spray of S-ABA

Seedling growth of foliar application was presented in Table 1. There was no significant difference in plant height among four treatments with values of 7.0 - 8.2 cm. For stem diameter, the mean value obtained from ones with treatment of S-ABA 0.01 ppm was 3.2 mm, higher than control ones (2.9 mm), while S-ABA 0.1 and 1 ppm did not affect stem diameter. More leaf

number (8.0) was obtained from ones with S-ABA 0.1 ppm treatment. Regarding root number, S-ABA 0.01 and 0.1 ppm significantly increased root number with 44.0 and 42.4 compared to control in 26.6. While S-ABA 1 ppm had no effect on root number. However, there was no significant average root length difference between treated and untreated seedlings.

Table 1. Effects of S-ABA by foliar spray on shoot and root growth of 'Tainung No. 2' papaya seedlings.

S-ABA (ppm)	Plant height (cm)	Stem diameter (mm)	Leaf number	Root number	Average length of root (cm)
Control	7.0 a[z]	2.9 b	7.3 b	26.6 b	6.7 a
0.01	8.2 a	3.2 a	7.8 ab	44.0 a	5.2 a
0.1	7.6 a	3.1 ab	8.0 a	42.4 a	6.3 a
1	7.2 a	2.9 b	7.8 ab	35.0 ab	6.3 a

z: Means in each column followed by the same letters are not significantly by Tukey range test at 5% level.

Fresh weight of papaya seedlings was shown in Table 2. Means of stem fresh weight from treatment of S-ABA 0.01 and 1 ppm (739.0 and 725.5 mg, respectively), were significantly higher than the control (545.6 mg). In the case of leaf, the result appeared that leaf fresh weight was similar to stem fresh weight. Highest fresh weight of leaves was obtained from treatment of S-ABA 0.01 (888.0 mg) and 0.1 ppm (832.9 mg). While fresh weight of control group and S-ABA 1 ppm treatment were 607.1 and 707.5 mg, respectively. For fresh weight of roots, the application of S-ABA had no effect on fresh weight of seedling roots. Means of fresh weight were between 1122.3 and 1612.1 mg. Fresh weight of whole plant was significantly increased by S-ABA 0.01-0.1 ppm compared to the control.

2. Medium supply of S-ABA

Seedling growth of medium supply method was shown in Table 3. There was no significant difference in plant height among four treatments with means between 7.1 and 7.9 cm. For stem diameter, only treatment of S-ABA 0.01 ppm resulted in significantly larger in stem diameter compared to control and S-ABA 0.1 ppm treatment. Other values of stem diameter obtained from treatment of S-ABA 1 ppm were not significantly different from the control. For leaf number, maximum leaf numbers of seedlings were obtained from S-ABA 0.01 and 0.1 ppm

treatment. Leaf numbers of those two treatments were 7.9, while leaf number of 7.5 and 7.3 were obtained from treatment of S-ABA 1 ppm and control, respectively. Root numbers and average length of root were also shown in Table 3. Root numbers obtained from supply of all treatment of S-ABA were significantly higher than the control, but there was no significant difference in average length of root with means of 4.29 – 4.91 cm.

Table 2. Effects of S-ABA by foliar spray on fresh weight of 'Tainung No. 2' papaya seedlings.

S-ABA (ppm)	Stem (mg)	Leaves (mg)	Roots (mg)	Whole plant (mg)
Control	545.6 c[z]	607.1 c	1122.3 a	2275.0 b
0.01	739.0 a	888.0 a	1612.1 a	3239.1 a
0.1	725.5 ab	832.9 ab	1472.7 a	3031.1 a
1	591.8 bc	707.5 bc	1267.8 a	2567.1 ab

z: Means in each column followed by the same letters are not significantly by Tukey range test at 5% level.

Table 3. Effects of S-ABA by medium supply on shoot and root growth of 'Tainung No. 2' papaya seedlings.

S-ABA (ppm)	Plant height (cm)	Stem diameter (mm)	Leaf number	Root number	Average length of root (cm)
Control	7.1 a[z]	2.8 b	7.3 b	30.0 b	4.75 a
0.01	7.3 a	3.0 a	7.9 a	48.3 a	4.29 a
0.1	7.5 a	2.8 b	7.9 a	48.1 a	4.91 a
1	7.9 a	2.9 ab	7.5 ab	52.4 a	4.78 a

z: Means in each column followed by the same letters are not significantly by Tukey range test at 5% level.

Fresh weight of papaya seedling was shown in Table 4. A significant difference in fresh weight of stem was found. Highest fresh weight was obtained from treatment of S-ABA 0.01 and 1 ppm with means of 717.5 and 769 mg. For leaves, the result showed that all treatments of S-ABA improved fresh weight of leaves but only S-ABA 1 ppm resulted in significantly higher

than control. For root fresh weight, the highest fresh weight was found from treatment of S-ABA 0.01 ppm with mean of 1463.3 mg, whereas means of S-ABA 0.1 and 1 ppm treatment did not different from control. However, all treatments of S-ABA affected to increase whole plant fresh weight compared to the control.

Table 4. Effects of S-ABA by medium supply on fresh weight of 'Tainung No. 2' papaya seedlings.

S-ABA (ppm)	Stem (mg)	Leaves (mg)	Roots (mg)	Whole plant (mg)
Control	533.1 c[z]	596.9 b	1006.1 b	2136.1 b
0.01	717.5 ab	701.2 ab	1463.3 a	2882.0 a
0.1	626.7 bc	723.0 ab	1255.6 ab	2605.3 a
1	769.0 a	763.0 a	1413.1 ab	2945.1 a

z: Means in each column followed by the same letters are not significantly by Tukey range test at 5% level.

Experiment II. Effects of S-ABA on anatomical characteristics of papaya seedlings

There was only one layer of palisade cell number in all treatments. In Table 5, palisade cell from treatment of foliar spray and medium supply were 17.8 and 14.9 cells/0.02 mm2, respectively, which were significantly higher than the control, 12.4 cells/0.02 mm2. Conversely, the widest in diameter of palisade cell was found from control leaves (19.8 μm) while diameter of palisade cell from foliar spray and medium supply of S-ABA were 15.8 and 18.0 μm, respectively. Diameter of palisade cell from treatment of foliar spray is smallest, but length of palisade cell of this treatment is longest with 54.2 μm compared to the control. Palisade cells with both two methods of S-ABA treatment were longer in length than the control. The result also showed that number of those palisade cells was more than the control. It led to be firm in cell arrangement, and cellular space was small. Maximum numbers of cell layer in cortex of stem and root were found from ones with S-ABA treatments. Cortex cells with both two methods of S-ABA treatment were small, and there was small intercellular space. Similarly cell size in root with medium supply treatment was smaller than that with foliar spray treatment and control.

Table 5. Effects of S-ABA on anatomical characteristics of 'Tainung No. 2' papaya seedlings.

S-ABA (0.1 ppm) treatment	Palisade cell of leaf			Cell layer number of cortex	
	Number/ 0.02 mm^2	Diameter (μm)	Length (μm)	Stem	Root
Control	12.4 c[z]	19.8 a	48.2 b	7.2 b	11.1 b
Foliar spray	17.8 a	15.8 c	54.2 a	9.9 a	12.8 a
Medium supply	14.9 b	18.0 b	48.8 b	9.0 a	13.5 a

z: Means in each column followed by the same letters are not significantly by Tukey range test at 5% level.

Discussion

Results showed seedling growth of papaya was enhanced by introducing S-ABA with both two methods. In foliar spray method, S-ABA 0.01 – 0.1 ppm affected to enhance shoot growth. These dosages resulted in high soluble sugar content in leaf (unpublished data). It was implied that S-ABA was assimilated by leaf and contributed to photosynthesis thereafter increased leaf and stem growth. In this case, root growth was indirectly promoted. Concentration of 1 ppm was excess dose for papaya seedling, however it did not inhibit growth. The fresh weight of leaves had relation with leaf number like several leaf numbers resulted in high fresh weight. Fresh weight of stem was imposed by stem diameter. This result was shown that S-ABA might be stimulated some growth activities in papaya seedlings. For medium supply method, S-ABA treatments with various concentration enhanced growth of seedlings, especially for leaves and roots. S-ABA treatment affected growth of root directly due to it enhance roots water absorption and nutrient uptake, which was supported by high nitrogen and potassium content (unpublished data). It led to numerous roots were found from seedlings with S-ABA treatment, which was conformed to Sghaier *et al*. (2009) reported low dosage of mixed ABA (20μM) affected to increase length and thickness of palm somatic embryos and it was more effective in protein enrichment. In additional, S-ABA might be interact with other growth hormones and resulted in promote plant growth (Popko *et al*., 2009). Moreover, this study showed that number of palisade cell in leaves and number of cell in stem and root cortex were increased by S-ABA treatment. In leaf of S-ABA treated seedlings showed that palisade cells were narrow and long in length. Arrangement of those cells was tight and intercellular space small hence supported in substance translocation. This result might be affected by S-ABA interact with other hormones in

plants. Several researchers mentioned that high number of palisade cells in leaves caused in high number of chloroplast therefore, rate of photosynthesis was high. Leaves are the direct site of photosynthesis action, and determine source supply of carbohydrate for allocation to other plant parts (Andersen, 2003). In stem and root which treated by S-ABA was also presented in small cell size then it was contributed to substance translocation as well. Lots of amyloplast was found in root cells of seedling from S-ABA treatment. Therefore, fresh weight of root was increased in those treatments. Some researches were mentioned elongation of mesocotyl segments of oats and maize was promoted by endogenous ABA (S-ABA). Racemic ABA significantly increased the starch level and total protein content of Camellia sinensis somatic embryo (Preeti *et al*., 2004). Crouch and Sussex (1981) demonstrated that endogenous ABA promoted embryo growth and protein accumulation in in-vitro cultured rape embryos. However, S-ABA using in these experiments was 90%, and other 10% was unknown substance. Therefore, it could not demonstrate that these positive effects were from S-ABA activity directly or indirectly, remains to be investigated.

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S-ABA 對番木瓜(*Carica papaya* L.)實生苗生長之影響

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關鍵字: 植物生長調節劑、離層酸、解剖

摘要: 為探討 S-abscisic acid (S-ABA)對番木瓜植株生長之影響，本研究以葉片噴施及介質施用不同濃度之 S-ABA 於'台農 2 號'番木瓜之幼苗，在處理後 2 週發現，葉面噴施者的莖粗、葉數及鮮重會因 S-ABA 的處理而增加。該種噴施處理亦會促進根數的增加，但是對平均根長並無影響。另外，亦發現高濃度 1 ppm 的噴施未影響其生長。至於介質施用，各種濃度的 S-ABA 處理皆會影響幼苗的生長，其中對根鮮重的促進明顯與葉噴施者不同。另外，由植株解剖的結果可知，S-ABA 處理會促使葉片柵狀細胞的寬度變小，而細胞數及細胞長則增加，該結果又以葉片噴施者較明顯。至於莖及根的皮層細胞層數會因 S-ABA 的處理而增加。由以上結果可瞭解，葉片噴施 S-ABA 會影響葉的生長，再間接促進莖及根的生長，而介質施用 S-ABA 則直接影響根的生長。

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