

Brassinolide induces stem growth of water spinach (*Ipomoea aquatica* Forsk.)

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Summary

This report is a preliminary study to clarify the effects of exogenous brassinolide (BL) on the stem tissue growth of water spinach. Local varieties in Taiwan were used for the indoor experiments. The clones were propagated and grown under fluorescent lamps for plant growth, and the clones with 5 leaves were cut off and grown in a water culture for 7 days. Distilled water was used for diluting the concentration of BL, and the BL concentrations used were 0, 1, 2.5, 5, and 10 ppb (*w/v*). Each clone was cultured in 80 ml of the water solution. The plant height and stem diameter at the centre of the first internode above the point of cutting were measured after 5 days of treatment. The stem diameter and pith thickness increased significantly as the BL concentration in the water culture increased, whereas the cortex thickness was not affected by the BL concentration. The cavity diameters in the 5 and 10 ppb plots were significantly larger than those in the other plots. There was no significant difference in plant height among the treatments, although the plant height tended to be higher with increasing BL concentration.

Key words : Cavity, Cortex, Pith, Stem, Vegetable

Introduction

Water spinach is an important vegetable in tropical and temperate zones and is also a valuable resource that can be grown effectively in plant factories because of its rapid growth in water culture and higher plant nutrient absorption.

In a previous paper (Sasaki *et al.*, 2012), we reported that the plant height of water spinach seedling was promoted by blue light under artificial light conditions. In addition, the stem diameter and pith thickness also increased significantly by increasing the blue light (450 ± 10 nm) / red light (660 ± 10 nm) energy ratio, but the cortex thickness was not affected (Sasaki *et al.*, 2013). However, the eco-physiological mechanism for the sensitive response of stem growth to blue light is not known.

Brassinosteroids (BR) are phytohormones that can be induced by blue light irradiation, and increasing their concentration promotes plant stem growth. For example, there were several reports that BR concentration levels and BR biosynthetic gene expressions were markedly increased when rice seedlings were exposed to blue light irradiation (Iwasaki, 2002 ; Fukuda *et al.*, 2005). The bioassay data also clarified that blue light induced endogenous BR activity in rice seedlings (Abe *et al.*, 2000). BR can be commonly detected in many plant organs, including leaves, stems, pollen, and seeds, and it has growth-regulating effects that facilitate germination and stem elongate and enlargement, promote leaf expansion, or encourage bending in many plant species.

From our previous results and those of other reports, it was suggested that blue light induces BR synthesis and endogenous BR encourages stem growth in water spinach. We investigated to verify the hypotheses that (1) blue light induces endogenous BR activity and (2) BR induces stem growth. This report

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Materials and Methods

Plant materials

Local varieties of plant materials commonly available in food and seed markets in Taiwan were used for the indoor experiments. The clones were propagated and grown under fluorescent lamps for plant growth (Biolux A40W, NEC, Japan). The clones with 5 leaves were cut off and grown in a water culture for 7 days.

Chemicals

In the experiments, BL, which is the most active BR (Turk *et al.*, 2003), was used. The BL solution in 50% ethanol (BL 10, lot. no. A121427) was provided from Brassino (Toyama, Japan). The structural and chemical formulae and molecular weight of the test reagent are shown in Fig. 1.

Treatments

Distilled water was used for diluting the BL, and the BL concentrations used were 0, 1, 2.5, 5, and 10 ppb (*w/v*). The clone developed and the elongated rhizomes were fixed in a sponge pad in a test tube. Each clone was cultured in 80 ml of the water solution for 5 days. The experimental solutions were changed at intervals of 3 days. The clones were grown under continuous fluorescent lighting, for which the light intensity was controlled on PPFD $200 \mu \text{mol m}^{-2} \text{s}^{-1}$ at the top of clones, in a room maintained at 27°C . Five clones were used for each treatment, and the experimental design was a randomized-block design with 3 replications.

Observations

The plant height and stem diameter at the centre of the first internode above the point of cutting were measured after 5 days of treatment. The internode samples were fixed in FAA solution (formalin : 80% ethanol : acetic acid = 1 : 8 : 1), and the cavity diameter (*Ca*), pith thickness (*Pi*), and cortex thickness (*Co*) of the cross-section were measured by light microscopy (Fig. 2).

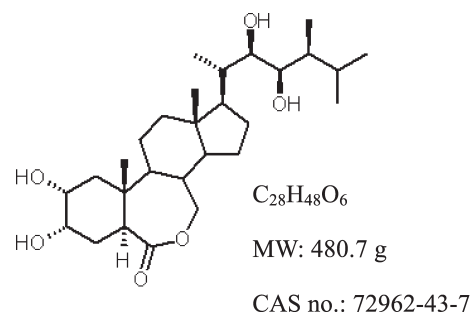


Fig. 1 Chemical information of brassinolide (BL).

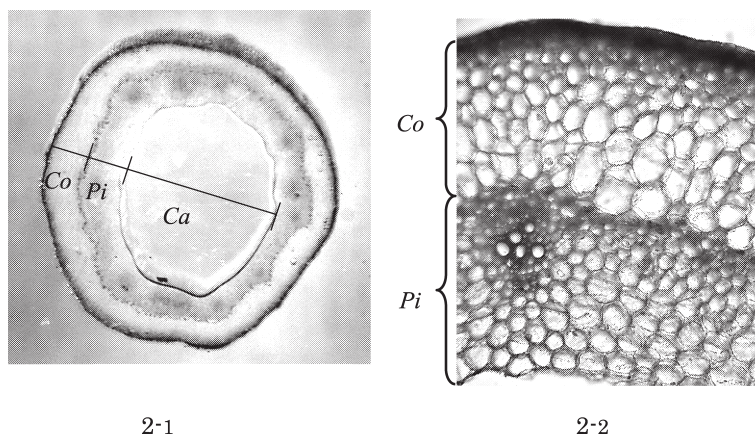


Fig. 2 Cross section of stem (2-1) and stem tissues (2-2) in water spinach.
Co : cortex, *Pi* : pith, *Ca* : cavity.

Results

The results for the stem diameter, cavity diameter, and pith and cortex thickness are shown in Fig. 3 and 4. The stem diameter and pith thickness increased significantly as the BL concentration in the water culture increased, whereas the cortex thickness was not affected by the BL concentration. The cavity diameters in the 5 and 10 ppb plots were significantly larger than those in the other plots. There was no significant difference in plant height among the treatments, although the plant height tended to be higher with increasing BL concentration.

Discussion

Based on the results in Fig. 3 and 4, we can suggest that BL enhanced stem growth. Hayat *et al.* (2001) noted that the effect of 28-homobrassinolide on plant growth was stronger than that of other phytohormones at lower concentrations, and BR had physiologically multifaceted effects at the molecular, cellular, tissue and individual levels. In water spinach, external BL appeared to promote stem growth as a phytohormone.

The stem diameter, pith thickness, and stem cavity size were influenced by different BL concentrations after only 5 days of treatment. By using ^{14}C -epibrassinolide, Nishikawa *et al.* (1994) reported that exogenous epibrassinolide applied to intact plants was acropetally transported in the leaves and roots of cucumber and wheat seedlings. The results in water spinach suggested that BL was quickly absorbed and transported from the root to the stem.

The stem diameter and pith thickness of water spinach was increased by higher ratios of blue light to red light (B/R), but the cortex thickness was not affected (Sasaki *et al.*, 2013). The effects of exogenous BL on the stem tissue (Fig. 3 and 4) were similar to those of the B/R ratio in the light spectrum. Abe *et al.* (2000) reported that the endogenous BR concentration in the rice seedlings was strongly increased by blue light irradiation and resulted in leaf unrolling. The previously reported effects of endogenous BR on rice and our results regarding the effects of the B/R ratio and exogenous BL on water spinach suggest that the B/R ratio or the intensity of blue light irradiation increased the endogenous BR concentration

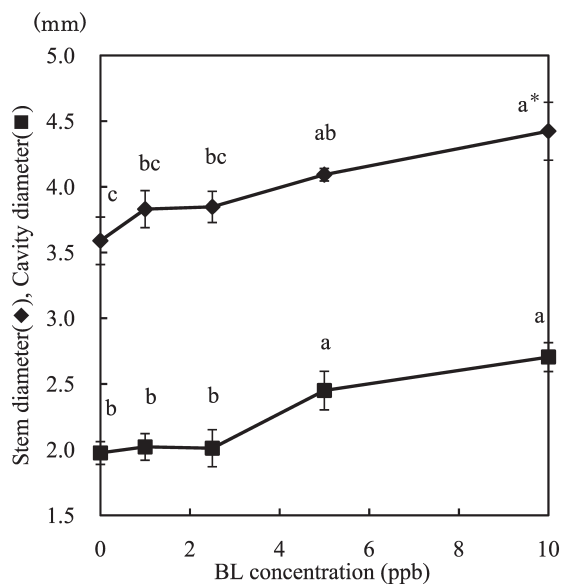


Fig. 3 Effects of BL concentration on stem diameter and cavity diameter.

*: The different alphabet indicates significant difference at the 5% level by Fisher's LSD.

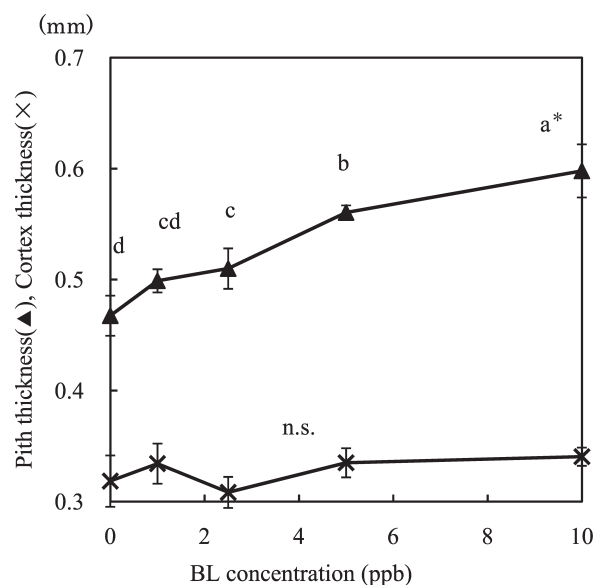


Fig. 4 Effects of BL concentration on pith thickness and cortex thickness.

*: The different alphabet indicates significant difference at the 5% level by Fisher's LSD.

n.s.: No significant.

in the stem of water spinach.

According to the results, cavity diameter as increased the avoidance to anaerobic stress by flooding was also increased by increasing the BR concentration of the water culture. Takematsu *et al.* (1986) reported that pre-treatment of plants with exogenous BR induced stress avoidance behavior in response to high temperature, drought, salt, flooding, and chemicals. Water spinach is an indigenous plant species around the shorefront of ponds, swamps, and rivers in South-eastern Asian countries in the tropical zone (Sharma, 1994), and this plant is frequently stressed by sudden water flooding. Flooding quickly changes the light condition of plants by altering the light intensity and B/R ratio because water effectively absorbs red light. The morphogenetic effects of the B/R ratio and endogenous BR on the pith growth and cavity diameter may have an important ecological significance for stem floating and air ventilation.

Further investigation will be required to clarify the relationships between the B/R ratio of light and the endogenous BR concentration.

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(* : in Japanese with English summary, ** : in Japanese)