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Short Report

Effect of Exogenous Ethylene on Leaf Morphology in Broadleaf Plantain (*Plantago major* L.)

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セイヨウオオバコの葉部形態に及ぼす外生エチレンの影響

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Introduction

Plantago major L. is a perennial forb with a rosette type of growth form which is distributed in the cool-temperate regions of the world[10], growing in more heavily trampled habitats than another trampling-tolerant forb, Asiatic plantain (*P. asiatica* L.), in Japan[6]. Several studies demonstrated that trampling changed leaf morphology of *Plantago* species[4,11]. However, a detailed mechanism underlying the induction of morphological changes has not been elucidated. JAFFE[5] termed the morphological changes of plants in response to mechanical stresses thigmomorphogenesis, and many studies have suggested that ethylene plays one of the important roles in thigmomorphogenesis[2,5,7]. In orchardgrass, artificial trampling induced the change of growth form and ethylene production[3]. Recently, we demonstrated that in *P. asiatica* human trampling increased the number of leaves and inflorescence per plant, the petiole diameter, and the leaf blade length to width ratio, and promoted ethylene production[8]. Moreover, a treatment with ethephon (2-chloroethylphosphonic acid), an ethylene-releasing agent, changed the leaf blade shape, i.e. the formation of relatively narrow leaf blades, in a manner similar to the change induced by trampling, suggesting that ethylene was involved in the trampling-induced morphological change in *P. asiatica*. However, there is no information about ethylene-induced morphological changes in other *Plantago* species. In this study, we focused on *P. major* as one of popular *Plantago* species and examined the effect of trampling on ethylene production as well as the effect of ethephon on leaf morphology of this species.

Materials and Methods

1. Plant materials

Seeds of *P. major* were purchased from Herbiseed Com. (Berkshire, UK). The seeds were sown in plastic trays containing Kasugai's nutrient solution and grown in a growth chamber at 30/20℃, with 14 h of daylight at 60 μmol m^{-2} s^{-1}. Seedlings at about the 5 th- or 6th-leaf stage were used for the subsequent experiments.

2. Effect of trampling on ethylene production

Trampling was carried out by a person weighing about 43.5 kg wearing flat, rubber-soled shoes. The seedlings were put on the ground and were subjected to trampling that involved pressing a foot 10 times directly on the whole plant for each seedling (approximately 0.24 kg cm^{-2}). Five seedlings were then transferred to a 35-ml glass vial containing 1 ml of distilled water. Non-trampled seedlings were also transferred to a glass vial containing 1 ml of distilled water as control. The glass vials were tightly sealed with a rubber plug and an aluminum stopper using a crimper, and incubated at 28℃ for 24 h in the dark. After incubation, 1 ml gas samples were collected from each glass vial using a syringe, and ethylene contents in the samples were determined by gas chromatography (Hitachi, G-5000A) as described

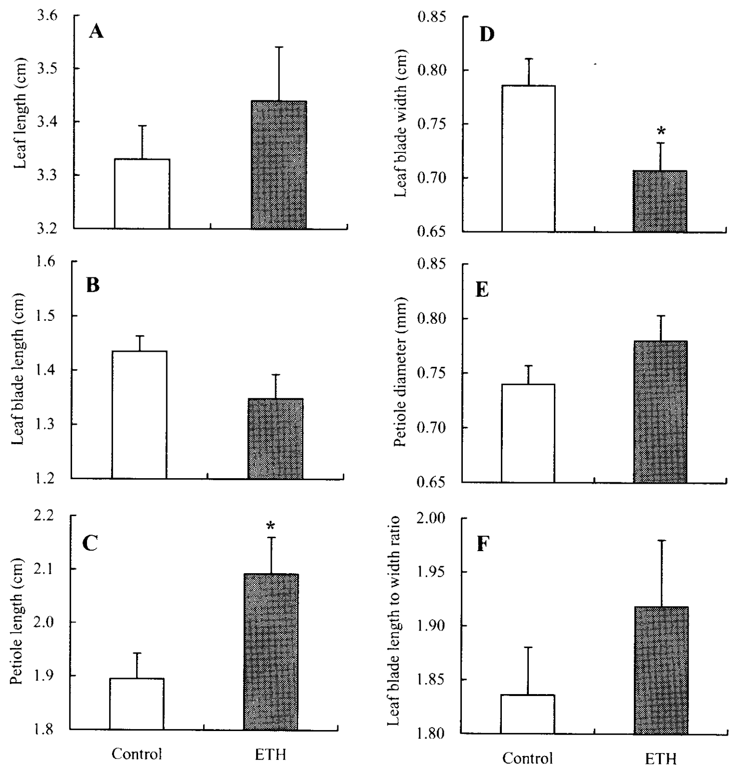

Fig. 1. Effect of 1 mM ethephon (ETH) on morphology of the largest leaf in *Plantago major* seedlings. Vertical bars indicate SE ($N=10$) of the means. * : $P<0.05$ (ETH vs. control).

previously[8,9]. The seedlings with roots were weighed after drying at 80℃ for 96 h.

3. Effect of ethephon on leaf morphology

Ten seedlings of *P. major* were transplanted into 300-ml beakers containing 100 ml of the nutrient solution, and sprayed with 1 mM ethephon solution, while control seedlings were not sprayed with the ethephon solution. The beakers were tightly sealed with two layers of polyethylene film and two layers of Parafilm™ and Sellotape™, then placed in the growth chamber. Spraying of the ethephon solution was repeated again four days after the first treatment. The length and width of leaf blades, the leaf length, and the diameter in the center of petiole were measured in the largest leaf of each seedling using a digital caliper before and seven days after the first treatment. The petiole length was calculated from the length of leaf and leaf blade. There were no significant differences in the initial leaf morphology between ethephon-treated and control seedlings.

4. Statistical analyses

The effects of trampling and ethephon on ethylene production and leaf morphology, respectively, were analyzed using *t*-test, after power transformations of the data using the slope from the regression of the log of standard deviations on the log of means. These statistical analyses were performed using SPSS 10.0J (SPSS Japan Inc., Hiroo, Tokyo, Japan), and relationships were considered significant when $P<0.05$.

Results and Discussion

We demonstrated ethylene production by trampling and morphological changes by ethephon treatment in *P. asiatica* in our previous study[8]. The occurrence of similar ethylene-related responses in other *Plantago* species was examined using *P. major*. *P. major* seedlings subjected to trampling produced 633±27 (SE, $N=5$) nl g DW^{-1} ethylene, while control evolved 414±29 (SE, $N=4$) nl g DW^{-1}, indicating that trampling significantly ($P<0.01$) promoted ethylene production in this species. Our previous study[8] showed that *P. asiatica* ramets subjected to trampling (pressing a foot 12 times) also produced more ethylene than those not subjected to trampling. However, the mean amount of ethylene from the trampled ramets of *P. asiatica* was 178 nl g DW^{-1}, and was markedly lower than that from *P. major* seedlings not subjected to trampling, suggesting that *P. major* may sensitively produce ethylene in response to stresses due to the ethylene measurement, such as touch by hand and cutting of lateral roots. Many studies have also shown that mechanical stresses induce ethylene production in other plants[2,7].

Ethephon, an ethylene releaser, increased the petiole length (Fig. 1 C ; $P<0.05$) and decreased the leaf blade width (Fig. 1 D ; $P<0.05$) in *P. major* seedlings, but there were no significant effects of ethephon on the leaf length (Fig. 1 A ; $P=0.37$), the leaf blade length (Fig. 1 B ; $P=0.12$), the petiole diameter (Fig. 1 E ; $P=0.18$), and the leaf blade length to width ratio (Fig. 1 F ; $P=0.29$). In our previous study[8] using mature plants of *P. asiatica* that had grown in a trampled habitat, 10 mM ethephon decreased the leaf blade width in a manner similar to the change in *P. major* seedlings treated with 1 mM ethephon. However, there were several inconsistencies in the ethephon-induced morphological changes between *P. asiatica* and *P. major* ; in mature *P. asiatica* 10 mM ethephon increased the leaf blade length to width ratio, and did not change the petiole length (data not shown in our previous study). These findings suggest that both species produced relatively narrow leaves in response to exogenous ethylene, but *P. major* and *P. asiatica* elongated petioles and leaf blades, respectively, more than widened their leaf blades. It is suggested that *P. major* and *P. asiatica* may have specific mechanisms to recognize ethylene as a signal for inducing the production of relatively narrow leaves. GRABHERR[1] reported that plants in the Austrian and German Alps which possess narrow and pliable leaves were capable of resisting the damage caused by being continually walked over. The morphological changes in leaf in response to ethylene that was induced by trampling as shown by the present study might be an important trait for plants to survive in heavily trampled habitats.

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