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Minimum Applied Ethylene Concentration as It Affects Seedling Growth in Several Legumes

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The minimum applied ethylene concentrations which have an influence on seedling growth were made clear in six epigeal leguminous species. The lowest ethylene concentration which inhibited hypocotyl elongation (LCe) significantly at 5% level ranged from 0.04 ppm (mung bean) to 0.16 ppm (soybean). On the other hand, the lowest concentration which increased hypocotyl thickness (LCt) significantly at 5% level ranged from 0.04 ppm (mung bean) to 0.38 ppm (soybean). The response of seedling growth to ethylene seemed to be classified into 3 types: 1) LCt was lower than LCe in kidney bean and hyacinth bean, 2) LCt was similar to LCe in mung bean, black gram and cowpea, and 3) LCt was higher than LCe in soybean. Neither LCe nor LCt correlated with seed weight or hypocotyl thickness. However, each LCe and LCt correlated positively with the endogenous ethylene production of a seedling ($r=0.841$ and 0.869, $n=9$, $P<0.01$). The results suggested that, in the seedlings, the high endogenous ethylene production may be discourage the response to applied ethylene.

INTRODUCTION

In some legumes, when a seedling elongation is restricted by soil particles or crust, the seedling produces more ethylene, resulting in both an inhibition of elongation and an increase in stem thickness (Goeschl *et al.*, 1966). As a result, the seedling elongation force increases, enabling seedlings to emerge through the crust (Zheng and Inouye,1989; 1990; Zheng, 1997). In a previous paper (Zheng and Inouye, 1991), we reported that the minimum vertical load which influenced seedling growth and ethylene production ranged from 5 to 160 g among leguminous species. The objective of this report is to make clear the minimum ethylene concentration which has an influence on seedling growth and how it differs among species.

MATERIALS AND METHODS

Nine varieties of six epigeal legume species were used. The seeds of each variety were grown at the experimental field of Kyushu University in 1993. Harvested seeds were stored for about 6 months at 5°C under dry conditions. Uniform seeds (within 10% of mean seed weight) were used for the experiment.

Cultivation of seedlings

Sandy loam soil adjusted for about 13% moisture by weight was used. Ten pregerminated seeds (radicle length: 2–5 mm) were sown in soil contained in a glass tube (10 cm × 7 cm i.d.). The sowing depth was about 2 cm. The glass tube was then moved into an incubator at 25 °C in darkness.

Ethylene application

The glass tube contained ten seedlings (cv. 1.5 cm in length) was sealed in a glass desiccator (cv. 8 liters in volume) with a rubber septum to permit gas sampling. To obtain various concentrations of ethylene, standard ethylene gas was injected into the desiccator through septum with a tight syringe. After air equilibrium was established in the desiccator, each ethylene concentration was determined by gas chromatograph (GC-12A, Shimadzu) with a flame ionization detector. Ethylene was separated on a 3 mm i.d.×2 m stainless steel column packed with Porapak Q operated isothermally at 110 °C, and using nitrogen as the carrier gas. The limit of detection of this operation was found to be 0.01 ppm. The abovementioned operation was performed quickly under dim green light. Forty-eight hours after ethylene application, the seedlings were taken out of the desiccator, and the hypocotyl length and diameters (short and long) were measured. A t-test was performed to check the significance between the plots of different concentrations of ethylene.

RESULTS AND DISCUSSION

The effects of ethylene concentrations on seedling growth were shown in Fig. 1. A decrease in hypocotyl elongation and an increase in hypocotyl thickness (cross sectional area) were accompanied by an increase in ethylene concentration in all the species. The lowest concentration which inhibited hypocotyl elongation (LCe) significantly at 5% level compared to the control was 0.04 ppm in mung bean, followed by 0.07 ppm in black gram and cowpea, 0.07 to 0.09 ppm in kidney bean, 0.10 ppm in hyacinth bean, and 0.12 to 0.16 ppm in soybean. On the other hand, the lowest concentration which increased hypocotyl thickness (LCt) significantly at 5% level compared to the control was 0.04 ppm in mung bean, followed by 0.05 ppm in hyacinth bean, 0.05 to 0.07 ppm in kidney bean, 0.07 ppm in black gram, 0.07 to 0.09 ppm in cowpea, and 0.19 to 0.38 ppm in soybean. The response of seedling growth to ethylene seemed to be classified into 3 types: 1) LCt was lower than LCe in kidney bean and hyacinth bean, 2) LCt was similar to LCe in mung bean, black gram and cowpea, and 3) LCt was higher than LCe in soybean.

In the previous report (Zheng and Inouye, 1991), the minimum vertical load which inhibited hypocotyl elongation significantly compared to the control was less in seedlings of smaller seed size and thinner hypocotyls. However, in this experiment, neither LCe nor LCt correlated with seed weight, hypocotyl thickness or minimum vertical load. It is reported that ethylene production by seedlings was lower in mung bean and cowpea than in soybean (Zheng and Inouye, 1990). In this experiment, both LCe and LCt were lower in mung bean than in soybean. Furthermore, each LCe and LCt correlated positively with the endogenous ethylene production of a seedling (Fig. 2). Though the mechanism of absorbing applied ethylene into the seedling is not well known, if it depends on natural gaseous diffusion, the differences in both LCe and LCt among species could be considered due to endogenous ethylene concentration in the hypocotyl tissue. At the end of this experiment, no ethylene was detected in any of the control lots except one soybean variety (0.039 ppm). Accordingly, it seems that, in the legume species used, evolved

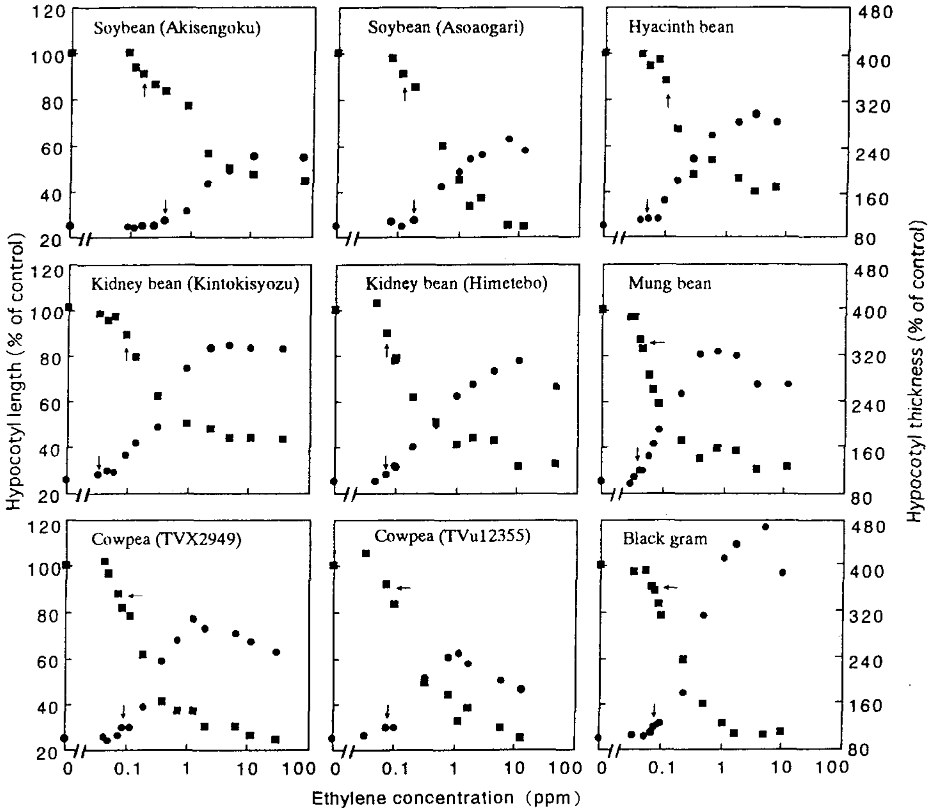

Fig. 1. Response of the seedling growth to applied ethylene concentration.
■: Hypocotyl length ●: Hypocotyl thickness
The arrows indicate the minimum ethylene concentrations which inhibited the hypocotyl elongation and increased the hypocotyl thickness significantly at 5% level.

ethylene from a seedling does not have influence on the seedling growth.

Moreover, the maximum effective applied ethylene concentrations were shown in table 1. The concentration which most inhibited hypocotyl elongation (MCe) was 1.7 ppm (black gram) to 11.6 ppm (cowpea), and the concentration which most enhanced hypocotyl thickness (MCt) was 0.8 ppm (mung bean) to 11.1 ppm (kidney bean). No significant relationships were found between LCe and MCe, or between LCt and MCt. However, for all species, the hypocotyl length was about 25% (soybean) to 40% (kidney bean) of control in MCe, whereas hypocotyl thickness was about 220% (soybean) to 460% (black gram) of control in MCt. It is suggested that the hypocotyl thickening mechanism induced by ethylene clearly differs among species. Cell wall structure may be a limiting factor since the inhibition of elongation and the increase of hypocotyl thickness by physical stress are due to individual cell elongation and thickening (Zheng *et. al.*, 1993). However, to test the above assertion, further physiological studies are needed.

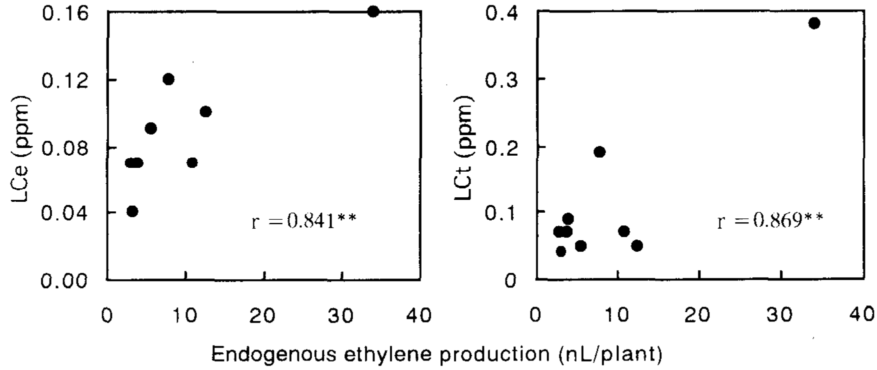

Fig. 2. Relationships among endogenous ethylene production, LCe and LCt.
LCe: The lowest ethylene concentration which inhibited hypocotyl elongation significantly.
LCt: The lowest ethylene concentration which increased hypocotyl thickness significantly.
The measurement of endogenous ethylene is delailed in Zheng and Inouye (1989).
**: Significant at 1% level.

Table 1. The maximum effective applied ethylene concentration and its effects on hypocotyl growth.

Species	Variety	Maximum Inhibition of hypocotyl elongation		Maximum enhancement of hypocotyl thickness	
		Concentration (ppm)	Hypocoly length (% Of control)	Concentration (ppm)	Hypocotyl thickness (% Of control)
Soybean	Akisengoku	10.8	42	10.8	221
	Asoaogari	6.6	25	6.6	252
Kidney bean	Himetebo	11.1	31	11.1	310
	Kintokisyozu	5.0	44	5.0	339
Cowpea	TVu12355	5.9	30	1.2	249
	TVX2949	11.6	26	1.3	309
Mung bean	Bundomame	3.6	31	0.8	328
Black gram	*Acc. 3061*	1.7	27	5.0	456
Hyacinth bean	Akabana	7.1	41	3.0	294

It was confirmed that ethylene is also evolved from soil (Smith and Russell, 1969; Smith and Restall, 1971). According to Otani and Ae (1993), the ethylene concentration in the soil by direct measurement over the seasons ranged from 0.03 to 0.38 ppm with peak levels during rainy summer. The value of ethylene concentration in the soil was higher than the LCe and LCt in some sensitive species. These results seem to indicate the possibility that the seedling growth of these species during the emerging process may be affected by the ethylene in the soil. Therefore, it is suggested that the concentration of ethylene in soil may also play an important role in seedling emergence under soil-crust conditions.

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