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Herbicidal Activity of δ-aminolevulinic Acid on Several Plants as Affected by Application Methods

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ABSTRACT : Herbicidal activity of δ-aminolevulinic acid (ALA), an intermediate for the biosynthesis of tetrapyrroles such as chlorophyll, heme, bacteriochlorophyll, and vitamin B_{12} analogues, was examined to determine the variation in phytotoxic potential against different plant species as affected by different application methods. Seed-soaking treatment, ALA at low concentrations did not affect shoot and root lengths of test plants while at highest concentration reduced them by 20 to 30%. Alfalfa showed the most tolerant response to ALA in both pre- and post-emergence application, and followed by rice. When applied with pre-emergence, cotyledons of Chinese cabbage were severely bleached with 0.5 mM of ALA at 24 hrs after application, and root growth of rice, barnyard grass, and alfalfa was significantly inhibited with increasing of concentration. With post-emergence application, ALA at 2 to 4 mM reduced shoot and root growths of Chinese cabbage and barnyard grass completely. Herbicidal effects of ALA were more enhanced in the treatment combined with 2,2-dipyridyl sthan single application in barnyard grass and Chinese cabbage. The results suggest that alfalfa was the most tolerant to ALA among the tested plants, and that post-emergence application of ALA exhibited greatest photodynamic activity against tested plants.

Keywords : δ-aminolevulinic acid, herbicidal activity, application methods

δ-aminolevulinic acid (ALA) has been well known as an intermediate for the biosynthesis of tetrapyrroles such as chlorophyll, heme, bacteriochlorophyll, and vitamin B_{12} analogues (Sasaki *et al.*, 1987) in human, plants, animals and microorganisms. Recently, ALA has received great attention as new biodegradable pesticides that are inhibitory to weeds or insects but are not harmful to crops, animals, and human (Rebeiz *et al.*, 1984, 1988a and b, and 1990; Sasikala *et al.*, 1994), and as a prodrug for photodynamic diagnosis and therapy of cancer (Mariet *et al.*, 1996; Schuitmaker *et al.*, 1999). In addition, ALA has been reported to promote the growth and yield of rice, corn, kidney bean, potato, garlic and *Vigna* species (Tanaka et al, 1992; Hotta *et al.*, 1997a and b; Roy and Vivekanandan, 1998) at low concentrations, and to improve salt tolerance of cotton seedlings (Watanabe *et al.*, 2000).

ALA was named as tetrapyrrole-dependent photodynamic herbicides (TDPH) that force green plants to accumulate undesirable amount of metabolic intermediates (protoporphyrin IX) of the chlorophyll and heme metabolic pathway in darkness, namely tetrapyrrole (Rebeiz *et al.*, 1990) or as a laser herbicide that is photodynamic (Rebeiz *et al.*, 1984). Under the light, the accumulated tetrapyrroles photosensitize the formation of singlet oxygen that kills the treated plants by oxidation of their cellular membranes as like diphenyl ether (DPE) herbicides. A variety of DPE herbicides such as acifluorfen-methyl, oxadiazone, and oxyfluorfen cause rapid peroxidative photobleaching and desiccation of green plant tissues (Duke *et al.*, 1991; Scalla and Matringe, 1994). The target site of action of these herbicides has been well known to be protoporphyrinogen oxidase (Protox), which catalyzes the oxidation of protoporphyrinogen IX (Protgen IX) to protoporphyrin IX (Proto IX), in the biosynthesis of hemes and chlorophylls (Duke *et al.*, 1991; Beale and Weinstein, 1990).

Development of ALA as a new herbicide involves the joint utilization of δ-aminolevulinic acid and activators of the chlorophyll biosynthetic pathway, such as 2,2'-dipyridyl, in order to induce treated plants to biosynthesize and accumulate massive amounts of tetrapyrrole intermediates of the chlorophyll biosynthetic pathway in the dark. During the subsequent light period the accumulated tetrapyrroles act as potent photodynamic sensitizers, which in turn results in the death of susceptible plants in a matter of hours (Rebeiz *et al.*, 1984).

The present study was conducted to determine biological activity of ALA against rice (*Oryza sativa* L.), alfalfa (*Medicago sativa* L.), Chinese cabbage (*Brassica rapa* L.) and barnyard grass (*Echinochloa crus-galli*, Beauv. var. *oryzicola* Ohwi.) by seed soaking, pre- and post-emergence application, and to know if photodynamic herbicidal damage are enhanced by combined treatment

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with 2,2'-dipyridyl. The fundamental bioassay would be useful for development of ALA as a new bioherbicide that is biodegradable, environmentally sound, and safe to human, animals and crops.

MATERIALS AND METHODS

Experimental Materials and Bioassay

ALA has been known to be produced from photosynthetic bacteria and been purified through bioengineering process. ALA used in this study was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and assayed to determine its phytotoxic effects on early seedling growth of alfalfa (cv. Vernal), barnyard grass, Chinese cabbage (cv. Sinjeonseung) and rice (cv. Dongjin). To determine differences in photodynamic effects of ALA as affected by different application methods, laboratory and greenhouse experiments were conducted. Visual rates of phytotoxic effects, plant height, root length and fresh weight were measured as needed. Data collected were transformed to percent of control for analysis. The experiments were duplicated, each with three replications.

Pretreatment of Seeds with ALA

Seeds of rice, barnyard grass, alfalfa and Chinese cabbage were soaked in varying aqueous solutions of ALA (0, 2, 4, 6, and 8 mM) in darkness for 12 hrs at 22°C, washed in tap water, and placed on filter paper wetted with distilled water in petri dishes. The petri plates were transferred in growth chamber held at 25°C during the 14-h light period and 22°C during dark period. The plates were illuminated with 180 mol photons $m^{-2}s^{-1}$ photosynthetically active radiation (PAR). Plant height and root length of test plants were measured on all seedlings in each petri-dish at 6 days after seeding. Data were transformed to percentage of control for analysis.

Pre-emergence Application of ALA

ALA stock solution was diluted with distilled water to give final concentration of 0.5, 1, 1.5, and 2 mM. Four milliliters of each diluted solution was pipetted into the petri dishes with Whatman No. 2 filter paper. The distilled water was used as the control. Seeds of alfalfa, barnyard grass, Chinese cabbage, and rice were surface sterilized with 0.525 g L^{-1} sodium hypochlorite for 15 min. Seeds were rinsed four times with deionized water, imbibed in deionized water at 22°C for 12 h, and carefully blotted using a folded paper towel. Twenty swelled seeds of each species were evenly placed on filter paper wetted with ALA solution in each petri dish. The petri dishes were covered, sealed by wrapping with parafilm, and placed flat in a growth chamber maintained at 24°C during the14-h light period and 22°C during the 10 h dark period. Plates were illuminated with 180 μmol photons m^{-2} s^{-1} PAR provided by a mixture of incandescent and fluorescent lamps. Shoot and root length were measured on all seedlings in each petri dish at 6 days after incubation.

Post-emergence Application of ALA

Imbibed seeds of rice, barnyard grass, alfalfa, and Chinese cabbage were seeded in small horticulture pot (10×10×5 cm) filled with silt-loam soil. Germinated seedlings were grown for 12 days under greenhouse conditions. Then ALA at 0, 2, 4, 6, and 8 mM mixed with Tween 80 was foliar applied. A 15 ml of ALA solution was applied with handy sprayer at 6:00 PM. At the time of application, leaf stage of test plants was about 2-leaves. After application, post-spray dark incubation period was kept for 14 hrs, and next morning exposed to the natural sunlight ranged from 1000 to 1500 μmol photons m^{-2} s^{-1} to elicit photodynamic damage. Shoot length, root length and fresh weight were measured on all seedlings 6 days after exposure to sunlight.

Photodynamic Effects of ALA+2,2'-dipyridyl

To know whether 2,2'-dipyridyl as a modulator enhance the photodynamic herbicidal properties of the ALA against 4 plant species at low concentrations, pot test was conducted in greenhouse as above described in post-emergence application. Rice, barnyard grass, alfalfa, and Chinese cabbage grown in greenhouse for 12 days were foliar sprayed with 1 or 2 mM of ALA+2,2'-dipyridyl solution, after which the treated plants were incubated in the dark for 14 hrs in order to induce the accumulation of tetrapyrroles. Controls were also sprayed with the surfactant Tween 80. After 3, 6, 12, and 24 hours of exposure to daylight (approx. 1000 μmol photons m^{-2} s^{-1}), visual evaluation was undertaken. Fresh weight of each plant was measured on all seedlings 6 days after exposure to sunlight.

RESULTS AND DISCUSSION

Pretreatment of Seeds with ALA

To determine the effects of seed pretreatment on early seedling growth of rice, barnyard grass, alfalfa and Chinese cabbage, ALA was applied to seeds by soaking in different solutions. Both plant height and root length of seedlings which had been soaked in lower concentrations of ALA, were not affected significantly. However, ALA with more than 2 mM significantly suppressed the plant height and root length of test plant seedlings (Fig. 1). The results corroborate partly with early conclusions that a foliar application of ALA at low concentrations increased up to 10-60 % over the control on the growth of kidney bean, barley, potato, and garlic (Hotta *et al.*, 1997b). However, ALA effect on seedling growth of test plants depended on the concentration and plant species. The stimulation effects at low concentration as well as the inhibition effects at high concentrations coincided with previous results (Roy and Vivekanandan, 1998; Hotta *et al.*, 1997a and b).

Pre-emergence Application of ALA

Results showed that shoot growth of test plant species was less sensitive to ALA than root growth. Chinese cabbage was the most susceptible plant while alfalfa the most tolerant. Cotyledone, shoot and root of Chinese cabbage treated with ALA in concentration of 0.5 mM were completely bleached within 24 h after light exposure. Shoot lengths of rice and barnyard grass with ALA of 0.5 mM were reduced by 33 and 27%, respectively, while root lengths of rice and barnyard grass by 73 and 70%, respectively, and were significantly inhibited with increase of concentration (Fig. 2). However, in alfalfa 0.5 mM ALA reduced shoot and root length by 20 and 28%, respectively.

Post-emergence Application of ALA

Symptoms of photodynamic injury within the first 1 hour after exposure to light after post-spray dark incubation period for 15 hrs become apparent. Initial symptoms

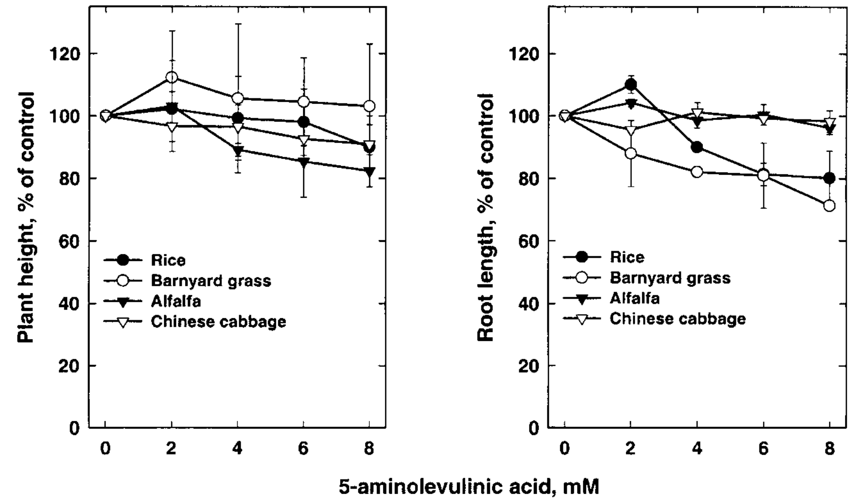

Fig. 1. Effects of pre-seed treatment by soaking with ALA on plant height and root length of rice, barnyard grass, alfalfa, and Chinese cabbage 6 days after seeding on filter paper wetted with distilled water.

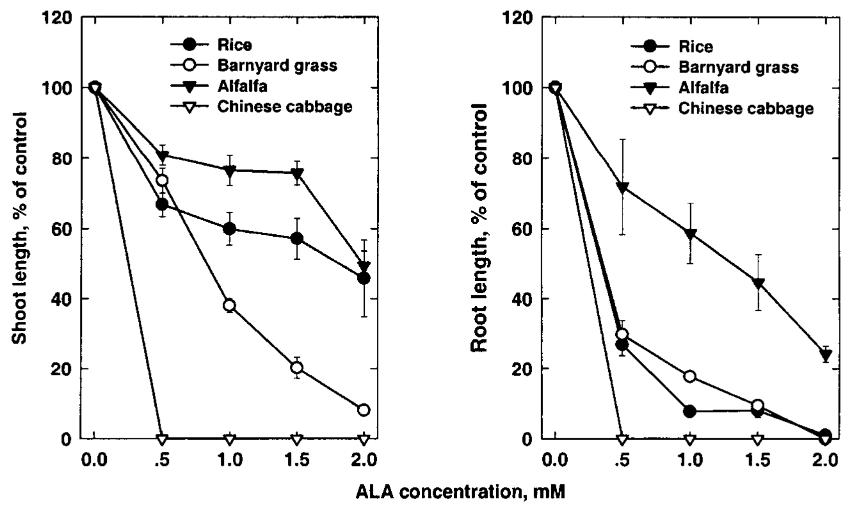

Fig. 2. Effects of pre-emergence application of ALA on plant height and root length of rice, barnyard grass, alfalfa, and Chinese cabba ge 6 days after placing on filter paper wetted with different ALA concentrations.

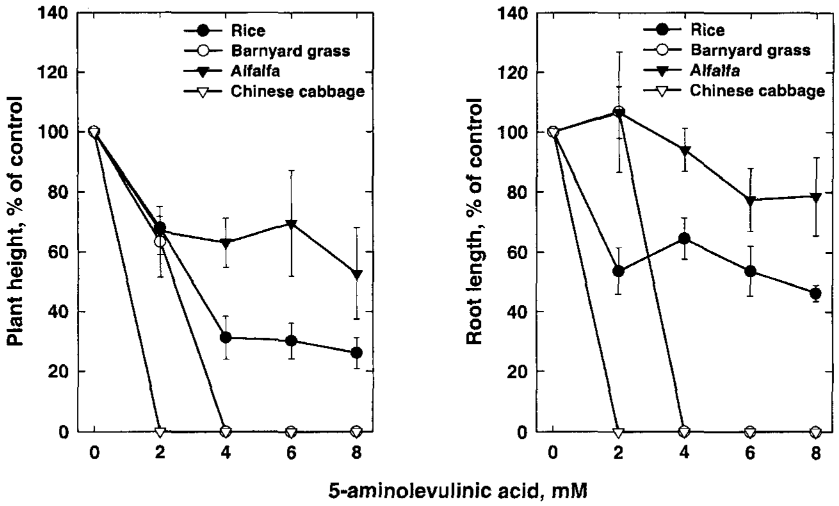

Fig. 3. Effect of post-emergence application of ALA on shoot and root lengths of rice, barnyard grass, alfalfa, and Chinese cabbage 6 d ays after application. The plants were grown in horticultural pots filled with silt-loam soil under greenhouse.

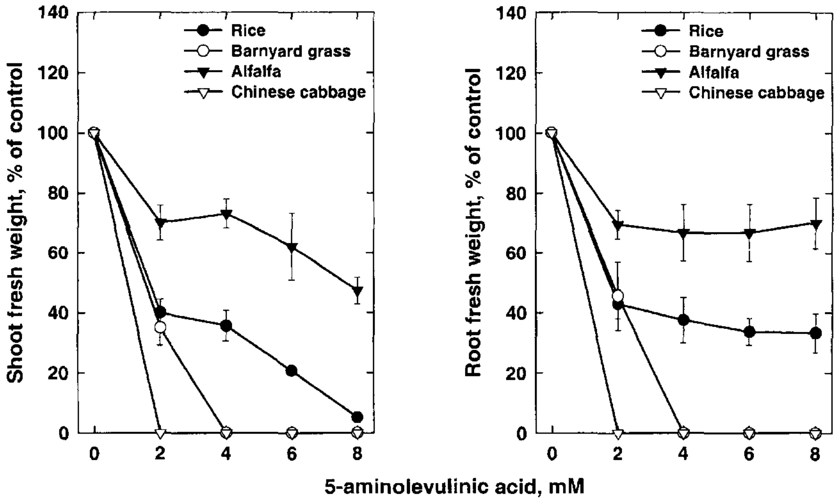

Fig. 4. Effect of post-emergence application of ALA on shoot and root fresh weight of rice, barnyard grass, alfalfa, and Chinese cabbag e 6 days after application. The plants were grown in horticultural pots with silt-loam soil under greenhouse.

appeared on green foliage of susceptible plants as isolated bleached spots contiguous. Bleaching is accompanied by severe loss of turgidity followed by desiccation. Within 24 hrs the green plant tissue turns into a brownish desiccated mass of dead tissue (Data not shown). In Chinese cabbage, 2mM ALA reduced shoot length completely at 6 days after application, in barnyard grass, 4mM ALA by 100%, and in rice and alfalfa 4mM ALA by 69 and 37%, respectively. Root length was less sensitive to ALA than shoot length of test plants (Fig. 3). It was thought that roots applied with pre-emergence were more directly contacted to ALA than seed treatment or post-emergence application. At 4mM ALA, root length of barnyard grass and Chinese cabbage were reduced completely, and at 6 mM that of alfalfa and rice by 33 and 46%, respectively. Fresh weight responses to ALA tended to be similar to plant height and root length. Shoot growth of test plants by ALA was more sensitive to ALA than was root. At 2 mM concentration, ALA significantly reduced shoot and root fresh weight compared with control (Fig. 4). Barnyard grass and Chinese cabbage were more sensitive to ALA than were rice and alfalfa. Rebeiz *et al.* (1983) suggested that photodynamic herbicides exhibit a very pronounced organ, age, and species-dependent selectivity. This contrasts with the conclusion based on findings against other plants (Rebeiz *et al.*, 1984) that dicotyledonous weeds such as mustard, red root pigweed, common purslane and lamsquarter are very susceptible while monocotyledonous plants such as corn, wheat, barley and oats are not.

Photodynamic Effects of ALA+2,2'-dipyridyl

Single treatment of 2,2'-dipyridyl as a synergy agent at

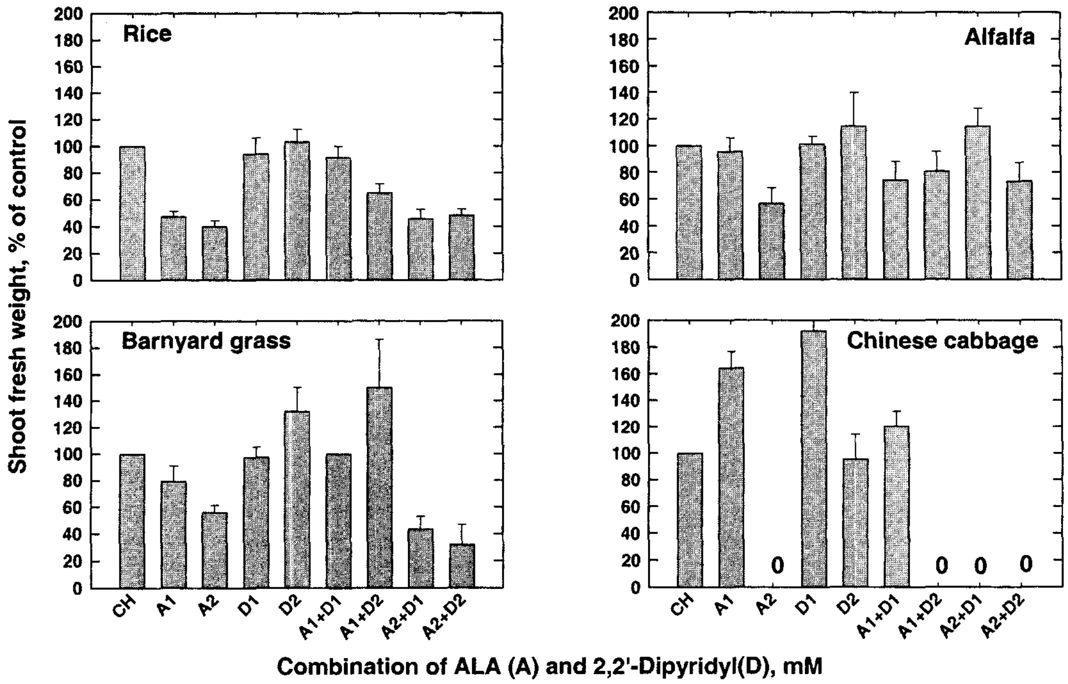

Fig. 5. Effect of combined application of 5-aminolevulinic acid and 2, 2-dipyridyl on fresh shoot weight of rice, barnyard grass, alfal fa, and Chinese cabbage 6 days after application. The plants were grown in horticultural pots filled with silt-loam soil under greenhous e.

both 1 and 2 mM did not affect seedling growth of 4 plant species treated. ALA treatment combined with 2,2'-dipyridyl also did not affect or even increased seedling growth of both rice and alfalfa up to 20%. However, shoot fresh weight of barnyard grass was more reduced by ALA 2mM+2,2'-dipyridyl than single treatment of ALA. ALA 1mM+2,2'-dipyridyl 2 mM and ALA 2 mM+2,2'-dipyridyl 2 mM completely reduced shoot fresh weight of Chinese cabbage (Fig. 5). Our results suggest that combination of ALA and 2,2'-dipyridyl at lower concentrations had the potential to enhance biological activity in susceptible plant species such as barnyard grass or Chinese cabbage through foliar application.

Originally photodynamic herbicides have been known to be nonselective in their mode of action. Rebeiz *et al.*, (1988a), in their laboratory and field experiment, reported that various ALA and modulator combinations exhibited a significant degree of photodynamic herbicidal selectivity. In our study the selectivity among plant species would be based on (a) the different tetrapyrrole accumulating capabilities of various plant tissues, (b) the differential susceptibility of various greening groups of plant to the accumulation of various tetrapyrroles and (c) the differential tetrapyrrole metabolism in various plant tissues (Rebeiz *et al.*, 1988a). Also, our results indicated that both pre- and post-emergence application of ALA exhibited complete photodynamic phytotoxicity, indicating that translocation of ALA from roots or leaves works.

CONCLUSION

In conclusion, ALA effect on early plant growth was greatly concentration dependant, suggesting that it promotes plant growth at very low concentration and inhibits at high concentration. Chinese cabbage and barnyard grass were very susceptible to ALA ranging 2 to 4 mM and alfalfa was very tolerant in both pre- and post-emergence application. ALA at some concentrations may have the potential to control weeds by both pre- and post-emergence application. To maximize herbicidal activity of ALA more researches have to be focused on searching for (a) a promising microorganisms which produces high amount of ALA, (b) an alternative competitive inhibitor which enhances the accumulation of ALA instead of levulinic acid (LA) during biological production of photosynthetic bacteria, and (c) a strong modulator which enhances photodynamic activity of the reduced amount of ALA. Further investigations also needed to determine dependence of photodynamic herbicidal activity of ALA upon the extent of tetrapyrrole accumulation by plant species or various plant tissues, and to confirm the selectivity of ALA between crops and weeds.

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