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The Effect of Abscisic Acid on Growth Promotion©

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The involvement of gibberellins (GA) in plant growth promotion, stem elongation by stimulating cell division and elongation, and stimulation of bolting and flowering response under long days are widely known functions. Another hormone, abscisic acid (ABA), originally known as a hormone responsible for stimulation of stomata closure because of water stress (environmental response) and induction and maintenance of seed dormancy, is considered a growth inhibitor. Recently, research has shown a reverse effect of the natural type of ABA [(S)-(+)-Abscisic acid], which has shown an early differentiation of bud and flower promotion depending on the timing of application. Until recently, the synthetic racemic mixture of ABA (RABA) was used in research studies examining its physiological response and/or effects. This has lead to potential incorrect information obtained regarding ABA function (Kamuro, 1994). Because the natural type of ABA (SABA) was expensive to produce and apply to evaluate its functions was difficult. However, with the development of an inexpensive technique to synthesize SABA, it can now be artificially produced on a large scale at a moderate price.

The effects of exogenous ABA differ with species. In this work a mixture of SABA and GA was applied at a low concentrations to long-day, short-day, and day-neutral plants. Flower bud differentiation and flowering in long-day plants was promoted by application of these hormones. However, in short-day plants flowering was not promoted, but vigorous plant growth was induced. This suggests that SABA and GA interact and have synergetic effects on growth and flowering in plants. We expect that these results are applicable to increases in plant propagation and production.

INTRODUCTION

Abscisic acid (ABA) is well known as a phytohormone that generally acts to inhibit metabolic functions and promote dormancy. On the other hand, gibberellins (GA) are growth regulators that cause or influence cell enlargement, flowering, and seed germination. Many experiments studying the actions of ABA and GA in plant growth have been conducted. However, the interaction between ABA and GA has not been investigated in great detail.

Most of the research examining the effects of ABA has been conducted using a synthetic racemic mixture of ABA, which is an unnatural type of ABA (here referred to as RABA). However, the natural ABA, (S)-(+)-abscisic acid (purity of natural type of abscisic acid is 90%) was found to show a somewhat different physiological activity from that of the racemic mixture (Gusta et al., 1992).

Up to now the natural type of ABA (here referred to as SABA) (Kamuro, 1994) was expensive to produce, and the application to evaluate its functions was limited. However, with the development of a technique to synthesize SABA, the synthetic production of SABA became possible on a large scale at a moderate price.

In a study to examine the effects of applying mixtures of SABA+GA on flowering in long-day and short-day plants, Kamuro et al. (2001) reported that the application of SABA and GA mixtures promoted the growth and flowering of long-day plants, while it was inhibitory to that of short-day plants. They suggested that SABA and GA had reciprocal actions on flowering and the exogenous application of SABA+GA mixtures had opposite effects on the timing of flowering in the long-day plants and short-day plants.

In this work, SABA mixed with GA (here represented by SABA+GA mixture), SABA, and GA solutions were applied exogenously at low concentration to long-day, short-day, and day-neutral plants to evaluate the interaction between SABA and GA, and different expression of gene responding to hormonal control.

MATERIAL AND METHODS

Plant Material. Three types of plant species were used in this study. There include: short-day plants, *Brassica rapa* (syn. *B. campestris)*, *Raphanus sativus*, *Oryza sativa*, *Glycine max*, *Lycopersicum esculentum*; long-day plants, *Spinacia oleracea* 'Aichi-jiromaru' and *S. oleracea*; and day-neutral plants, *Nicotiana tabacum* and peaton [a graft hybrid between yatsubusa and Spanish paprika (Taller et al, 1998)].

Seeds were sterilized in 1% sodium hypochlorite solution containing one drop of detergent, rinsed, and sown in vinyl pots containing soil. The seeds and seedlings were cultivated under natural conditions.

Hormone Treatment and Growth Evaluation. The leaf surfaces of short-day, long-day, and day-neutral plants were sprayed with low hormone concentrations of (SABA + GA mixture, SABA, and GA) to determine their growth promotion effects.

Solutions of SABA+GA, SABA, and GA were exogenously applied as a spray. When the plant reaches a certain growth (true-leaf stage depending on the plant type) the leaves of plants were sprayed one time, equally with adequate dose of mixture. The hormone concentrations used was 10 ppm for the SABA+GA mixture, 20 ppm for GA, and 20 ppm for SABA. Growth was evaluated weekly by measuring the leaf number index and the height of the plant once each month.

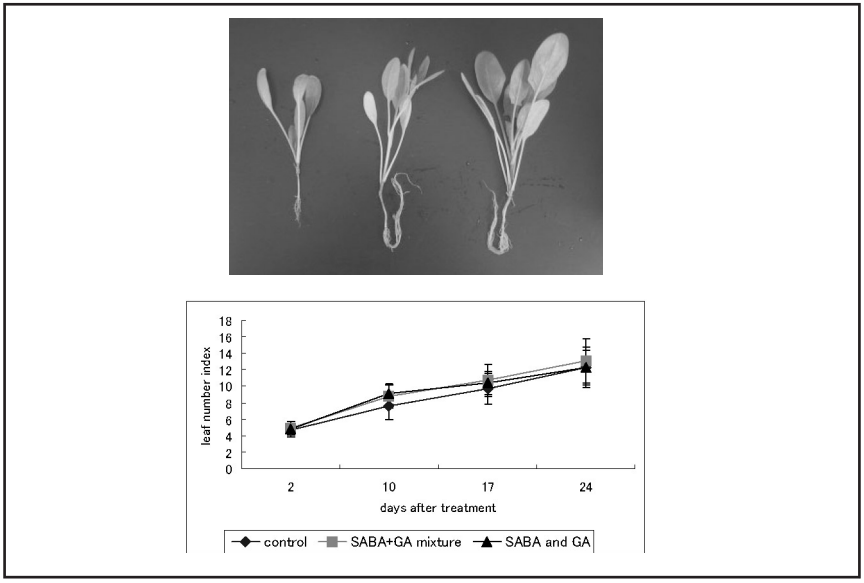

Figure 1. Leaf number index comparison between spinach control and hormone treated. The picture above represents left to right; control plant, spinach treated with GA+SABA mixture and GA and SABA solution; 10 days after treatment. Ten days after hormone treatment, the internodes elongation of treated plant could be observed but in control plant could not be observed. After hormone treatment the leaf number index was evaluated at one-week intervals as shown in the graph. According to these data, the leaf number index between control and treatment did not shown much difference.

Growing Point Sections. The growing point of plants, which were treated with the hormones, was cut into sections using a microslicer and observed**.**

Gene Searching. The DNA of hormonally treated and control plants was extracted using the CTAB extraction (hexadecyltrimethylammonium bromide) method. Random amplified polymorphic DNA analysis (RAPD), using a common primers (BEX and Operon Co.), was carried out. The polymerase chain reaction (PCR) was performed in a total volume of 25 μl with common primers. The program used included an initial denaturing step 94 ºC for 1 min, 40 cycles of denaturation at 94 ºC for 1 minute, annealing at 37 ºC for 1 minute, extraction at 72 ºC for 2 min, and a final extraction step was carried out at 72 ºC for 10 min. The tubes were stored at 4 ºC until electrophoresis.

RESULT

Hormone Application Experiment. Only spinach, a long-day plant, out of all treated plants showed growth promotion, that is, the leaf number index and height increased when compared with control plants. At the fourth-true-leaf stage SABA+GA mixture, SABA, and GA solutions were applied with the leaf number index shown in Fig. 1 and weight in Fig. 2. The pictures of treated and control plants were taken 10 days after treatment.

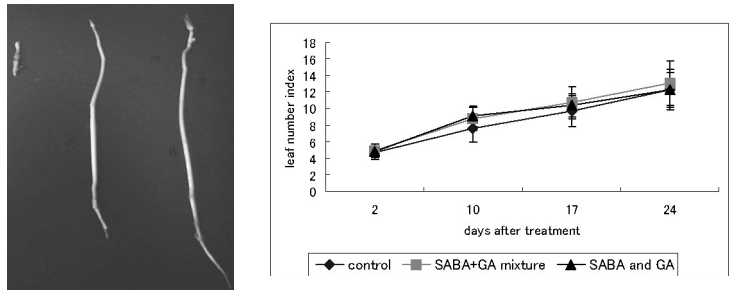

Figure 2. Growth promotion evaluation compares height of spinach control and hormone treated plants. In the picture above, 10 days after treatment the leaves of control spinach and treated spinach was took off to evaluate the height. Like in the figure 2 left to right; control plant, spinach treated with GA+SABA mixture and GA and SABA solution was arranged. After hormone treatment the height was evaluated at one-week intervals as shown in the graph. The height (vegetative growth) treated was promoted shown in the picture and graph. Ten days after hormone treatment, the internodes elongation of treated plant could be observed; in contrast, this elongation could not be seen in the control plant (still rosette stage).

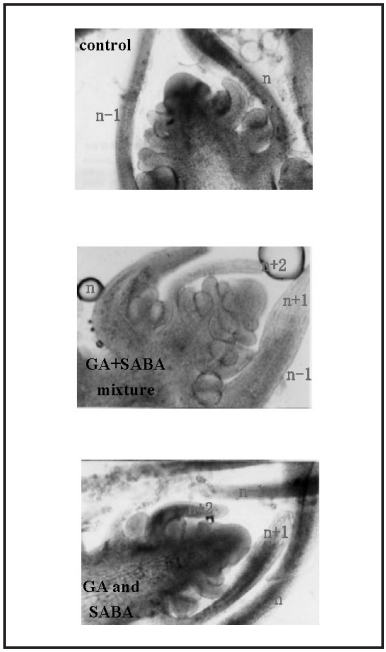

Figure 3. Morphology at growing point in the sections of control, GA+SABA mixture and GA and SABA treatments, respectively. In treated plants the one leaf stage development was promoted, resulting in a spinach plant growth promotion.

The leaf number index between control and treated plants showed little difference (Fig. 1), but the height (vegetative growth) average of plants treated was promoted (Fig. 2).

This promotion can be conformed in the Figs. 1 and 2. Ten days after hormone treatment, internode elongation of treated plants could be observed; in contrast, this elongation was not observed in control plants (still in rosette stage).

Growing Point Sectioning. After 10 days of treatment the growing points of treated and control plants were sectioned and showed that the leaf stage of treated plants was more developed than that of control plants (Fig. 3).

Gene Searching. After the DNA extraction of spinach, RAPD an analysis was done, but no difference was noted between control and treated plants at DNA level (Fig. 4).

DISCUSSION

As a whole, the effect of exogenous SABA+GA mixture, and separate SABA and GA solution applications varied according to plant species. In this work SABA+GA mixture was applied to long-day, short–day, and day-neutral plants at low concentration. As the result, SABA+GA mixture application promoted the flower bud differentiation and the flowering in long-day plants. In the other words, the vegetative growth and consequently the flowering of long-day plants were promoted with the application of SABA+GA mixture. However, in short-day plants the flowering was not promoted but vigorous plant growth resulted (data not shown), like the heterosis phenomenon observed in hybrid plants. This indicates that the SABA+GA mixture exhibits a synergetic effect on growth and flowering in some plants.

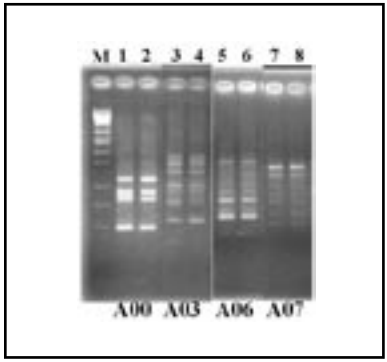

Figure 4. RAPD analysis using random primer (A00, A03, A06 and A07) of BEX Co. was done, but any difference was noted between control and treatment, DNA. 1, 3,5,7 represent control plants and 2,4,6,8 represent plant treated with SABA+GA mixture.

In spinach, the vegetative growth and flowering promoted by application of SABA+GA mixture was only temporary, about 3 or 4 weeks. Spinach is an "annual plant" and its life cycle is short, about 2 months. Thus the difference between treated plants and control plants could be observed only for about 3 weeks after treatment (data not shown). According to this, we propose that the appropriate genetic analysis during the 3-week period for treated spinach can be found at the RNA levels. This stimulation and promotion natures may lead to key point for heterosis expression mechanism.

From the results presented above, it is possible that the SABA + GA mixture may have a role in increasing plant production and needs to be further tested.

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