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Inhibition of Abscission of Bean Petiole Explants by Lepidimoide

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Abstract. The effect of lepidimoide on the process leading to abscission was studied in bean (*Phaseolus vulgaris* L. cv. Masterpiece) petiole explants. The assays, involving observations on the junction of the petiole of primary leaves and the pulvinus, were conducted in the light. Lepidimoide, at concentrations of 1 μM or higher, delayed the abscission process; however, the progression of abscission proceeded at normal rates, and complete abscission resulted. On the other hand indoleacetic acid inhibited the normal senescence resulting in greatly decreased abscission during the observation period. These observations show that lepidimoide only delays abscission, and the kinetics seem to indicate that lepidimoide and indoleacetic acid affect abscission through different mechanisms.

Key Words. Abscission—Bean petiole—Indoleacetic acid—Lepidimoide—*Phaseolus vulgaris*

Lepidimoide was isolated and characterized as a novel allelopathic substance from the mucilage of cress (*Lepidium sativum* L.) seeds. The compound promoted shoot growth but inhibited root growth in different plant species (see in Fig. 1) (Hasegawa et al. 1992a, 1992b, 1992c). Lepidimoide-like activity was found in the exudates of seeds from various plant species (Hasegawa et al. 1993, Shioya et al. 1995). The occurrence of lepidimoide was also shown in a variety of weeds and crops (Yamada et al. 1995). These facts suggest that lepidimoide is widespread in the plant kingdom.

Preliminary physiologic observations on the effects of lepidimoide showed promotion of shoot growth and inhibition of root growth in seedlings of various plant species (Hasegawa et al. 1992a, 1992b, 1992c, 1993) and acceleration of flowering and increased seed production in *Arabidopsis* plants (Goto et al. 1995). These facts together with the wide distribution of lepidimoide in the plant kingdom suggest that lepidimoide may control various physiologic phenomena in plants and may be considered a new type of endogenous plant growth regulator or phytohormone.

In this paper we report that lepidimoide substantially inhibits the formation of abscission during leaf senescence in bean petiole explants.

Materials and Methods

Chemicals

Natural lepidimoide was isolated from the exudates of germinated cress seeds as the sodium salt of 2-O-rhamnopyranosyl-4-deoxy-α-L-threo-hex-enopyranosiduronate (Fig. 1) (Hasegawa et al. 1992c). However, a study of structure-activity relationships of lepidimoide and its analogs in the *Amaranthus* hypocotyl elongation test revealed that the free carboxylic acid form of natural lepidimoide had the same growth-promoting activity (Yamada et al. 1996). Therefore, in the present study the free carboxylic acid form of not original (natural) but synthesized lepidimoide, which was synthesized from D-glucose and L-rhamnose (Kosemura et al. 1993), was used. In some experiments synthetic and natural lepidimoide were used to compare physiologic effects.

Abscission Assays

The abscission assay, using bean (*Phaseolus vulgaris* L. cv. Masterpiece) explants, was carried out according to the method of Ueda et al. (1991) with some modifications. Bean seeds were soaked in running tap water for a few hours and germinated in sand that was washed with water. Seedlings were grown under continuous white fluorescent light (8 W/m^2) at 25°C for 10 days. Explants (segments) containing the first abscission layer between the pulvinus and the petiole were prepared from the primary leaves of the seedlings. The lengths of the

Abbreviation: IAA, indoleacetic acid.

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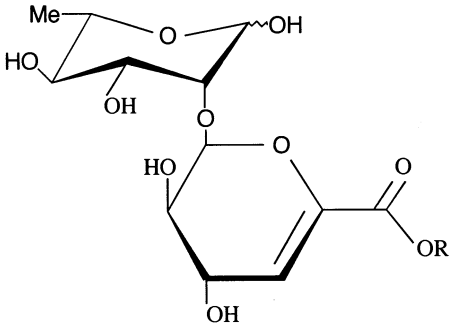

Fig. 1. Chemical structure of lepidimoide. *R* = Na in lepidimoide; R = H in carboxylic acid form.

pulvinus and petiole were 3 and 7 mm, respectively. Sixteen or 10 explants were placed on two layers of Toyo no. 2 filter paper, moistened with 5 mL of the test solution in a sterilized 9-cm Petri dish. The Petri dishes were kept in the light at 25°C in a humid chamber to avoid evaporation.

Abscission was quantitated by application of a constant light pressure to the pulvinar end of the explants, after appropriate incubation periods.

Allof the experiments were repeated at least three times except for the kinetics experiment, which was repeated twice.

Results and Discussion

Abscission of plant parts is considered to be the last and most dramatic phenomenon of plant senescence. In leaves as soon as the petiole explant is prepared, symptoms of senescence appear in the pulvinus, resulting in the onset of the abscission process (Osborne 1973).

Bean petiole explants were incubated in various concentrations of the carboxylic acid form of lepidimoide for 48 and 60 h under constant light (Fig. 2). The carboxylic acid form of lepidimoide at concentrations higher than 1 μM inhibited abscission significantly. After 60 h of incubation about 80% of the explants incubated with water abscised; however, only 30% of the explants treated with 1 mM lepidimoide did so.

To investigate of inhibitory mechanism of abscission, kinetic changes were examined using a 10 μM concentration of the free carboxylic acid form of lepidimoide (Fig. 3). After 60 h of incubation, abscission of the explants treated with lepidimoide was suppressed by about 40% relative to control. However, the lepidimoide-treated explants appeared healthy, and almost complete abscission occurred after 72 h, resulting in parallel kinetic curves of nontreated and treated explants. This fact suggests that lepidimoide-induced inhibition of abscission is due to deceleration of processes leading to abscission.

To study the structural requirement of sodium for the inhibition of abscission, the effect of the sodium salt of lepidimoide was compared with that of the free carboxylic acid form. As shown in Table 1, no significant differences were found between the effects of the two forms of lepidimoide, indicating that sodium is not a structural requirement for inhibition of abscission.

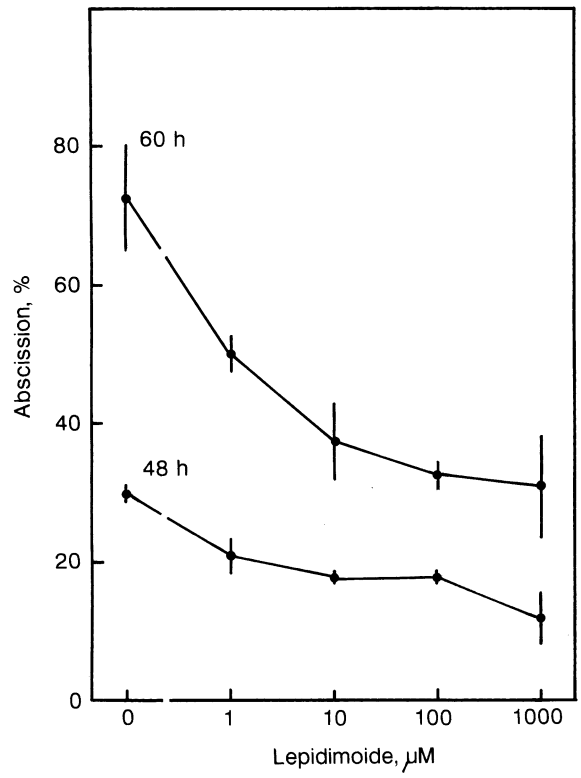

Fig. 2. Effect of lepidimoide on the abscission of bean petiole explant in the light. Bean petiole explants were incubated with or without the carboxylic acid form of lepidimoide for 48 and 60 h in the light.

Auxin has been reported to inhibit abscission strongly in bean petiole explants (Osborne 1968, Reddy et al. 1988, Ueda et al. 1992). As shown in Fig. 3, IAA caused not only dramatic delays in the onset of abscission but also in its progression. This fact suggests that the mode of action of lepidimoide is different from that of IAA.

It is well known that plant growth regulators such as ethylene, abscisic acid, or jasmonic acid promote abscission, but IAA is inhibitory (Osborne 1968, Reddy et al. 1988, Ueda et al. 1991, 1992). In the process of leaf abscission, cellulase is an important enzyme for the degradation of cell wall polysaccharides (Osborne 1973, Ueda et al. 1996). Measurement of cellulase activity and endogenous levels of plant growth regulators in response to lepidimoide applications remain problems to be investigated.

We have already reported that lepidimoide inhibits the loss of total chlorophyll synergistically with cytokinin in excised *Avena* leaf segments during leaf senescence (Miyamoto et al. 1996). Together with the finding that lepidimoide delayed abscission in bean petiole explants, it is suggested that this compound plays an important role in the regulation of leaf senescence.

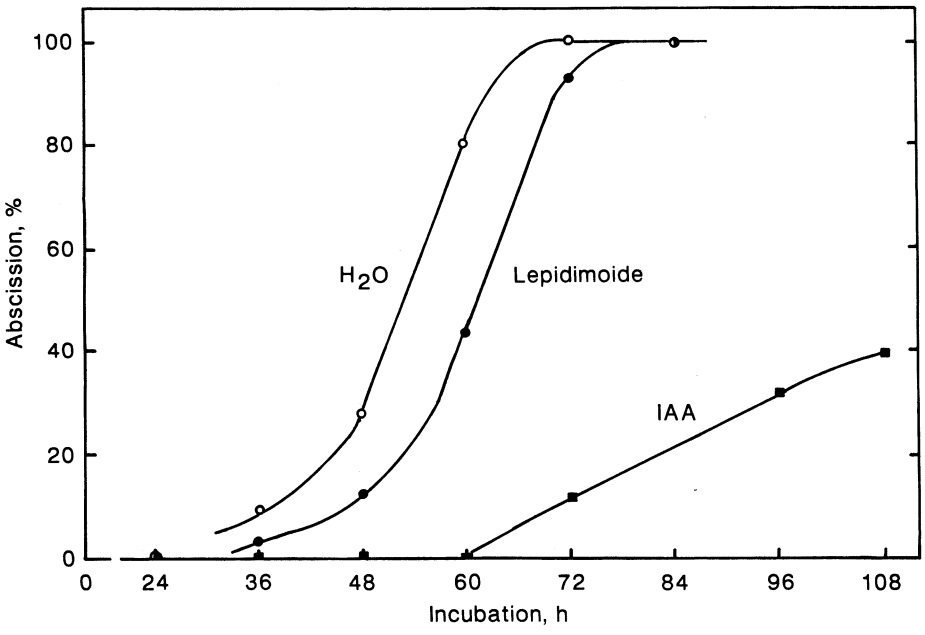

Fig. 3. Effects of lepidimoide and IAA on the abscission of bean petiole explants in the light. Bean petiole explants were incubated with a 10 μM concentration of the carboxylic acid form of lepidimoide or 10 μM IAA.

Table 1. Effect of sodium salt and free form of lepidimoide on abscission of bean petioles. Bean petiole explants were incubated with or without a 100 μM concentration of the sodium salt or free carboxylic acid form of lepidimoide for 48 and 60 h in the light. Values are the mean ± S.E. (n = 3).

	Abcission (%)	
Treatment	48 h	60 h
None	47 ± 7	80 ± 10
Sodium form	25 ± 11	52 ± 6
Carboxylic acid form	33 ± 3	53 ± 7

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