

Mitigation of Ethylene-promoted Leaf Senescence by a Natural Lipid, Lysophosphatidylethanolamine

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Abstract. Mitigation of ethylene promoted leaf senescence by lysophosphatidylethanolamine (LPE) was studied. Micropropagated ‘Russet Burbank’ potato (*Solanum tuberosum* L.) plantlets were grown on MS media in sterile culture tubes. After 2 weeks of growth, tubes were sealed and ethylene gas was applied to obtain 5 nL·L⁻¹ final concentration in the culture tubes. Observations and measurements were taken two weeks after ethylene injection. Potato plantlets treated with ethylene showed severe leaf senescence symptoms such as epinasty, lack of growth, yellowing and axillary shoot formation. These observations indicate that apical dominance has been lost with ethylene treatment. The same experiment was repeated with different concentrations of LPE in the MS medium. Inclusion of 50 or 100 mg·L⁻¹ of LPE in the medium mitigated the damage normally caused by applied ethylene. Leaves of plantlets exposed simultaneously to LPE and ethylene had significantly higher chlorophyll content and more healthy leaves compared to plantlets grown on medium lacking LPE. Results of this study suggest that LPE may have the potential to retard ethylene-promoted leaf senescence and may mitigate ethylene induced loss in apical dominance of micropropagated potato plantlets.

Lipids are known to be important in membrane structure and energy balance. It is now evident that lipids and lipid-derived metabolites also play a role in critical cellular processes. Studies have shown that membrane lipid-based signaling mediated by phospholipases, such as phospholipase A₂ (PLA₂), phospholipase C (PLC), and phospholipase D (PLD) and their products constitutes a crucial step in plant growth and development (Exton, 1994; Lis-covitch and Cantley, 1994; Nishizuka, 1992; Scherer, 1996; Ryu et al., 1997; Wang, 1999). Substances classified among lipids (vitamins and their precursors, and a number of hormones) have intense biological activity. For example, PLA₂-derived lysophospholipids were found to act as growth regulators that retard senescence of plant tissues (Frag and Palta, 1993; Ryu et al., 1997; Kaur and Palta, 1997; Ozgen et al., 2005). Interestingly, PLA₂ products such as lysophosphatidylethanolamine (LPE) inhibited the activity of PLD (Ryu et al., 1997), a key enzyme thought to be responsible for membrane lipid breakdown leading to plant senescence (Ryu et al., 1997; Thompson, 1988). Ryu et al. (1997) concluded that lysophospholipids such as LPE serve as signaling molecule in retarding plant senescence. Foliar applications of LPE have also been found to retard senescence of intact or detached leaves, flowers, and fruits (Frag and

Palta, 1993; Kaur and Palta, 1997; Ozgen et al., 2005). Similarly, Ozgen and Palta (2002) found that LPE can protect cranberry fruit and foliage from injury by chlorothalonil-based fungicides. To date, no reports are available regarding the effects of LPE application through the root system, particularly in micropropagated plants.

In a two-part study, we investigated the potential retardation of ethylene-promoted senescence by LPE using a model system of micropropagated potato plantlets. We aimed to stimulate leaf senescence through exposure to exogenous ethylene and to explore whether LPE in the rooting medium would affect plant response to ethylene. The role of ethylene in inducing leaf senescence has been well documented in many studies (Gepstein and Thimman, 1987; Lewington et al., 1967). Ethylene accelerates senescence in the leaves of most species and promotes processes characteristic of leaf senescence, such as decline in chlorophyll and photosynthesis, reduction in the levels of proteins and starch, and increase in the activities of many hydrolytic enzymes (Abeles et al., 1992; Ferguson and Simon, 1973; Mattoo and Aharoni, 1988). Obvious symptoms of ethylene induced leaf senescence include malformed leaves, thickened stems and leaves, lack of growth (stunting), and epinasty (curling and bending downward of leaves). This study marks the first attempt to explore the inhibitory effects of LPE absorbed through the root system on ethylene action.

Materials and Methods

Plant material, medium, and growth conditions. MS media was prepared according to

Murashige and Skoog (1962), with pH adjusted to 5.6 ± 0.02. Test tubes were then filled with 10 mL of media and capped before autoclaving at 132 °C for 15 min. LPE containing culture tubes were prepared by adding sterile LPE solutions with three different concentrations 0, 25, 50 and 100 mg·L⁻¹ when the medium had cooled to about 70 °C. Stem cuttings of potato plantlets were then, micropropagated in these culture tubes. The 2nd and 3rd nodes of one month old micropropagated ‘Russet Burbank’ potato (*Solanum tuberosum* L.) plantlets grown on MS medium were excised as single node cuttings and returned to sterile culture tubes (20 × 150 mm, Pyrex no. 9820) containing MS medium with or without LPE.

LPE derived from egg lecithin was used in our experiments and was obtained from Doosan Serdary Research Lab (Englewood, N.J.). LPE was suspended in water by using a sonicator (Sonic Dismembrator model 550; Fisher Scientific, Pittsburgh, Pa.).

Leaf senescence was stimulated by the treatment with exogenous ethylene after 2 weeks of growth. Tubes were sealed and ethylene gas was applied to obtain 5 nL·L⁻¹ final concentration in the culture tubes. The study was conducted in two steps: In step 1, the damaging effects of ethylene on the plantlets were observed to confirm that the expected action of ethylene was evident in our system. In step 2, the potential retardation of leaf senescence by different concentrations of LPE was assessed. Observations were taken on axillary bud growth, leaf chlorophyll content and number of healthy leaves 2 weeks after ethylene injection.

Cultures were arranged in a randomized complete block design under continuous light with about 60 μmol·m⁻²·s⁻¹ photosynthetic photon flux from cool white fluorescent lamps. Each treatment consisted of 15 plantlets. Experiments were repeated three times and similar results were obtained. Results from one experiment are summarized here.

Chlorophyll determination. Leaf disk samples from five plantlets in each of three replications were collected for chlorophyll extraction. Samples were incubated in vials containing 10 mL of absolute methanol. Vials were continuously shaken in a covered water bath at ambient temperature to facilitate chlorophyll extraction. Absorbance was read at 650 and 665 nm and chlorophyll concentration was calculated (25.5 [A₆₅₀] + 4.0 [A₆₆₅]) as mg chlorophyll/g fresh weight (Crafts-Brandner et al., 1984; Holden, 1976).

Data collection. In step 1 of the experiment, micropropagated potato plantlets grown on regular MS media were incubated in 0 and 5 nL·L⁻¹ ethylene concentrations. The number of axillary buds and healthy (no epinastic appearance) leaves were counted after two weeks of incubation. In stage 2 of the experiment, single node cuttings of potato plantlets were grown on different concentrations of LPE containing media. After two weeks of growth, these plantlets were incubated in 5 nL·L⁻¹ ethylene concentrations. Two weeks later, the number of axillary shoots and healthy leaves were counted and chlorophyll content of leaves was determined.

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Table 1. Influence of ethylene, on micropropagated potato plantlets. Tubes were injected with either 5 nL·L⁻¹ ethylene or air. The number of axillary shoots and healthy leaves for each plantlet were counted 2 weeks after injection.

Treatment	Axillary shoots (no.)	Healthy leaves (no.)	Healthy leaves (%)
Untreated control	0.87	6.93	92.6
Ethylene	3.67	2.80	39.8
Significance	**	**	**

**Significantly different from control at $P \leq 0.01$ by *t* test.

Table 2. Influence of LPE on ethylene promoted senescence of micropropagated potato plantlets. Tubes were injected 5 nL·L⁻¹ ethylene and axillary shoots, leaf growth and chlorophyll content measurements were taken 2 weeks after ethylene injection.

LPE concn (mg·L ⁻¹)	Axillary shoots (no.)	Healthy leaves (no.)	Chlorophyll concn (mg·g ⁻¹ fresh wt)
0 (with ethylene)	3.53 a ²	2.73 c	0.45 d
25	1.87 b	4.60 b	0.63 c
50	1.07 bc	6.27 ab	0.89 ab
100	1.67 bc	5.80 ab	0.82 b
0 (without ethylene)	0.73 c	6.73 a	1.02 a

²Number of axillary shoots and normal leaves from 15 separate plantlets: chlorophyll data from 3 replications of composite samples of 5 plantlets. Means were compared by Fisher's protected LSD ($P < 0.05$); within each column means followed by the same letter do not differ significantly.

Results

Effect of ethylene on leaf senescence.

Exposure to ethylene in step 1 of the experiment significantly affected potato plantlets in the culture tubes (Table 1). Potato plantlets treated with 5 nL·L⁻¹ ethylene showed severe leaf senescence symptoms including thickened stems and leaves, axillary shoot growth, lack of growth, yellowing and epinasty (curling and bending downward of leaves) after two weeks. About 60% of the leaves of each plantlet were damaged by exposure to ethylene. Ethylene application also resulted in formation of an average of 3.7 axillary shoots per plantlet while untreated control plants had only 0.9 axillary shoots per plantlet.

Effect of LPE on ethylene-promoted leaf senescence. Incorporation of LPE into the rooting medium mitigated ethylene-promoted leaf senescence in micropropagated potato plantlets (Table 2). Plantlets grown in medium containing 50 or 100 mg·L⁻¹ LPE had showed significantly higher chlorophyll content compared to control and plant grown on medium containing 25 mg·L⁻¹ LPE, after 2 weeks of ethylene exposure. Also, these plantlets had significantly more healthy leaves and less axillary shoot formation compared to plantlets grown in medium lacking LPE. Plantlets grown on medium containing 50 mg·L⁻¹ LPE had three times less axillary shoot formation, more than twice the number of healthy leaves and 98% higher chlorophyll content in their leaves than plantlets grown on medium lacking LPE but exposed to ethylene.

Discussion

Application of exogenous ethylene to tubes containing micropropagated potato plantlets had effects that were expected based on literature: epinasty, yellowing and lack of growth. However, potato plantlets grown on medium

containing 50 or 100 mg·L⁻¹ of LPE showed dramatically less damage typical of ethylene exposure. Results from this and previous studies (Farag and Palta, 1993; Kaur and Palta, 1997; Ozgen et al., 2005; Ryu et al., 1997) demonstrate that the protective effect of LPE is concentration dependent. In the current study, concentrations of 50 and 100 mg·L⁻¹ LPE protected against ethylene damage while lower concentrations did not and higher concentrations produced no toxic effects (data not shown). Furthermore ethylene treatment resulted in the formation of axillary shoots (Table 1) suggesting that ethylene, when introduced in the environment of tissue cultured plantlets, can cause a loss in the apical dominance. Our results show that this loss in the apical dominance by ethylene was mitigated by the presence of LPE in the media (Table 2). Similar observations on axillary shoot formation, in tissue cultured plantlets, have been made when shoot apex is injured due to calcium deficiency (Busse et al., 2004; Ozgen and Palta, 2003). Results of the present study suggest that inclusion of LPE in the media to reduce the incidence of axillary shoot growth may have value in the maintenance of healthy shoot cultures.

The exact mode of action of LPE is not known. However, in previous studies, we established that LPE maintains plant cell health by protecting membranes during senescence and aging presumably by regulating PLD activity in a highly specific manner (Ryu et al., 1997). PLD expression levels are positively correlated with the rate of leaf senescence (Ryu and Wang, 1995). Therefore, it is reasonable to speculate that the protective effects of LPE against damage due to ethylene exposure drives from the inhibitory effect of LPE on the PLD enzyme. Results from this study have important implications based in part on the fact that, for the first time, the action of LPE absorbed through the root system has been shown to parallel that of foliar application.

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