Plant Growth Promotive Allelochemicals

Kosumi Yamada[1], Katsutoshi Hirose[2], Hideyuki Shigemori[1], Koji Hasegawa[1, 2]
[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan
[2]KNC Laboratories Co., Ltd., Hyogo 651-2271, Japan
E-mail: kosumi@sakura.cc.tsukuba.ac.jp

Abstract: The term "allelopathy" has been defined as both detrimental and beneficial biochemical interactions among all classes of plants (including microorganisms) through the production of chemical compounds that are released into the environment. Previously, the shoot growth of cockscomb (*Celosia cristata*) seedlings was significantly promoted when various plant species were cultured together with cockscomb seeds in a Petri dish. This phenomenon led to the finding of stimulatory allelopathy in which germinating plant seeds secrete growth-promoting substance(s) to their environment as allelopathic factors. Candidates for stimulatory allelopathic factors include lepidimoide (LM) from the mucilage of germinating cress (*Lepidium sativum*) seeds, lepidimoic acid (LMA) from Arabidopsis (*Arabidopsis thaliana*) seeds, and both arctigenin and arctigenic acid from burdock (*Arctium lappa*) seeds. During the past decade, many significant advances concerning the biological activities of LM and LMA in several developmental stages of plants have been made. In addition, a convenient method for synthesis of LMA from okra (*Hibiscus esculentus*) mucilage has also been developed. LM and LMA promote chlorophyll accumulation in sunflower (*Helianthus annuus*) and cucumber (*Cucumis sativus*) cotyledons by affecting the level of 5-aminolevulinic acid (ALA), leaf development, flowering and seed production in Arabidopsis as well as shoot growth in seedlings of various plant species, and inhibits the loss of total chlorophyll in oat (*Avena sativa*) leaf segments and the formation of abscission in bean (*Phaseolus vulgaris*) petiole explants. On the basis of these information, LM and LMA appears to be a novel plant growth regulator with multiple physiological functions for the regulation of growth and development in plants. A summary of this work as well as the possible application of these allelochemicals in agriculture will be presented.
Keywords: Mucilage, Plant Growth Regulator, Seed Exudates, Stimulatory Allelopathy

Introduction

Allelopathy has been defined as the term covering both detrimental and beneficial biochemical interactions among all classes of plants (including microorganisms) through the production of chemical compounds that are released into the environment [1]. However, researchers involved in allelopathy and allelochemicals, have generally ignored the stimulatory effects, possibly because stimulatory effects are often not as spectacular as inhibitory effects. Nonetheless, there are some reports of stimulation of plants by other plants, of plants by microorganisms and vice-versa and of microorganisms by other microorganisms [2]. Multiple cropping, with careful attention to the selected plant components, could be expected to provide higher yields and reduce weed and pests. Seed germination stimulants from host and non-host plants and microbially produced ethylene are highly efficacious and low-environmental impact tools to control angiospermous plant parasites. Here we provide several examples of stimulatory allelopathy during seed germination observed in the very early stages of plant development.

Results and Discussion

When two kinds of seeds or seedlings of various plant species were cultured together in a Petri dish, the shoot growth of neighboring plants, especially that of cockscomb (*Celosia cristata*), was significantly promoted with several combination [3]. This phenomenon led to the finding of stimulatory allelopathy in which germinating seeds secrete growth-promoting substance(s) to their environment as allelopathic factors. As a candidate of allelochemical observed in the cress (*Lepidium sativum*) and cockscomb combination, a potent novel growth-promoting substance was isolated from the mucilage of germinating cress seeds [4]. And its structure was determined to be 4-deoxy-β-L-*threo*-hex-4-enopyramunosyl-(1→2)-L-rhamnopyrano se sodium salt (named lepidimoide, LM) by spectral analyses and total synthesis (Fig. 1). LM-like activity was found in the exudates from the seeds of various plant species [5]. The occurrence of LM in the exudates was determined using a physicochemical assay among the tested weed and crop seeds, although its amounts did not differ greatly among genera or families [6]. It was therefore suggested that LM acts as a phytohormone-like substance as well as allelochemicals. Subsequently, the free carboxylic acid form of LM

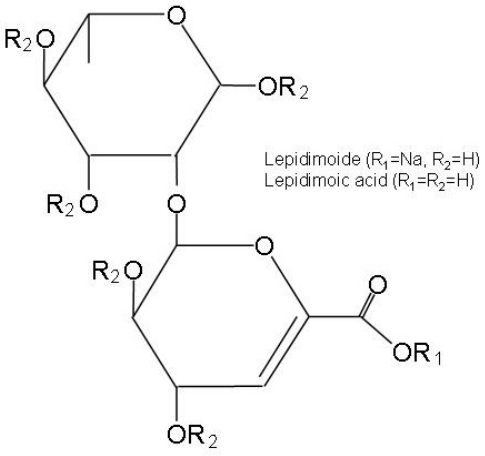

Figure 1. Chemical structures of lepidimoide (LM) and lepidimoic acid (LMA)

(named lepidimoic acid, LMA) was isolated from the exudates of germinating Arabidopsis (*Arabidopsis thaliana*) seeds (Fig. 1) [7], followed by arctigenin (4-[(3,4-dimethoxyphenyl) methyl] dihydro-3-[(4-hydroxy-3-methoxyphenyl) methyl]-2(3*H*)-furanone) and arctigenic acid (2-(3-methoxy-4-hydroxybenzyl)-3-(3,4-dimethoxybenzyl)-4-h ydroxybutanoic acid) from the burdock (*Arctium lappa*) seeds by the method of mixed culture (Fig. 2) [8]. Among the identified stimulatory allelochemicals, the mode of exudation in both LM and LMA (designated as LM(s)) from the germinating seeds has been vigorously studied. Interestingly, both LM presented in the dry seeds as well as produced in the kernels following imbibition, has been released into the environment in relation to seed germination [9]. These findings, together with the wide distribution of LM in the plant kingdom, suggested that LM(s) may be a novel type of endogenous plant growth regulator, like phytohormones.

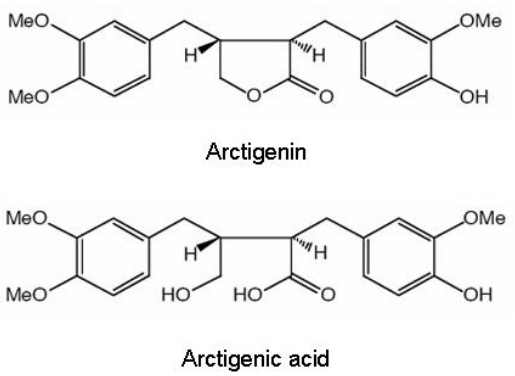

Figure 2. Chemical structures of arctigenin and arctigenic acid

To determine the structural requirement of LM(s), the structure–activity relationship of LM, its analogues and some sugars were investigated using the hypocotyl elongation test in cockscomb [10]. Firstly, LMA had as high as the growth-promoting activity of LM, suggesting that sodium salt is not the structural requirement for plant growth-promoting activity. Secondly, the structural requirements of LM for the activity were the uronic acid derivative bearing an α,β-unsaturated carboxylate bonded to rhamnose via a α-glucoside linkage, and a double bond in the C-4,5 position in the uronic acid.

The shoot elongation is the most typical phenomenon observed in the LM treatment. However, it exhibited further multiple functions. The effect of LM on some developmental stages has been shown in several wild types of Arabidopsis [11]. Among the ecotypes of wild type studied, Columbia (Col) and Wassilewskija (WS) were generally sensitive to LM. From the present study, it was concluded that LM promotes the growth of the shoot and leaf area, and increases the fresh and dry weights of plants, although the effect is greatly different between ecotypes. At the same time, LM hastened the life cycle of Arabidopsis through the acceleration of leaf and flower development. Activation of the reproductive growth with LM treatment, such as seed production, was also strongly indicated.

Although LM(s) promoted the shoot growth among several plant species, this growth promotion may be due to the acceleration of respiratory metabolism to produce metabolic energy and biosynthetic precursors. The interaction of pyrophosphate-dependent phosphofructokinase (PFP) and fructose 2,6-bisphosphate (Fru-2,6-P_2), which is an important cytosolic signal metabolite, is considered to be an important mechanism on the regulation of sugar metabolism between glycolysis and gluconeogenesis in plants [12]. In general, it is believed that PFP activity was measured in the glycolytic direction from Fru-6-P to Fru-1,6-P_2 with various amounts of exogenously applied Fru-2,6-P_2. Fru-2,6-P_2 markedly activated PFP activity in a dose-response manner [13]. Judging from the increased level of Fru-2,6-P_2 with LMA application in Amaranthus (*Amaranthus caudatus*) seedlings, the increased activity of PFP may be the effect of Fru-2,6-P_2, suggesting a promoting activity of LMA on glycolytic metabolism [14].

In our previous experiment, the promotion of greening was observed in the cotyledon of sunflower (*Helianthus annuus*) seedlings with LM(s) under the low light intensity [15]. It is well known that 5-aminolevulinic acid (ALA) is a rate-limiting step in the formation of chlorophyll (Chl) (Fig. 3) [16]. LM(s), at concentrations higher than 100 μM, significantly promoted the light-induced ALA accumulation as well as the light-induced Chl accumulation in the cotyledon of sunflower seedlings, suggesting the LM(s)-induced enhancement of Chl accumulation is related to the enhancement of ALA synthesis [17]. Cytokinin can also exhibit a similar promoting effect on Chl accumulation in various plants [18]. The effect of LM(s) on the accumulation of ALA and Chl with cytokinin seemed somewhat an additive one. These results suggested that LM(s), in cooperation with cytokinin, causes light-induced Chl accumulation by affecting the level of ALA. It was then investigated whether LMA up-regulates the gene expression

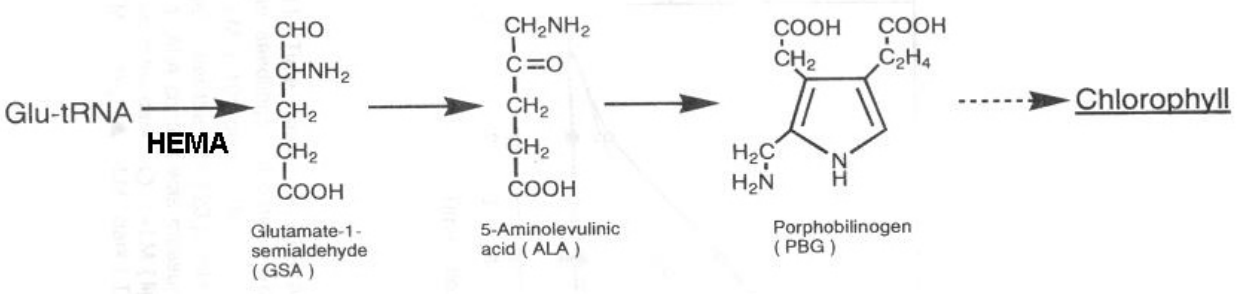

Figure 3. Biosynthetic pathway of chlorophyll (Chl)

of a key enzyme (glutamyl-tRNA reductase, HEMA) associated with ALA formation [19]. As expected, LMA could affect the transcriptional level of *hemA* gene of cucumber cotyledons in the light [20].

The term "senescence" may be associated with the complex deterioration processes that naturally terminate the functional life of plants. In this process, an important contributing factor is the decrease in total Chl content and/or the formation of abscission in the leaves [21]. The effects of LM(s) on Chl preservation in oat (*Avena sativa*) leaf segments was studied in the dark condition [22]. In this bioassay system, LM(s) greatly inhibited the loss of total Chl at concentrations higher than 10 μM, tested 24 h after the incubation. Most of the Chl (about 90% of the initial level) treated with LM(s) was preserved, whereas about 35% of the initial Chl was degraded in the control leaf segment. LM(s) synergistically preserved the Chl content with cytokinin. These results suggest that LM(s) have inhibitory effects on leaf senescence, which is characterized by the loss of total Chl in the leaves in cooperation with cytokinin. On the other hand, abscission is considered to be the last phenomenon during the senescence period in plants. When the petiole explant is excised from an intact plant, cells at the base of the leaf immediately begin to form an abscission layer [23]. The effect of LM(s) on the formation of abscission was studied in bean (*Phaseolus vulgaris*) petiole explants in the light [24]. LM(s) at concentrations higher than 1 μM significantly affected the inhibition of abscission. Sixty hours after the incubation, about 80% of the control explants formed abscission, whereas only 30% of the explants treated with LM(s) were abscised. Together with the inhibiting effect of LM(s) on the leaf senescence, these results indicated that LM(s) has a cytokinin-like activity. Interestingly, LMA treatment also induced the root emerge in detached cucumber cotyledons, suggesting the different target site between LM(s) and cytokinin [20]. On the basis of these observations, LM(s) appears to be a novel plant growth regulator with multiple physiological functions for controlling various aspects of physiological growth and development in plants. The application of LM(s) as a plant growth regulator has been expected in the agricultural field.

During the past decade, significant advances concerning the chemical synthesis of LM(s) have been made. Previously, LM was synthesized from D-glucose and L-rhamnose in 22 steps [25], but this method was complicated and the yield was not so high. Therefore, it was deemed necessary to develop a simplified, large-scale production method for the synthesis of LM(s). In view of the biosynthetic origin of LM by Fry et al., it was suggested that LM could be produced in seeds by the cleavage of a pectic polysaccharide such as rhamnogalacturonan [26]. The screening of the materials for the preparation of LM(s) has been performed and the mucilage isolated from immature okra (*Hibiscus esculentus*) fruit was selected as a favorable pectic polysaccharide [27]. On the basis of this information, the convenient and the highly efficient preparation of LMA from the okra mucilage has been succeeded by sequential chemical degradation reactions (Fig. 4) [28].

The greatest concentration of microorganisms in soil is found in the vicinity of plant roots. In this rhizosphere, plants secrete plenty of nutrients such as organic acids, sugars, vitamins or amino acids [29]. The presence of these nutrients in the rhizosphere allows an intense microbial life. It has been reported that out of 50 fungi and 8 bacteria, 29 endophytic fungi produced an oligosaccharide that has the same R_f value on thin-layer chromatography as LM from okra mucilage [30]. Although the mechanism behind the enzyme reaction has not been clarified, these observations would provide the cues for understanding the possible role of LM(s) between the endosymbiotic microorganisms and plants. This might lead to the identification of the enzymes which can degrade polysaccharides from okra mucilage into LM(s).

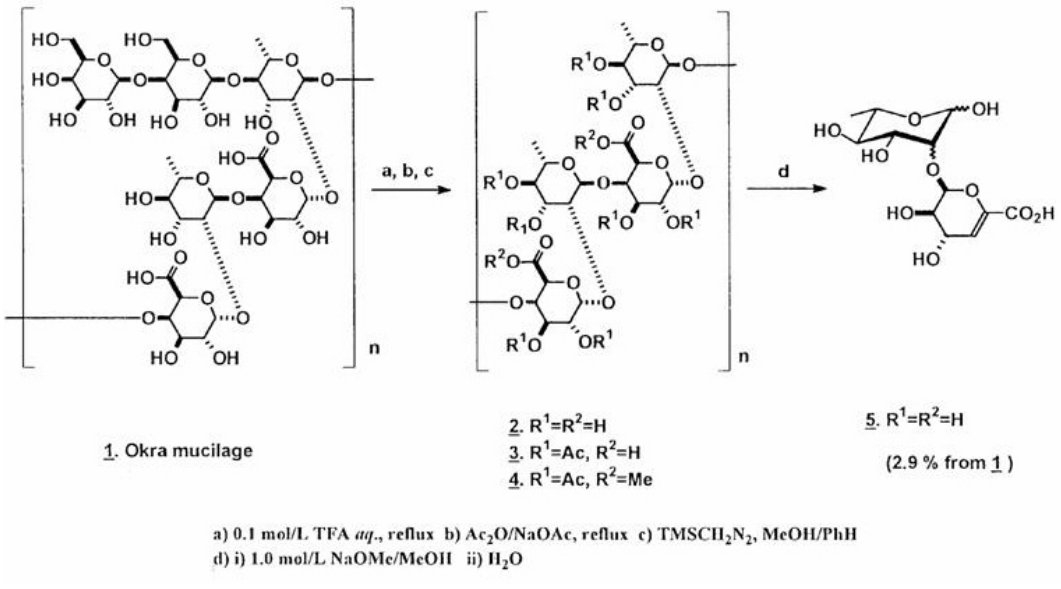

Figure 4. Convenient synthesis of lepidimoic acid (LMA) from okra mucilage F

As described above, significant advances concerning the biological activities of LM(s) have been made. Despite the progress, however, the biosynthetic pathway of LM(s) and the biological meaning for their exudation into the environment remain to be determined. It is of great interest to identify the enzymes concerning the degradation of polysaccharides from okra mucilage. Several kinds of technical approaches may be needed to elucidate the mode of action of LM(s), including the discovery of novel biological effects alone and in collaboration with other phytohormones, and more thorough physiological descriptions of its known effects. With a view to exploit this stimulatory allelopathy where feasible in agriculture and biological research, mutant analysis in Arabidopsis might lead to breakthroughs in the studies of transport, binding, action and turnover of LM(s) in plants, since this plant is now the most important tool in genetic and biochemical studies. If carefully exploited, stimulatory allelochemicals could be used to enhance crop yield, reduce reliance on synthetic pesticides and promote sustainable agriculture.

References

[1] Rice, E. L. 1984. *In*: Allelopathy, 2nd ed. Academic Press, Orlando, Florida.

[2] Mallik, M. A. B., Williams, D. R. 2005. Allelopathic Growth Stimulation of Plants and Microorganisms. *Allelopathy J*., 16: 175-198.

[3] Hasegawa, K., Amano, M., Mizutani., J. 1992. Allelopathic Substances Produced from Cress Seedlings. *Weed Res. Japan* (in Japanese), 37: 71-77.

[4] Hasegawa, K., Mizutani, J., Kosemura, S., Yamamura, S. 1992. Isolation and Identification of Lepidimoide, a New Allelopathic Substance from Mucilage of Germinated Cress Seeds. *Plant Physiol*., 100: 1059-1061.

[5] Hasegawa, K., Amano, M., Asakawa, C., Kakuta, H., Mizutani, J. 1993. Elongation-Promoting Activity of *Amaranthus* Hypocotyls in Mucilage of Germinated Seeds. *Weed Res. Japan* (in Japanese), 34: 109-111.

[6] Yamada, K., Anai, T., Hasegawa, K. 1995. Lepidimoide, an Allelopathic Substance in the Exudates from Germinated Seeds. *Phytochemistry*, 39: 1031-1032.

[7] Yokotani-Tomita, K., Goto, N., Kosemura, S., Yamamura, S., Hasegawa, K. 1998. Growth-Promoting Allelopathic Substance Exuded from Germinating *Arabidopsis thaliana* Seeds. *Phytochemistry*, 47: 1-2.

[8] Higashinakasu, K., Yamada, K., Shigemori, H., Hasegawa, K. 2005. Isolation and Identification of Potent Stimulatory Allelopathic Substances Exuded from Germinating Burdock (*Arctium lappa*) Seeds. *Heterocycles*, 65: 1431-1437.

[9] Yamada, K., Kosemura, S., Yamamura, S., Hasegawa, K. 1997. Exudation of an Allelopathic Substance Lepidimoide from Seeds during Germination. *Plant Growth Regul*., 22: 189-192.

[10] Yamada, K., Anai, T., Kosemura, S., Yamamura, S., Hasegawa, K. 1996. Structure-Activity Relationship of Lepidimoide and Its Analogues. *Phytochemistry*, 41: 671-673.

[11] Goto, N., Sando, S., Sato, Y., Hasegawa, K. 1995. Effects of Lepidimoide on Growth and Development of *Arabidopsis thaliana*. *Weed Res. Japan*, 40: 87-94.

[12] Plaxton, W. C. 1996. The Organization and Regulation of Plant Glycolysis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 185-214.

[13] Kesy, J., Kowalczyk, S. 1987. Comparison of the Activity of the Enzymes Participating in Fructose-6-Phosphate/Fructose-1,6-Bisphosphate Interconversion during Early Stages of Mung Bean (*Phaseolus coccineus* L.) Development. Measurement of Pyrophosphate and Fructose-2,6-Bisphosphate Content. *J. Plant Physiol*., 127: 411-421.

[14] Kato-Noguchi, H., Yamada, K., Hasegawa, K. 2001. Lepidimoic Acid Increases Fructose 2,6-Bisphosphate in *Amaranthus* Seedlings. *Phytochemistry*, 56: 499-503.

[15] Yamada, K., Anai, T., Yokotani-Tomita, K., Hasegawa, K. 1996. Physiological Function of Lepidimoide. *Plant Cell Physiol*., (supplement): s150.

[16] Beale, S. I., Weinstein, J. D. 1990. Tetrapyrrole Metabolism in Photosynthetic Organisms. *In*: Biosynthesis of Heme and Chlorophylls, Dailey, H. A. (ed), McGraw-Hill, New York, pp. 287-391.

[17] Yamada, K., Matsumoto, H., Ishizuka, K., Miyamoto, K., Kosemura, S., Yamamura, S., Hasegawa, K. 1998. Lepidimoide Promotes Light-Induced Chlorophyll Accumulation in Cotyledons of Sunflower Seedlings. *J. Plant Growth Regul*., 17: 215-219.

[18] Davies, P. J. 1987. The Plant Hormones: Their Nature, Occurrence and Functions. *In*: Plant Hormones and Their Role in Plant Growth and Development, Davies , P. J. (ed), Martinus Niihoff, Dordrecht, The Netherlands, pp. 1-11.

[19] Masuda, T., Ohta, H., Shioi, Y., Tsuji, H., Takamiya, K. 1995. Stimulation of Glutamyl-tRNA Reductase Activity by Benzyladenine in Greening Cucumber Cotyledons. *Plant Cell Physiol*., 36:1237-1243.

[20] Yamada, K., Hondo, M., Minami, E., Hirose, K., Hasegawa, T., Shigemori, H., Hasegawa, K. 2005. Effect of a Novel Allelopathic Substance, Lepidimoide, on the Chlorophyll Biosynthesis, *PACIFICHEM 2005*, (Abstracts) Agrochemistry 73.

[21] Beevers, L. 1976. Senescence, *In* Plant Biochemistry Ed. 3, Bonner, J., Varner, J. E. (eds) Academic Press, New York, pp. 775-790.

[22] Miyamoto, K., Ueda, J., Yamada, K., Kosemura, S., Yamamura, S., Hasegawa, K. 1997. Inhibitory Effect of Lepidimoide on Senescence in *Avena* Leaf Segments. *J. Plant Physiol*., 150: 133-136.

[23] Osborne, D. J. 1973. Internal Factors Regulating Abscission, *In* Shedding of Plant Parts. Kozlowski, T. T. (ed), Academic Press, New York, pp. 125-147.

[24] Miyamoto, K., Ueda, J., Yamada, K., Kosemura, S., Yamamura, S., Hasegawa, K. 1997. Inhibition of Abscission of Bean Petiole Explants by Lepidimoide. *J. Plant Growth Regul*., 16: 7-9.

[25] Kosemura, S., Yamamura, S., Kakuta, H., Mizutani, J., Hasegawa, K. 1993. Synthesis and Absolute Configuration of Lepidimoide, a High Potent Allelopathic Substance from Mucilage of Germinated Cress Seeds. *Tetrahedron Lett*., 34: 2653-2656.

[26] Fry, C. S., Aldington, S., Hetherington, P. R., Aitken, J. R. 1993. Oligosaccharides as Signals and Substrates in the Plant Cell Wall. *Plant Physiol*. 103: 1-5.
[27] Hirose, K., Kosuge, Y., Otomatsu, T., Endo, K., Hasegawa, K. 2003. Preparation of a Growth-Promoting Substance, Lepidimoic Acid, from Okra Pectic Polysaccharide. *Tetrahedron Lett*., 44: 2171-2173.
[28] Hirose, K., Endo, K., Hasegawa, K. 2003. A Convenient Synthesis of Lepidimoide from Okra Mucilage and Its Growth-Promoting Activity in Hypocotyls. *Carbohydr*. *Res*. 339: 9-19.
[29] Dunn, K. A., Handelsman, J. 2002. Toward an Understanding of Microbial Communities through Analysis of Communication Networks, *Antonie van Leeuwenhoek*, 81: 565-574.
[30] Tanaka, M., Yoshimura, M., Suto, N., Yokota, A., Asano, K., Sukara, E., Tomita, F. 2002. Production of Lepidimoide by an Endophytic Fungus from Polysaccharide Extracted from *Abelmoschus* sp.: Identification of the Product and the Organism Producing It, *J*. *Biosci*. *Bioengin*., 93: 531-536.