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Effects of 5-Aminolevulinic Acid on Growth and Inhibition of Various Plant Species

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ABSTRACT: The purpose of this study is to investigate the positive or negative effects of 5-aminolevulinic acid (ALA) on the growth of several crops and weeds, by applying a seed soaking treatment, foliar treatment, and application timing, while comparing biological activity between ALA produced by chemical synthesis (Synthetic-ALA) and extracellularly-accumulated ALA by overexpressing the *hemeA* gene isolated from *Bradyrhizobium japonicum* (Bio-ALA). Seed soaking treatment of ALA in barley (five cultivars) and wheat (five cultivars) had not shown positive effects at lower concentrations, 0.05 to 0.5 mM as well as negative effects at higher concentrations, 1 to 30 mM. In rice, there also was no positive effect by seed soaking treatment of ALA at lower concentrations, although the rice became damaged by an application of 5 and 10 mM ALA. Growth in barley cultivars, Ganghossalbori, Naehanssalbori, Songhakbori, Saessalbori, and Daehossalbori were increased up to 14%, 19-51%, 17-64%, 18-23%, and 22-38% by ALA foliar application at lower concentrations, 0.05 to 0.5 mM, respectively. On the other hand, the growth in barley cultivars was inhibited by ALA foliar application at higher concentrations. Barley responded more positively to ALA foliar application than wheat and rice. The growth stimulation caused by ALA seed soaking treatment was less than by ALA foliar treatment. ALA treatment at the 1.5-leaf stage increased growth of barley by 19-58%, while pretreatment to seeds, post-emergence treatment at 3 days after seeding, 3-leaf stages, and 5-leaf stages had not shown positive effects. Thus, the positive effects of ALA on barley were dependent greatly upon the timing of application and its concentration. Monocots weeds were more sensitive to ALA foliar treatment than dicotyledonous weeds. A monocot weed, *Setaria viridis* L. was the most susceptible plant to ALA while a dicotyledonous weed, *Plantago asiatica* L. was the most tolerant. No significant difference in biological activity between bio-ALA and synthetic ALA on barley, wheat, rice, and weed, *Ixeris dentate* tested was observed. Thus, ALA produced by microorganisms would be a potent substance to be used effectively in agricultural production.

Keywords : 5-Aminolevulinic Acid, barley, herbicidal activity, positive effect, rice, wheat

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5-Aminolevulinic acid (ALA) is a precursor in the biosynthesis of porphyrins such as chlorophyll and heme in animals and plants (Avissar & Moberg, 1995; Granick & Sassa, 1971). ALA formation is the rate limiting step in chlorophyll biosynthesis (Beale, 1990) and very low levels of ALA are maintained *in vivo* (Chereskin & Castelfranco, 1982; Weinstein & Beale, 1985).

ALA promotes the growth and yield of crops and vegetables at low concentrations by 10-60% over the control as observed in cases of crops of radish, kidney beans, barley, potatoes, garlic, rice, *Vigna* species, and corn (Hotta *et al.*, 1997a & b; Roy & Vivekanandan, 1998). These positive effects may be induced through the increased chlorophyll content, enhanced photosynthetic activity, and the inhibition of respiration by ALA treatment (Hotta *et al.*, 1997a). In addition, ALA improves cold resistance in rice seedling (Hotta *et al.*, 1998) and salt tolerance of cotton seedling (Watanable *et al.*, 2000).

In plants treated with ALA at relatively high concentrations, the herbicidal properties of ALA are well documented. In ALA-treated plants, an accumulation of several chlorophyll intermediates such as protoporphyrin , Mg- protoporphyrin , and protochlorophyllide to abnormally high levels have been reported (Castefranco *et al.*, 1974; Matsumoto *et al.*, 1994; Sundquvist, 1969). Excess tetrapyrrole compounds are photosensitized and consequent photodynamic reactions injure plants (Rebeiz *et al.*, 1984). It is assumed that the accumulated tetrapyrroles act as a photosensitizer for the generation of singlet oxygen, which results in peroxidation of membrane lipids (Chakraborty & Tripathy, 1992). Hence, photodynamic compounds have received attention as potential herbicides (Towers & Arnason, 1988), among which the diphenyl ethers have been well studied (Johnson *et al.*, 1978), practically applied, and put to commercial use (Duke *et al.*, 1991). ALA also can be applied as a photodynamic herbicide. Although ALA is harmless for humans and animals (Rebeiz *et al.*, 1984), and as a prodrug for photodynamic diagnosis and therapy of cancer (Schuitmaker *et al.*, 1999), it could not be applied widely as a com-

mercial product because of its low availability and high cost. ALA is expensive because it is synthesized via many complex reactions (Tschudy & Collins, 1959). In contrast, microbiological production of ALA involves simple reactions. Therefore, biological production using microorganisms has been suggested as an inexpensive way to produce ALA. Sasaki *et al.*, (1987) observed the extracellular accumulation of ALA by *Rhodobacter sphaeroides* up to 16 mM at neutral pH with levulinic acid addition using a volatile fatty acids medium. Another approach to achieving ALA production is by metabolic pathway engineering. Extracellular accumulation of ALA by an *E. coli* overexpressing ALA synthase was achieved by inserting a *hemeA* gene from *Bradyhizobium japonicum* and expressed under the control of T7 promoter (Choi *et al.*, 1999). ALA production by this method was 30 mM. The object of the present study is to investigate positive or negative effects of ALA on the growth of several crops and weeds by using seed soaking treatment, foliar treatment, and application timing, and to compare biological activity between ALA produced by chemical synthesis and extracellular accumulation of ALA by overexpressing the *hemeA* gene isolated from *Bradyrhizobium japonicum*.

MATERIALS AND METHODS

Chemicals

ALA produced by chemical synthesis (synthetic-ALA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and ALA produced by overexpressing the *hemeA* gene isolated from *Bradyrhizobium japonicum* (Choi *et al.*, 1999) was supported from Environgen Co., Korea (Bio-ALA).

Seed soaking treatment with ALA

Seeds of barley (cv. Ganghossalbori, Naehanssalbori, Songhakbori, Saessalbori, Daehossalbori) and wheat (cv. Geumgangmil, Geurumil, Urimil, Olmil, Donghaemil), and rice (cv. Dongjinbyeo) were soaked in various aqueous solutions of ALA (0, 0.001, 0.005, 0.01, 00.5, 0.1, 0.5, 1, 5, 10, and 30 mM) in darkness for 12 hrs at 25°C. Seeds of rice were soaked in water for 3 days at 25°C before ALA soaking treatment. The seeds were washed with distilled water and sowed 15 seeds in pots (200 cm^2) containing commercial potting mix (peat moss, vermiculite, and zeolite) soil substrate (Boo-Nong Soil, Seoul Korea). The pots were transferred to a greenhouse. The greenhouse was maintained at 25°C day/15°C night temperature and 10 hrs photoperiod. Seedlings were watered daily. Plant height and shoot fresh weight were measured 10 days after application. Data collected was transformed to percent of control and was analyzed using a variance (ANOVA) procedure in SAS program (SAS, 2000). Treatment means were separated using Fisher's protected LSD (P=0.05).

Foliar treatment with ALA

Barley and wheat of the same cultivars described in the section above were used for this study. Seeds of barley and wheat were seeded in pots (200 cm^2) containing commercial potting mix (peat moss, vermiculite, and zeolite) under greenhouse temperatures at 25°C day/15°C night. At the two-leaf stages (13 days after seeding), the seedlings were given an ALA application. Seeds of rice were soaked in water for 4 days at 25°C, sown in trays containing commercial soil substrate (Boo-Nong Soil, Seoul Korea), and placed in the greenhouse. At 8 days after seeding, rice seedlings were transplanted to pot (200 cm^2). Two-leaf seedlings, growing in 3 cm deep simulated flood, were applied with ALA. For foliar spray treatment, ALA aqueous solutions of 0, 0.05, 0.1, 0.5, 1, 5, 10, and 30 mM containing Tween 20 (0.1%) as a surfactant were sprayed with handy sprayers in amounts of 10 ml/pot. ALA aqueous solutions were applied at 8:00 p.m. and kept in darkness for 10 hrs. Thereafter, the seedlings were exposed to natural sunlight (about 1000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD) under greenhouse conditions. Plant height and shoot fresh weight were measured 10 days after application. The data was analyzed as described in the section above.

Effects of ALA application timing

Barley (cv. Naehanssalbori) and wheat (cv. Geumgangmil) were used for this study. Growth conditions were the same as those described in the section above. Plants were sprayed with 10 ml/pot ALA aqueous solutions of 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mM containing Tween-20 (0.1%) as a surfactant at five different growing stages; seed, five days after seeding, 1-leaf, 3-leaf, and 5-leaf. Other procedures were the same as those described in the section above.

Effects of ALA on weed species

Monocots weeds, *Setaria viridis* L., *Echinochloa crus-galli* (L.) var. *oryzicola*, *Echinochloa crus-galli* (L.) Beauv., *Panicum dichotomiflorum* Michx., and *Digitaria ciliaris* (Retz.) and dicotyledons weeds, *Ixeris dentate* (Thunb.), *Phtheirospermum japonicum* (Thunb.), *Xanthium strumarium* L., *Aeschynomene indica* L., *Chenopodium glaucum* L.,

Mosla punctulata (Gmel.), *Achyranthes japonica* (Miq.), *Plantago asiatica* L., *Leonurus japonicus* Houtt., and *Clinopodium chinense* (Benth.) were used for this study. These seeds were sown in cups (200 ml). At two-leaf stages, the plants were sprayed with 3 ml/cup ALA aqueous solutions of 0, 1, 3, 5, 10, and 30 mM containing Tween-20 (0.1%) as a surfactant. Ten days after application, the plants were cut at the soil surface, and the shoot fresh weight was recorded. Experiments were repeated two times. Data was expressed as a percentage of untreated control. Logistic equation:

Equation: $Y = a/\{1 + (X/X_0)^b\}$

was used to describe the responses of various weed species to ALA, by using Statistical Analysis System (SAS) software (2000), where Y is the shoot fresh weight expressed as a percentage of the untreated control, X is the ALA rate, a is the coefficients corresponding to the lower and upper asymptotes, b is the slope of the line, and X_0 is the rate at the point of inflection midway between the upper and lower asymptotes. Fitted regression equations are shown in Table 4. GR_{50} values were calculated from the regression equations.

Comparison of biological activity between bio-ALA and synthetic ALA

Barley (cv. Naehanssalbori), wheat (cv. Geumgangmil), rice (cv. Dongjinbyeo), and weed, *Ixeris dentata* were tested for the difference of biological activity between bio-ALA and synthetic-ALA. These cultivars had shown the most positive or negative effects on ALA. Barley, wheat, and rice at three leaf stages and weed, *Ixeris dentate* at two leaf stages, were applied bio- and synthetic-ALA aqueous solutions of 0, 0.05, 0.1, 0.5, 1, 5, 10, and 30 mM containing Tween 20 (0.1%) as a surfactant. Other procedures were the same as those described in the section above.

RESULTS AND DISCUSSION

Seed soaking treatment with ALA

To elucidate the effects of ALA in barley (five cultivars) and wheat (five cultivars), and rice (one cultivar), ALA was applied to seeds by soaking at 0.001, 0.005, 0.01, 0.5, 1, 5, and 10 mM (Table 1). Plant height and shoot fresh weight of five cultivars of barley at 10 days after seed soaking treatment with lower or higher concentrations of ALA, were not affected significantly. Plant height and shoot fresh weight of four cultivars (Geumgangmil, Geurumil, Urimil, and Donghaemil) of wheat at 10 days after seed soaking treatment with lower or higher concentrations of ALA, were also not affected significantly. The plant height of Olmil cultivar of wheat caused by ALA applied at 0.001-10 mM to seeds, were found to be increased up to 9-17%, but shoot fresh weight was not found to be increased by ALA application. In rice, there were no positive effects on plant height and shoot fresh weight by seed soaking treatment with ALA at lower concentrations. However, at seed soaking application of 5 and 10 mM, plant height and shoot fresh weight of rice were partially damaged, possibly caused by the herbicidal properties of ALA. This result suggested that ALA seed soaking treatment had not shown the positive effects at lower concentrations as well as negative effects at high concentrations. It was thought that seeds applied were not responsive enough to ALA than foliar application. However, in rice seedlings, ALA at 0.1-1.0 ppm by root-soaking treatment increased the dry weight of seedling to 111-115% (Hotta *et al.*, 1997c).

Foliar treatment with ALA

The effects of ALA resulting from foliar application at 1.5-leaf stage using five cultivars of barley, five cultivars of wheat, and one cultivar of rice are shown in Table 2. The plant height in 'Naehanssalbori', 'Songhakbori', 'Saessalbori', and 'Daehossalbori' increased by 16-25%, 8-21%, 12-18%, and 11-31% compared to control by ALA at lower concentrations, respectively. However, plant height in Ganghossalbori was not affected by ALA at lower concentrations. Although the negative effects of plant height by ALA resulting from foliar application were different with cultivars, ALA with more than 30 mM significantly suppressed the plant height. The shoot fresh weight in 'Ganghossalbori', 'Naehanssalbori', 'Songhakbori', 'Saessalbori', and 'Daehossalbori' were increased up to 14%, 19-51%, 17-64%, 18-23%, and 22-38% by ALA at lower concentrations, 0.05 to 1 mM, respectively. However, the effects of ALA in 'Ganghossalbori', 'Naehanssalbori', 'Songhakbori', 'Saessalbori', and 'Daehossalbori' were inhibited by 29-77%, 45-89%, 29-63%, 28-92%, and 32-91% at higher concentrations 5, 10, and 30 mM, respectively. Plant height was less sensitive to ALA than shoot fresh weight. For further study, we selected the seeds with the most positive effects for 'Naehanssalbori'. Plant height in wheat cultivars except for 'Olmi' was not affected by ALA at lower concentrations, but the plant height was reduced by ALA at 10 and 30 mM in all tested cultivars. Shoot fresh weight in wheat cultivars except for 'Geurumil' was increased by ALA at lower concentrations, and was inhibited by ALA at higher concentrations in all tested cultivars. We selected 'Geumgangmil' as a test plant for further study. Rice plants also showed positive or negative effects by ALA at lower or higher concentrations. Barley was more response to ALA than were wheat and rice. Stimulation in plant height and shoot fresh weight caused by ALA seed

Table 1. Effects of ALA on the plant height and shoot fresh weight of barley, wheat, and rice resulting from seed treatment. Parameters were recorded 10 days after application.

Cultivar	ALA concentration (mM)																				
	0	0.001	0.005	0.01	0.05	0.1	0.5	1	5	10	LSD (0.05)	0	0.001	0.005	0.01	0.05	0.1	0.5	1	5	10	LSD (0.05)
	Plant height (% of untreated)											Shoot fresh weight (% of untreated)										
Barley																						
Ganghossalbori	100^{b}	105^{ab}	107^{ab}	108^{ab}	106^{ab}	111^{a}	105^{ab}	111^{a}	113^{a}	110^{a}	9	100^{a}	91^{a}	82^{a}	85^{a}	93^{a}	99^{a}	99^{a}	119^{a}	114^{a}	87^{a}	40
Naehanssalbori	100^{a}	98^{ab}	98^{ab}	100^{a}	98^{ab}	97^{ab}	103^{a}	100^{a}	97^{ab}	91^{b}	7	100^{ab}	88^{b}	103^{ab}	107^{ab}	107^{ab}	87^{b}	99^{ab}	99^{ab}	120^{a}	92^{b}	26
Songhakbori	100^{ab}	96^{ab}	91^{ab}	102^{a}	90^{ab}	97^{ab}	99^{ab}	97^{ab}	88^{b}	96^{ab}	13	100^{bc}	115^{ab}	88^{b}	110^{ab}	119^{ab}	110^{ab}	100^{ab}	103^{ab}	103^{ab}	129^{a}	36
Saessalbori	100^{bc}	104^{b}	103^{bc}	101^{bc}	118^{a}	107^{ab}	107^{ab}	107^{ab}	102^{bc}	92^{c}	12	100^{b}	122^{ab}	119^{ab}	110^{ab}	124^{ab}	125^{ab}	144^{a}	123^{ab}	144^{a}	93^{b}	37
Daehossalbori	100^{bc}	103^{abc}	112^{a}	100^{bc}	104^{abc}	95^{c}	104^{abc}	109^{ab}	102^{abc}	100^{bc}	11	100^{a}	110^{a}	111^{a}	91^{a}	111^{a}	98^{a}	98^{a}	87^{a}	111^{a}	90^{a}	38
Wheat																						
Geumgangmil	100^{ab}	105^{a}	96^{b}	88^{c}	104^{a}	105^{a}	99^{ab}	101^{ab}	103^{ab}	104^{a}	7	100^{ab}	104^{ab}	72^{cd}	53^{d}	122^{a}	92^{bc}	70^{cd}	86^{bc}	97^{b}	105^{ab}	24
Geurumil	100^{abcd}	91^{d}	96^{cd}	108^{a}	106^{ab}	100^{abc}	96^{cd}	99^{cd}	97^{cd}	104^{abc}	8	100^{bc}	105^{abc}	88^{c}	133^{a}	119^{abc}	110^{abc}	105^{abc}	118^{abc}	114^{abc}	127^{ab}	31
Urimil	100^{a}	101^{a}	76^{b}	95^{ab}	102^{a}	103^{a}	98^{ab}	96^{ab}	100^{a}	94^{ab}	22	100^{abc}	110^{a}	59^{d}	96^{abc}	101^{abc}	108^{ab}	89^{bc}	98^{abc}	92^{abc}	87^{c}	19
Olmil	100^{b}	112^{a}	109^{a}	117^{a}	110^{a}	109^{a}	108^{ab}	111^{a}	115^{a}	114^{a}	9	100^{bcde}	104^{abc}	119^{a}	103^{abcd}	117^{ab}	84de	110^{abc}	82^{e}	114^{ab}	93^{cde}	19
Donghaemil	100^{abc}	99^{abc}	97^{abc}	105^{a}	102^{ab}	95^{bc}	95^{bc}	92^{c}	94^{bc}	93^{c}	8	100^{ab}	80^{bc}	100^{ab}	114^{a}	105^{ab}	99^{ab}	85^{bc}	83^{bc}	69^{c}	67^{c}	27
Rice																						
Dongjinbyeo	100^{a}	-	-	91^{a}	88^{ab}	92^{a}	92^{a}	88^{ab}	68^{bc}	58^{c}	20	100^{a}	-	-	69^{a}	76^{a}	85^{a}	78^{a}	99^{a}	32^{b}	12^{b}	32

Table 2. Effects of ALA on the plant height and shoot fresh weight of barley, wheat, and rice resulting from foliar application at the 1.5-leaf stage. Parameters were recorded 10 days after application.

Cultivar	ALA concentration (mM)																				
	0	0.05	0.1	0.5	1	5	10	30	LSD (0.05)	0	0.05	0.1	0.5	1	5	10	30	LSD (0.05)		
	Plant height (% of untreated)									Shoot fresh weight (% of untreated)										
Barley																					
Ganghossalbori	100^{ab}	110^{a}	110^{a}	102^{ab}	120^{a}	105^{a}	73^{bc}	67^{c}	30	100^{bc}	104^{abc}	114^{a}	91^{c}	113^{ab}	71^{d}	57^{e}	23^{f}	14			
Naehanssalbori	100^{c}	116^{b}	124^{ab}	125^{a}	117^{ab}	103^{c}	97^{c}	30^{d}	8	100^{c}	119^{bc}	151^{a}	138^{ab}	121^{b}	55^{d}	57^{d}	11^{e}	21			
Songhakbori	100^{c}	108^{b}	113^{b}	121^{a}	111^{b}	108^{b}	89^{d}	62^{e}	7	100^{d}	102^{cd}	117^{bc}	164^{a}	121^{b}	107^{bcd}	71^{e}	27^{f}	16			
Saessalbori	100^{b}	118^{a}	112^{a}	117^{a}	114^{a}	89^{c}	100^{b}	21^{d}	9	100^{bc}	118^{ab}	119^{ab}	123^{a}	114^{ab}	72^{c}	59^{c}	8^{d}	20			
Daehossalbori	100^{d}	104^{d}	118^{bc}	124^{ab}	131^{a}	111^{cd}	76^{e}	10^{f}	11	100^{b}	122^{a}	128^{a}	138^{a}	130^{a}	68^{c}	50^{c}	9^{d}	18			
Wheat																					
Geumgangmil	100^{ab}	103^{ab}	107^{a}	108^{a}	107^{a}	99^{ab}	93^{b}	56^{c}	9	100^{bc}	91^{c}	121^{a}	98^{bc}	110^{ab}	68^{d}	64^{d}	40^{e}	18			
Geurumil	100^{ab}	102^{ab}	102^{ab}	103^{a}	104^{a}	95^{bc}	91^{c}	67^{d}	7	100^{a}	100^{a}	96^{ab}	97^{ab}	91^{ab}	67^{bc}	70^{abc}	48^{c}	31			
Urimil	100^{b}	102^{ab}	100^{b}	106^{ab}	114^{a}	85^{c}	73^{c}	44^{d}	13	100^{b}	105^{b}	111^{b}	116^{b}	169^{a}	66^{c}	64^{c}	17^{d}	17			
Olmil	100^{c}	109^{ab}	110^{a}	110^{ab}	105^{b}	97^{c}	85^{d}	49^{e}	4	100^{b}	95^{b}	176^{a}	106^{b}	126^{b}	95^{b}	38^{c}	32^{c}	44			
Donghaemil	100^{a}	95^{ab}	98^{ab}	103^{a}	98^{ab}	95^{ab}	91^{b}	75^{c}	8	100^{b}	97^{b}	82^{cd}	115^{a}	91^{bc}	76^{d}	57^{e}	38^{f}	12			
Rice																					
Dongjinbyeo	100^{a}	97^{ab}	101^{ab}	103^{a}	99^{ab}	87^{bc}	75^{c}	23^{d}	15	100^{cd}	107^{bc}	124^{ab}	128^{a}	107^{bc}	81de	66^{e}	39^{f}	19			

soaking treatment was smaller than by ALA foliar treatment, and the effects of ALA were shown to be greater on the shoot fresh weight than on plant height. Although the effects of ALA differed with cultivars, crops, concentrations, and application methods, we found that ALA had positive effects on the growth of barley, wheat, and rice. Similar to our result, ALA has been reported to promote the growth and yield of corn, kidney bean, potato, garlic, and *Vigna* species at low concentrations (Tanaka *et al.*, 1992; Hotta *et al.*, 1997a & b; Roy & Vivekanandan, 1998). These positive effects of ALA on the growth of several crops could be explained by the increased photosynthetic activity and the suppression of respiration in the plants (Hotta *et al.*, 1997a & b).

Effects of ALA application timing

The appropriate timing of ALA application was examined using barley and wheat. Various solutions of ALA were foliar-applied to barley and wheat at five different stages. ALA treatment at 1.5-leaf stage increased the plant height of barley by 16-25%, while pretreatments to seeds, post-emergence treatment at 3 days after seeding, 3-leaf stages, and 5-leaf stages had not shown positive effects on the plant height of barley. ALA treatment at the 1.5-leaf stage also increased

Table 3. Effects of ALA application timing on the plant height and shoot fresh weight of barley and wheat. Parameters were recorded 10 days after application.

Crop	Application timing	ALA concentration (mM)																			
		0	0.001	0.005	0.01	0.05	0.1	0.5	1	5	10	0	0.001	0.005	0.01	0.05	0.1	0.5	1	5	10
		Plant height (% of untreated)										Shoot fresh weight (% of untreated)									
Barley (Naehanssalbori)	Seed	100^{a}	98^{ab}	98^{ab}	100^{a}	98^{ab}	97^{ab}	103^{a}	100^{a}	97^{ab}	91^{b}	100^{ab}	88^{b}	103^{ab}	107^{ab}	107^{ab}	87^{b}	99^{ab}	99^{ab}	120^{a}	92^{b}
	3 DAS	100^{a}	-	-	-	100^{a}	92^{a}	99^{a}	97^{a}	-	-	100^{a}	-	-	-	88^{b}	92^{b}	82^{b}	86^{b}	-	-
	1.5-leaf	100^{c}	-	-	-	116^{b}	124^{ab}	125^{a}	117^{ab}	103^{c}	97^{c}	100^{c}	-	-	-	119^{bc}	151^{a}	138^{ab}	121^{b}	55^{d}	57^{d}
	3-leaf	100^{a}	-	-	-	107^{a}	102^{a}	104^{a}	98^{a}	84^{b}	72^{c}	100^{a}	-	-	-	101^{a}	107^{a}	109^{a}	98^{a}	78^{b}	49^{c}
	5-leaf	100^{a}	-	-	-	103^{a}	102^{a}	99^{a}	98^{a}	-	-	100^{b}	-	-	-	108^{a}	106^{a}	110^{a}	111^{a}	-	-
Wheat (Geumgangmil)	Seed	100^{ab}	105^{a}	96^{b}	88^{c}	104^{a}	105^{a}	99^{ab}	101^{ab}	103^{ab}	104^{a}	100^{ab}	104^{ab}	72^{cd}	53^{d}	122^{a}	92^{bc}	70^{cd}	86^{bc}	97^{b}	105^{ab}
	1.5-leaf	100^{ab}	-	-	-	103^{ab}	107^{a}	108^{a}	107^{a}	99^{ab}	93^{b}	100^{bc}	-	-	-	91^{c}	121^{a}	98^{bc}	110^{ab}	68^{d}	64^{d}
	3-leaf	100^{a}	-	-	-	96^{b}	94^{b}	93^{b}	83^{b}	82^{b}	66^{c}	100^{a}	-	-	-	98^{a}	109^{a}	99^{a}	90^{a}	78^{b}	45^{c}

[a]DAS, days after seeding

Table 4. Effect of ALA on GR_{50} of monocotyledons and dicotyledons weeds resulting from foliar application at the 2-leaf stage. Parameter was recorded 10 days after application. GR_{50} values were the ALA concentrations that reduce shoot fresh weight by 50%.

	Weeds	Equation	R^2	GR_{50}
Monocotyledons weeds	*Setaria viridis* L.	$Y= 99.4/\{1+(X/1.68)^{1.003}\}$	0.95	1.6
	Echinochloa crus-galli (L.) var. *oryzicola*	$Y= 109.5/\{1+(X/4.3)^{5.46}\}$	0.92	4.4
	Echinochloa crus-galli (L.) Beauv.	$Y= 101.6/\{1+(X/1.74)^{1.92}\}$	0.96	1.7
	Panicum dichotomiflorum Michx.	$Y= 100.7/\{1+(X/4.01)^{1.22}\}$	0.96	4
	Digitaria ciliaris (Retz.)	$Y= 97.7/\{1+(X/2.82)^{5.88}\}$	0.99	2.8
Dicotyledons weeds	*Ixeris dentate* (Thunb.)	$Y= 106/\{1+(X/3.8)^{4.99}\}$	0.98	3.9
	Phtheirospermum japonicum (Thunb.)	$Y= 105.7/\{1+(X/4.04)^{1.85}\}$	0.98	4.3
	Xanthium strumarium L.	$Y= 97.4/\{1+(X/9.25)^{2.25}\}$	0.99	8.9
	Aeschynomene indica L.	$Y= 98.7/\{1+(X/11.87)^{1.75}\}$	0.92	11.7
	Chenopodium glaucum L.	$Y= 135/\{1+(X/6.02)^{3.03}\}$	0.87	7.1
	Mosla punctulata (Gmel.)	$Y= 114.7/\{1+(X/9.5)^{4.1}\}$	0.95	10.1
	Achyranthes japonica (Miq.)	$Y= 95.7/\{1+(X/12.6)^{0.9}\}$	0.91	11.3
	Plantago asiatica L.	$Y= 109.3/\{1+(X/16.6)^{2.5}\}$	0.93	17.7
	Leonurus japonicus Houtt.	$Y= 90.1/\{1+(X/17.96)^{3.96}\}$	0.92	16.9
	Clinopodium chinense (Benth.)	$Y= 108/\{1+(X/12.8)^{2.7}\}$	0.95	13.4

the shoot fresh weight of barley by 19-58%. Similar to plant height, other ALA application timings had shown poor or no positive effects on shoot fresh weight in barley. The positive effects of ALA on plant height and shoot fresh weight of wheat at 1.5-leaf stages application also observed, but not to seeds or 3-leaf stage application. Thus, the positive effects of ALA on barley and wheat depended greatly on the timing of application and its concentration. The positive effects of ALA on other crops also depended on the timing of application (Bingshan, 1998; Hotta *et al.*, 1997b). Further study is necessary to determine the precise effects on yield through ALA treatment at different stages.

Effects of ALA on weed species

In addition to the very good growth stimulator of ALA, it contains a herbicidal property when used at higher concentrations (Tanaka *et al.*, 1992). To determine the effects of ALA on various weed species, ALA was applied to foliar by spray in different solutions (Table 4). Based on GR_{50}, monocots weeds were more sensitive to ALA than dicotyledonous weeds except for *Ixeris dentate* (Thunb.) and *Phtheirospermum japonicum* (Thunb.). Monocots weed, *Setaria viridis* L. were the most susceptible plants (GR_{50}: 1.6 mM) while dicotyledonous weed, *Plantago asiatica* L. were the most tolerant (GR_{50}: 17.7 mM). However, dicotyledonous weeds such as lambsquarter, mustard, redroot pigweed, and common purslane were highly susceptible to the tetrapyrrole-induced photodynamic damage, monocots such as corn, wheat, oats, and barley were not adversely affected by the spray. Furthermore, dicotyledonous economic crops were either unaffected by the spray at an early stage of development, as in soybean, or fully recovered from a rapid destruction of the primary leaves by producing new healthy leaves, as was observed for kidney bean, soybean, and cotton (Rebeiz *et al.*, 1984). On the other hand, our studies on barley, wheat, and rice were also less affected than weeds by ALA foliar spray at early seedling stages.

The physiological actions of ALA at high concentrations suggests that ALA increases the levels of porphyrin intermediate such as protochlorophyllide, protoporphyrin , and Mg-protoporphyrin abnormally, and the accumulated tetrapyrroles act as a photosensitizer for the formation of singlet oxygen triggering photodynamic damage (Askira *et al.*, 1991; Rebeiz *et al.*, 1984). Thus, in our study the selectivity among plant species would be based on tetrapyrrole accumulating capabilities and the tetrapyrrole metabolism in various plant species (Rebeiz *et al.*, 1988).

Comparison of biological activity between bio-ALA and synthetic ALA

ALA has received great attention as a growth regulator at low concentrations and as a new biodegradable herbicide at high concentrations. In addition, ALA is not harmful to crops, animals, and humans, and used as a prodrug for photodynamic diagnosis and therapy of cancer (Schuimaker *et al.*, 1999). However, ALA is very expensive because it is usually synthesized chemically via complex processes. Therefore, biological production using microorganisms has been suggested as a less expensive way to produce ALA. For this study, we used 30 mM of ALA accumulated by overexpressing the *hemA* gene isolated from *Bradyrhizobium japonicum* (Choi *et al.*, 1999). There was no significant difference on biological activity in barley, wheat, rice, and weed, *Ixeris dentata* tested between bio-ALA and syn-

Table 5. Effects of bio (B)- and synthetic (S)-ALA on biological activity of barley, wheat, rice and weeds, *Ixeris dentate*. Barley, wheat, and rice at three leaf stages and weed, Ixeris dentate at two leaf stages, were applied bio- and synthetic ALA. Parameters were recorded 10 days after application.

Crop	ALA concentration (mM)																	
	ALA	0	0.05	0.1	0.5	1	5	10	30	LSD (0.05)	0	0.05	0.1	0.5	1	5	10	30	LSD (0.05)
		Plant height (% of untreated)									Shoot fresh weight (% of untreated)								
Barley (Naehanssalbori)	S	100	107	102	104	98	84	72	53	ns	100	101	107	109	98	78	49	18	ns
	B	100	109	104	100	98	93	87	50		100	107	106	85	83	84	74	23	
Wheat (Geumgangmil)	S	100	96	94	93	83	82	66	42	ns	100	98	109	99	90	78	55	17	ns
	B	100	102	99	98	94	89	82	51		100	94	93	98	81	84	66	23	
Rice (Dongjinbyeo)	S	100	91	88	83	83	79	75	51	ns	100	114	85	101	84	64	44	13	ns
	B	100	93	95	98	92	86	78	60		100	102	100	97	87	53	40	29	
Weed (*Ixerisdentata*)	S										100	-	97	106	88	30	12	0	ns
	B										100	-	93	89	86	38	15	0	

thetic-ALA (Table 5). Thus, ALA produced by microorganisms has the potential to become an industrial process, provided appropriate technologies are developed for making industrial production technically feasible. In addition, to maximize biological activity of ALA, more research has to be focused on searching for promising microorganisms which produce high amounts of ALA, and further studies of ALA are needed before it can be used effectively in agricultural production.

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