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Effect of LAB 173 711, an ABA Analogue, on Low-Temperature Resistance of Mung Bean Seedlings

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Abstract. The effect of LAB 173 711, a synthetic analogue of abscisic acid, has been evaluated on chilling-sensitive mung bean (*Vigna radiata* L. cv. Local V.) seedlings. Electrical conductivity was used for assessing the degree of chilling injury. Exposure of 8-day-old mung bean seedlings to 4°C for 35 h resulted in a 50% electrolyte leakage and induced irreversible chilling injury. The seedlings gained the best protection against chilling injury by pretreatment with LAB 173 711 (5×10^{-4} M) for 3 days. The protection effect could be sustained for 4 days. The LAB 173 711 pretreatment at 28°C did not cause a significant difference in the electrolyte leakage over the ambient temperature (28°C) control. Application of LAB 173 711 at 28°C reduced visible injury and the treated seedlings had higher ethylene production and respiration rate over the untreated control. LAB 173 711 helped maintain the integrity of the cell membrane and thus reduced the leakage of soluble sugar and amino acids. These combined effects led to a higher chilling tolerance in the mung bean seedlings.

The level of ABA has been previously found to increase in plants under a low-temperature stress (Rikin and Richmond 1976, Rikin et al. 1975). The protective effect of exogenous application of ABA in reducing low-temperature injury has been confirmed by subsequent studies (Rikin et al. 1975, Robertson and Gusta 1986, Keith and Mackersie 1986). Practical application of ABA or ABA analogues in agricultural production is currently limited. This is due to its relatively complicated synthesis and rapid metabolic deactivation. A pressing demand therefore requires synthesis of an ABA analogue as a protectant against chilling and freezing damage in agriculture. Many efforts have failed up to now owing primarily to low compound activity. A new ABA analogue, LAB 173 711, has recently been synthesized (Jung and Grossmann 1985). The dienoic side chain of ABA in this compound was replaced by a conjugated alkene-alkyne unit; the carboxyl group was substituted by an acetal function; and the 4′-keto group in the cyclohexenyl ring was replaced by a cyclic ketal. The biological activity in diverse physiological processes has been investigated by using technical preparations of this compound. Inhibition of seed germination and growth, promotion of abscission, and induction of stomatal closure have previously been reported (Grossmann and Jung 1984). The biological activity of LAB 173 711 is similar to that of the natural hormone (Jung and Grossmann 1985, Flores et al. 1988, Flores and Dörffling 1990); its effective duration, however, seems to be longer than that of ABA.

Examining the response of mung bean seedlings under low-temperature stress, as well as the effect and mechanism of LAB 173 711 as a chilling-protectant, are the primary goals of this investigation.

Materials and Methods

Mung bean (*Vigna radiata* L. cv. Local V.) seeds were obtained from Asian Vegetable Research and Development Center (AVRDC) (Tainan, Taiwan). Seeds were germinated after soaking in running water for 2 h in vermiculite in a growth chamber (28 ± 1°C; relative humidity, 80 ± 5%; photoperiod, 12 h; light intensity, 150 $\mu E\ m^{-2}\ s^{-1}$). After 5 days of germination, the first pair of leaves was fully expanded. Seedlings with uniform growth were chosen for the following treatments:

(1) Maintained at 28°C for 3 days, then 28°C for 2 days as ambient temperature control. Referred to as 28°C.
(2) Received LAB 173 711 treatment and maintained at 28°C for 3 days, then brought to 4°C for an additional 2 days as chilling treatment. Referred to as LAB 173 711 + 4°C.
(3) Identical to conditions as in (2), except that LAB 173 711 treatment was omitted. Referred to as 4°C.

LAB 173 711 (5-[2-6,6-trimethyl-1-hydroxy-4-(propylene-1,2-dioxy)-cyclohex-2-en-l-yl]-3-methylpent-2-en-4-in-1-al-dimethyl-acetal) used in this investigation was a gift from BASF (Limburgerhof, Germany).

For LAB 173 711 treatment, the leaves were sprayed until runoff of the test solutions (1×10^{-6} M to 5×10^{-3} M) occurred. All treatments took place with six replications, and each treatment was repeated at least three times.

Evaluation of Low-Temperature Injuries

The Leakage of Electrolytes. The method previously described by Sukumaran and Weiser (1972) was used for the measurement of electrolyte leakage of the leaves. Segments of 0.5 g in fresh weight of excised first leaf pair were put into a 25 ml plastic cup containing 10 ml double-distilled water, and incubated with shaking (Yamato, Water Bath Incubator Model BT-25) at 120 strokes/min for 2 h. Afterward, the first conductance reading was taken with a conductivity meter (Suntex Model SC-17A, Taiwan). The segments were then frozen at −70°C for 12 h, thawed again to leak out all electrolytes, and the conductivity was measured once more. The ratio of conductivity before and after freezing was used for assessing the percent of leakage.

Cell Sap Assay. Cell sap was obtained by filtering the frozen and thawed leaf segments without homogenization through a 100 mesh/in^2 stainless net. The filtrate was used for the following assays.

Free amino acid contents were measured with a ninhydrin reagent (Moore and Stain 1954), using L-leucine as a standard.

Soluble sugar contents of the cell sap were determined by the Anthrone method (Morris 1948), using glucose as a standard.

Determination of Respiration. A Clark type oxygen electrode (model 10, Rank Brother, England) was employed for the determination of leaf respiration rate. An aliquot of 0.2 g leaf segments was placed in an oxygraph incubation chamber containing 5 ml buffer (50 mM KH_2PO_4, pH 5.8). All measurements were conducted at 28°C with three replications. The rate of oxygen consumption was calculated on a fresh weight basis and expressed as nmol 0_2/g · min.

Ethylene Determination. Leaf segments of 0.5 g were collected and immediately sealed into 5.4 ml serum tubes with serum caps for 2 h. Gas samples from each tube were taken with a gas-tight hypodermic syringe and analyzed for ethylene concentration with a Shimadzu GC-14A gas chromatograph (with an alumina column packing; 3.0 mm I/D × 2.5 m stainless steel) fitted with a flame ionization detector. Pure ethylene was used as a standard.

Results and Discussion

Response of First Leaf to Chilling Temperature

The percent leakage of the first leaf of 5-day-old seedlings was stable (4 ± 1%) under an ambient temperature of 28°C for up to 168 h. A comparison of seedlings of two different ages, receiving 4°C treatment for 0–168 h, revealed that they had different chilling sensitivity. Eight-day-old seedlings reached 50% electrolyte leakage in 35 h, which was significantly faster than that of 80 h for 5-day-old seedlings (Fig. 1).

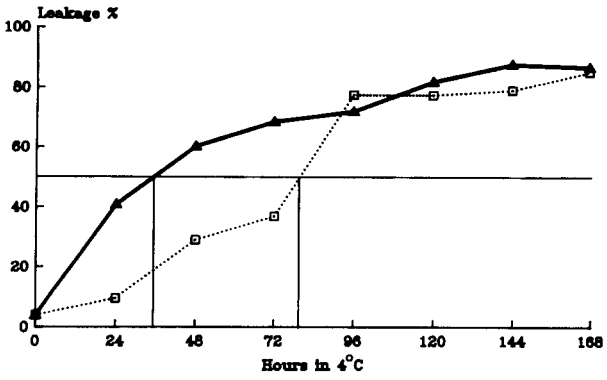

Fig. 1. Development of chilling injury in mung bean seedlings with time of incubation at 4°C. Five-day-old (□) and 8-day-old (▲) mung bean seedlings at the onset of chilling treatment were used in this experiment.

The percent leakage of 8-day-old mung bean seedlings under a 4°C low-temperature stress for 24 h was four times greater than that of 5-day-old seedlings. No visible symptom of chilling injury was observed. Thus, the percent leakage of electrolytes may be regarded as an early symptom of chilling injury. The sensitivity to chilling stress increased with the seedling age. Eight-day-old seedlings have therefore been adopted for all experiments throughout this report.

Effect of LAB 173 711 on Electrolyte Leakage of Chilled Leaf Tissues

The effect of LAB 173 711 pretreatment on membrane stability of leaf tissue of mung bean seedlings subjected to 4°C is shown in Fig. 2. Treatment at 4°C induced strong electrolyte leakage; LAB 173 711 pretreatment at 10°C remarkably reduced electrolyte leakage. The reduction of electrolyte leakage owing to LAB 173 711 pretreatment was proportionally correlated with the dosage applied. Application of 5×10^{-4} M LAB 173 711 3 days before exposure to 4°C for 2 days resulted in a 86% reduction in electrolyte leakage, as compared to control or 10°C pretreatment. Treatment with 5×10^{-4} M LAB 173 711 has therefore been adopted throughout this investigation. Following 4°C chilling stress, the increase in leakage was accompanied by an apparent visual injury (i.e., less turgid appearance, wilted leaves, and stunted growth). No visible dif-

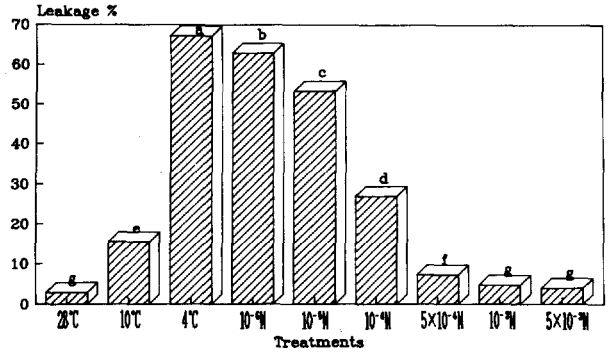

Fig. 2. A comparison of 10°C acclimation and LAB 173 711 pretreatment at different concentrations on the percent leakage of mung bean seedlings at 4°C for 2 days. Columns with a common letter are not significantly different. Mean separation by least significant difference test (LSD) at 5% level.

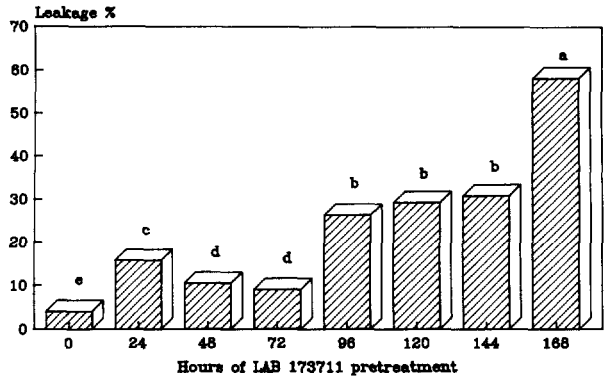

Fig. 3. Effect of LAB 173 711 (5×10^{-4} M) pretreatment of different duration on percent leakage of seedlings chilled subsequently at 4°C for 2 days. Columns with a common letter are not significantly different. Mean separation by least significant difference test (LSD) at 1% level. The "zero-hour" bar gives percent leakage of a high-temperature (28°C) control.

ference was observed in the morphology of the seedlings treated with LAB 173 711 at 5×10^{-4} M and exposed to 4°C, as compared to that of control, with the exception that treated plants were shorter. These results indicate that LAB 173 711 has maintained membrane stability in chilled mung bean leaves and has consequently minimized leakage of electrolytes.

Pretreatment with LAB 173 711 (5×10^{-4} M) at 28°C did not cause a significant difference in electrolyte leakage as compared to that of ambient temperature (28°C) control (data not shown). With application of LAB 173 711 at 5×10^{-4} M, the protective effect against membrane leakage was sustained at a level below 20% for up to 72 h. It was maintained at approximately 30% for the next 3 days (Fig. 3), and then increased drastically after 6 days.

Respiration

The effect of chilling and LAB 173 711 pretreatment on respiration was assessed. At 4°C, LAB 173 711 pretreatment caused a higher (180.7 O_2 nmol/min/g · fw) respiration rate over the untreated control at 28°C (118.8 O_2 nmol/min/g · fw) and the chilled control (77.3 O_2 nmol/min/g · fw). In a separate experiment, the plants received LAB 173 711 pretreatment for 3 days. Their respiration rate reached 96.7 O_2 nmol/min/g · fw, which means a 50% increase over the 28°C control (64.7 O_2 nmol/min/g · fw). ABA has previously been reported to enhance the respiration rate in potato (Hourmant and Penot 1979) and in *Lemna* (Tillberg et al. 1981). The ABA level increased linearly with respiration rate in ripening grapefruit (Niimi and Torikata 1979). ABA has been reported also to have enhanced respiration rate and NADP-specific isocitrate dehydrogenase activity in mitochondria which had been previously isolated from castor bean cotyledons (Tezuka et al. 1990). Higher metabolic activity was indicated to be present as a result of enhancement of oxygen consumption.

Ethylene Production

The effect of chilling and LAB 173 711 pretreatment on ethylene production was also assessed. Seedlings which received LAB 173 711 pretreatment alone or in combination with a chilling treatment had a higher ethylene production rate over the control at 28°C. Ethylene has been considered as stress hormone. Chilling has been shown to inhibit the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. ACC was therefore found to accumulate upon returning to an ambient temperature, and a surge of ethylene evolution occurred (Wang and Adams 1982, Etani and Yoshida 1987). In our study, plants receiving LAB 173 711 pretreatment for 3 days with or without chilling obtained a higher ethylene release rate of 0.012 nmol O_2/g · fw/h over the 28°C control (0.009 nmol O_2/g · fw/h). This is an indication that the treated plant had the capacity to sustain chilling stress.

Effect of LAB 173 711 on Leakage of Soluble Carbohydrates and Free Amino Acids

The effect of LAB 173 711 on leakage of soluble carbohydrates and free amino acids is shown in Table 1. In plants chilled at 4°C for 2 days, the leakage

Table 1. Leakage of soluble sugars and free amino acids from leaves of mung bean sprayed with LAB 173 711 and chilled at 4°C for 2 days, and without LAB 173 711 pretreatment.

Treatments	Soluble sugars (μg/g · fw)	Free amino acids (μg/g · fw)
28°C	11.0 ± 1.4[b]*	28.5 ± 2.1[b]
LAB 173 711 + 4°C	28.4 ± 2.4[b]	28.7 ± 2.9[b]
4°C	608.4 ± 14.8[a]	69.3 ± 4.3[a]

* Mean (X ± SE). Means followed by the same letter are not significantly different by Duncan's mean separation test at the 1% level (N = 10).

of soluble carbohydrate increased 55.3-fold and that of free amino acids increased 2.4-fold over the 28°C control. In plants pretreated with LAB 173 711, the level of soluble carbohydrates and free amino acids remained constant as in the 28°C control during the period observed. Soluble carbohydrates seem to be among the first components that leak out of the cell during or after chilling treatment. LAB 173 711 maintained membrane stability in chilled mung bean leaves and consequently minimized leakage of soluble carbohydrates and free amino acids. An increase of the level of soluble carbohydrates has often been observed during the process of acclimation (Chen et al. 1992). Sugars have been previously postulated to increase the osmotic molarity that leads to an increase of chilling resistance (King et al. 1988, Trudel and Gosselin 1982). Accumulation of free amino acids has a similar effect. Proline level increased from 2–4% to 60% over the control in cabbage leaves during the process of acclimation (Crawford 1989).

The low level of soluble carbohydrates and free amino acids in the leaf segment suspension of LAB-treated plants indicates that the solutes remained within the cells. This may also contribute to a higher chilling tolerance.

Plant water balance under chilling stress is regulated by both membrane stability and stomata opening. Foliar spray or application via the root system of the ABA analogue reduced transpiration of intact barley leaves (Jung and Grossmann 1985). Both ABA and the ABA analogues caused a decrease in stomatal pore width (Rademacher et al. 1987, Schubert et al. 1991). Application of LAB 173 711 at 10^{-6} M reduced water consumption of tomato plants by approximately 30% (Rademacher et al. 1987). Whether application of LAB 173 711 induces an increase in the level of endogenous ABA in mung bean warrants further investigation.

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