Studies of Stimulation of Early Growth of Rice Seedlings and Effects of Rice Seeds and Seedlings on Seasonal Variations in Emergence and Growth of *Monochoria vaginalis* in Direct-sowing Cultivation

Yasutomo Takeuchi

Rice cultivation by direct-sowing is advantageous for saving labor and cost in rice production. Direct-sowing was commonly practiced in the beginning of the history of rice production thousand years ago, but the seeding system has been changed to a seedling transplanting system in order to avoid weed problems and the unstable performance of the early growth of rice seedlings in direct-sown paddy fields.

Weeds can be controlled by herbicides in direct-sowing paddy fields at present, and therefore to improve unstable performance of the early growth of rice seedlings is an important requirement for the promoting a direct-sowing system. We are attempting to improve this problem in direct-sowing systems using plant growth regulators.

In recent years, *Monochoria vaginalis*, an aquatic weed in paddy fields, has become a serious problem in Japan, because it seems to multiply in direct-sown cultivation. The seasonal variations in the emergence of weeds have been known to be related to the characteristics of dormancy and germination of the weed seeds in paddy fields. It might also be affected by rice seeds and seedlings. This study attempts to clarify the effects of rice seeds and seedlings on the seasonal variations in the emergence and growth of *M. vaginalis*.

1. Study of stimulation of rice seedlins in direct-sowing system.

There are two types of direct-sowing rice cultivation in paddy fields in Japan : (A)sowing rice seeds on the soil surface and (B)sowing rice seeds at a 1~2cm depth in the soil. However, there are some problems such as lodging of the seedlings and bird depredation in type (A) and unstable emergence and establishment of the seedlings especially under low oxygen conditions in type (B). In paddy fields, oxygen is present at as low as 10ppm in water and less than 1ppm in soil. Rice seeds seeded in submerged soil can germinate and elongate coleoptiles into the water on the soil. After sufficient oxygen is supplied from the water to the seeds by translocation through the coleoptiles, the first leaf emerges from the soil and soon passes through the water. This phenomenon is defined as seedling establishment. Soon after the appearance of the first leaf from the water, the seminal roots elongate into soil.

Japonica rice can not easily elongate coleoptiles under extreamly low oxygen conditions such as in submerged soil, especially at low temperatures. Elongation of the coleoptiles in Japonica rice is promoted by ethylene evolved from the seeds and the surrounding soil in paddy fields[6], but elongation of the mesocotyl can not be easily

Utsunomiya University, Weed Science Center, Utsunomiya 321, Japan
<'96. 5. 10 접수>

induced by ethylene. The elongation of the coleoptile and mesocotyl also can not be promoted by gibberellins. Ethylene is produced aerobically from methionin in plant tissues through the biosynthetic pathway as known in Fig. 1. In the pathway, ACC is one of the main control sites of ethylene biosynthesis. The conversion is promoted by such stress as flooding and plant growth regulators such as IAA. The enzymatic converttsion of ACC to ethylene, which needs oxygen, is another key control site of ethylene biosynthesis. When ACC is converted to ethylene, HCN is also produced. HCN can be metabolized in healthy tissues in tolerant plants.

Therefore, manipulation of ethylene biosynthesis and ethylene actions in rice seeds might be possible techniques for improving unstable emergence and establishment of rice seedlings direct-sown in soil in paddy fields.

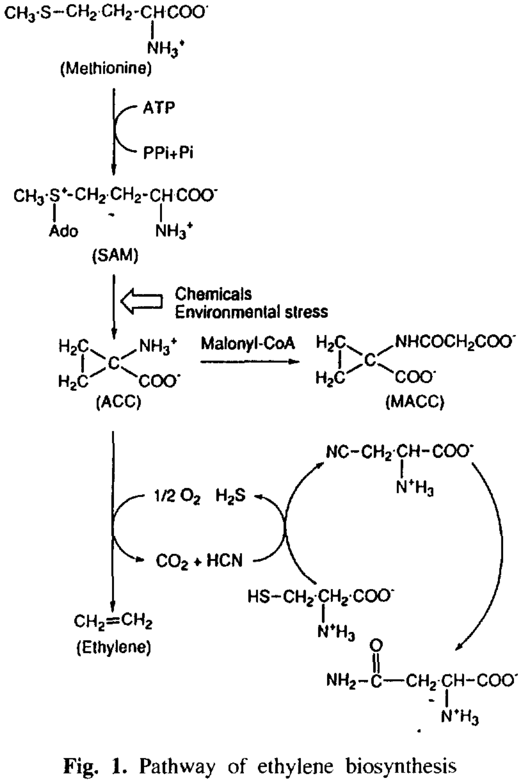

Fig. 1. Pathway of ethylene biosynthesis

(1) Effects of plant growth regulators on emergence and establishment of seedlings seeded in submerged soil.

The promotive effectiveness of plant growth regulators on the emergence and establishment of seedlings seeded in submerged soil was studied. Rice seeds of cv. Sasanishiki(Japonica-type), cv. Hitomebore(Japonica-type) and Milyang 23(Indica X Japonica-type hybrid) were selected by specific gravity with NaCl(1.1sq). The seeds were soaked in water for 48h at 28℃, and then soaked in water or solutions of plant growth regulators for 24h. Ten seeds were placed at 1 or 2cm depth in soil(clay loam) in a 15-cm pot and flooded with water to 2cm depth, then grown in a greenhouse regulated at 16℃ at night and at 26℃ in the daytime.

When the seeds of cv. Sasanishiki were soaked in water for 72h before seeding, they emerged 82% and established 55%. Seeds treated with GA_3 at 10~20ppm emerged 100% and established 52~63%(Table 1). These results indicate that methionin and ACC at 70~200ppm increased the rate of emergence, but that they did not show significantly promotive effectiveness on establishment of the seeds. GA_3 at 10~20ppm

Table 1. Emergence and establishment of direct-seeded rice(cv. Sasanishiki) seedlings grown in pots after the treatment with methionin, ACC, GA_3 and ethephon.

Treatment		Emergence (%)*	Establishment(%)*
Water		82±4	55±3
Methionin	70ppm	100	60±2
Methionin	200ppm	90±4	51±3
ACC	70ppm	100	65±5
ACC	200ppm	85±3	52±6
GA_3	10ppm	100	52±3
GA_3	25ppm	100	63±2
Ethephon	10ppm	100	91±5
Ethephon	20ppm	100	95±4

* Emergence and establishment were determined 8 and 14 days after the seeding, respectively.

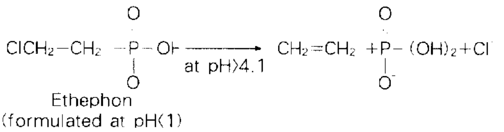

Fig. 2. Conversion of ethephon to ethylene in pots.

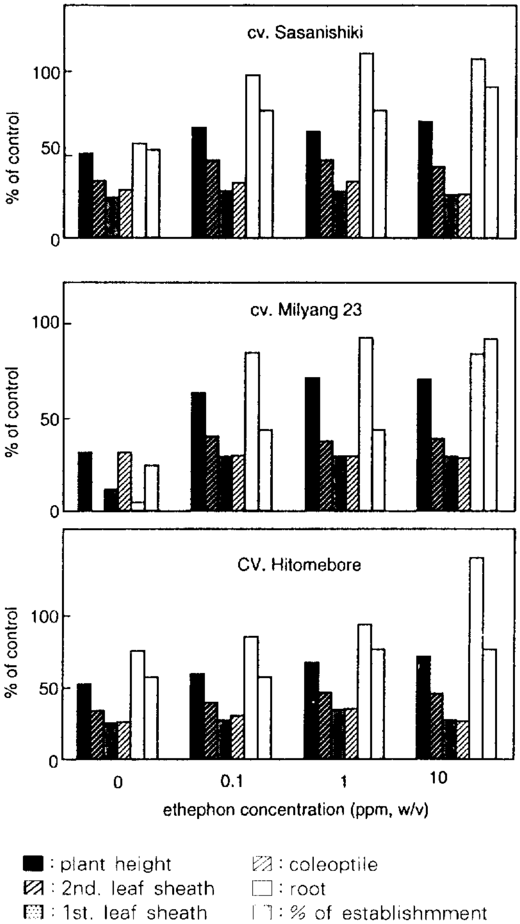

Fig. 3. Effect of ethephon on emergence, establishment and growth of rice seedlings sown in submerged soil.

increased the rate of the emergence, but did not increase the rate of the establishment. The use of ethephon, an ethylene-releasing compound, however, showed promotive effectiveness. Ethephon is fomulated at a pH of less than 1 in 10% commercial solution and releases ethylene non-enzymatically when it contacts with water at a pH of more than 1.1. Ethephon can easily release ethylene in plant tissues having a pH of more than 4(Fig. 2). In the case of cv. Hitomebore, the rate of effectiveness of these chemicals on the emergence and establishment was almost the same as that on Sasanishiki(data not shown).

In other experimint, when the seeds of cv. Sasanishiki were soaked in water, they emerged 75% and established 57%, respectively. Seeds treated with ethephon at 10ppm, they emerged 100% and established 91%, respectively. Also the growth of above and below-ground parts of the cultivar was promoted by the treatment. In the case of cv. Hitomibore, the rate of effectiveness on the emergence and establishment was almost the same as that on cv. Sasanishiki. Seeds of cv. Milyang 23 soaked in water emerged 42% and established 24%. However, when the seeds were treated with ethepon at 10ppm, they emerged 100% and established 90%(Fig. 3). The growth of the seedlings was also greatly promoted. TFIBA [4,4,4-trifluoro-3-(indole-3)-butyric acid], an auxin compound, showed promotive effectiveness on the establishment and growth of the seedlings of cv. Sasanishiki in the same experiment[1](Table 2).

An experimint was conducted to determine the promotive effectiveness of the early growth of rice seedlings under low temperature condition regulated at 14℃ at night and 22℃ in the day-time. Rice seeds of the cultivar Sassanishiki were seeded at a 2cm depth in soil(sandy loam soil) and grown for 14 days. When seeds were soaked in water before seeding, they established 46%. However, seeds were treated with 20ppm ethepon, 25ppm GA_3, 「20ppm ethephon+25ppm GA_3」 and 「20ppm ethephon+25ppm GA_3+5ppm pestalotin (gibberellin synergist)」, they established 58%, 53%, 75% and 83%, respectively(Table 3). The promotive effectiveness of ethephon on the establishment was greatly enhanced by GA_3 and 「GA_3+pestalotin」. The synergistic effectiveness in the

Table 2. Establishment of direct-seeded rice seedlings grown in pots for 12 days after the treatment with or without TFIBA.

Treatment		Establishment of seedlings(%)	Plant height(%)	Weight of above-ground part(%)
Water		63±5	100	100
TFIBA	0.01ppm	80±7	100	125±4
TFIBA	0.03ppm	90±4	125±5	140±5
TFIBA	0.1ppm	75±5	120±8	123±7

Table 3. Establishment of direct-seeded rice seedlings(cv. Sasanishiki) grown in pots under low temperature condition after the treatment with water, ethephon, GA_3, pestalotin and the combinations of ethephon with GA_3 or with GA_3 and pestalotin.

Treatment		Establishment of seedlings(%)	Plant height(%)	Root length(%)	Number of roots(%)
Water		46±4	100	100	100
Ethephon	20ppm	58±6	110±5	110±5	106±2
GA_3	25ppm	53±1	115±3	105±3	104±4
Pestalotin	5ppm	48±0	102±4	98±1	97±7
Ethephon	20ppm+$GA_3$5ppm	75±2	121±1	113±5	115±6
Ethephon	20ppm+GA_3 +Pestalotin 5ppm	83±5	138±0	123±6	113±1

* 2 leaf-stage
** 6.0cm
*** 5

combination of ethephon and GA_3 might be induced by GA_3 through increasing the physiological activity of rice seeds and coleoptiles, especially under low temperature conditions.

GA_3 and GA_4+GA_7 have been reported to promote germination of rice seeds at low temperatures[3], while growth of the seedlings was also promoted synergistically by the combination of ethephon and gibberellin.

(2) Ethylene evolution from rice seeds

Rice seeds were selected by specific gravity with NaCl(1.1sq). The seeds were then soaked in water for 72h, or soaked in water for 48h and in solutions of plant growth regulators for 24h. Thirty seeds each were placed in 30ml sealed glass tubes with a wet filter paper on the bottom and filled with gases containing different concentrations of oxygen. The tubes were kept in the dark at 20℃ or 30℃. The gas in the tubes was exchanged for new gas every 24h. The ethylene in the gas samples was chromatographed at regular intervals. A high level of ethylene is produced during the first 24h of the experiment just prior to radicle protrusion and thus may be important for germination. This is conceivable because ethylene promotes the release of hydrolase from the endosperm cell wall of *Hordeum vulgare* seeds[2].

Table 4. Ethylene evolution from rice seeds incubated at 20℃ for 6h after the treatment with water, methionin, ACC and ethephon.

Treatment		Ethylene evolution (nl/30seeds)
Water		2
Methionin	70ppm	4
Methionin	200ppm	6
ACC	70ppm	4
ACC	200ppm	8
Ethephon	20ppm	21

The seeds soaked in water, or treated with methionin, or ACC at 20~70ppm produced ethylene at 2, 4~6 and 4~8nl/30seeds during the first 6h(Table 4). However, these compounds did not significantly promote the establishment of the seedlings in the pot-test. These results suggest that methionin and ACC might have not been efficiently metabolized to ethylene in the seeds. The results also suggest that HCN, a by-product of the ethylene biosynthetic pathway, might have not been metabolized in unhealthy rice seeds seeded in submerged soil.

Ethephon produced ethylene in the seeds and also might have stimulated ethylene evolution from the seeds treated with or without ethephon, and was larger at high temperatures and under high oxygen conditions(Table 5, 6). GA_3 increased ethylene production slightly compared to non-treatment and did not increase the rate of ethylene production induced by ethephon(Table 7).

However, the combination of GA_3 and ethephon showed a synergistic effect on the promotion of both the elongation of the coleoptile and the establishment of the seedlings. These results suggest that GA_3 promoted ethylene action probably through increasing physiological activity of the seeds and coleoptiles under low temperature conditions. Utilization of ethephon, gibberellins and TFIBA might be a possible technique for the promotion of the emergence and establishment of rice seeds seeded in soil in paddy fields in Japan.

Brassinosteroids, ABA and jasmonic acids showed promotive effectiveness on the emergence and establishment of rice seedlings probably through increasing ethylene production in the seeds[1]. These compounds need more detailed study for their utilization.

Direct-sowing cultivation can also be promoted using compounds such as slow ethylene-releasing type compounds, temperature dependant ethylene producers like TFIBA, and physiological activity-increasing type compounds such as GA_3. It is to be desired that these compounds should not produce a lot of HCN in a short term in the seedlings[5,8].

Table 5. Ethylene evolution from rice seeds(cv. Sasanishiki) incubated for 0-6h, 24-30h and 48-52h at 20℃ and 30℃ after the treatment with or without ethephon.

Incubation time after the treatment	Ethephon evolution(nl/30seeds)			
	20℃		30℃	
	Water	Ethephon	Water	Ethephon
0-6h	1.0	7.8	2.7	11.5
24-30h	0.5	3.8	2.1	7.5
48-52h	0.3	3.5	1.7	6.0

Table 6. Ethylene evolution from rice seeds(cv. Sasanishiki) incubated in gas containing different oxygen-concentrations for 6h at 20℃ after the treatment with or without ethephon.

Composition of gas	Ethylene evolution (nl/300seeds)	
	Water	Ethephon
Oxygen 0% + N_2 100%	2.5	13
Oxygen 10% + N_2 90%	4.3	14
Air(Oxygen 21%)	4.5	18
Oxygen 90% + N_2 10%	5.8	20

Table 7. Ethylene evolution from rice seeds incubated at 20℃ for 6h after the treatment with water, GA_3 and the combination of GA_3 with ethephon.

Treatment	Ethephon evolution (nl/30 seeds)
Water	2
GA_3 1ppm	3
GA_3 10ppm	4.5
Ethephon 20ppm	18
Ethephon 20ppm + GA_3 1ppm	20
Ethephon 20ppm + GA_3 10ppm	18

2. Effects of rice seeds and seedlings on seasonal variations in emergence and growth of *Monochoria vaginalis.*

M. vaginalis is a very serious aquatic weed in paddy fields, growing vigorously and causing a reduction in rice yields because of its heavy competition with the crop for nutrients, light, and space. When the seeds were harvested in late October, they did not germinate in light or dark, with or without submerged conditions because of the mechanical restriction of the seed coats and the dormancy of the embryo. Seeds collected in a paddy field in early March, the dormancy of the embryo was broken as a result of exposure to low temperature during the winter, The seeds were dried and stored 2 to 3 months at 5℃. They germinated in the light under flooded condition, but they did not germinate in the dark under flooded or unflooded conditions. When the seed coats were punctured near the radicle, seeds germinated in the light and dark without flooding. Seed germination was not induced in the dark by auxins, gibberellins, cytokinins, ethylene or brassinosteroids, with or without flooding. However, germination was induced by ethylene in the light without flooding[9]. Seed germination was also induced by cotylenins in the dark without flooding[9]. Then effects of rice seeds and seedings on seasonal variations in the emergence and growth of *M. vaginalis* were studied in detail and the following results were obtained.

(1) Greenhouse experiment

An experiment using plastic trays(35×28.5×25cm) was conducted in a greenhouse in early summer. Plastic trays were filled with paddy field soil, and 22 seeds/tray of *M.vaginalis* seeds were sown at a 1cm depth in the soil. Pregerminated 3 day-old rice seeds(cv. Tsukinohikari), and seedlings grown for 10 days(2.0~2.2 leaf stage), 20 days(3.8~4.0 leaf-stage) and 30 days(4.2~4.5 leaf stage) were planted at densities of 5, 10 and 20 plants per tray. The depth of irrigation water in the plastic trays was maintained at 5cm during incubation. These trays were placed in the greenhouse and the number of the emerged weeds was counted 1, 2, 3, 4, 5 and 7 weeks after planting.

In the absence of rice plants, *M. vaginalis* emerged at 4.3, 15.4, 17.3, 21.1, 21.6 and 21.9% after incubation for 1, 2, 3, 4, 5 and 7 weeks, respectively. By contrast, as shown in Figs. 4-7, the number of the emerged weeds increased in the presence of rice seeds and 10 day-old(infant) rice seedlings, in particular during 1 to 3 weeks of the incubation period. After incubation for 7 weeks, the number of weeds increased in the presence of rice seeds and 10 day-old rice seedlings by 22~47% and by 17~23%, respectively, These results show that rice seeds and 10 day-old rice seedlings promoted the emergence of *M.*

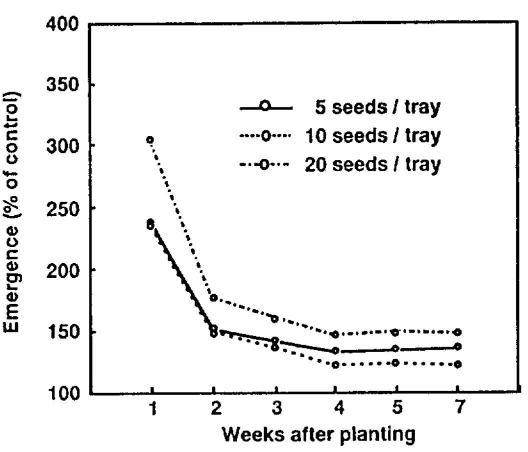

Fig. 4. Effect of pregerminated rice seeds on emergence of *M. vaginalis*.

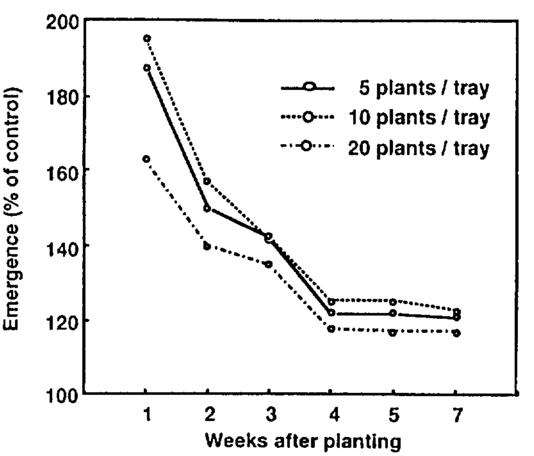

Fig. 5. Effect of 10 day-old rice seedlings(2.0-2.2 leaf stage) on emergence of *M. vaginalis*.

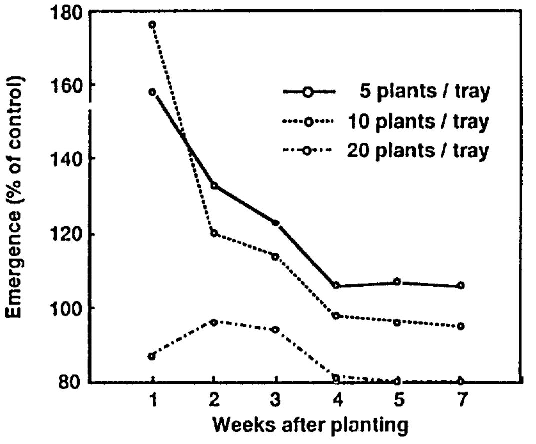

Fig. 6. Effect of 20 day-old rice seedlings(3.8-4.0 leaf stage) on emergence of *M. vaginalis.*

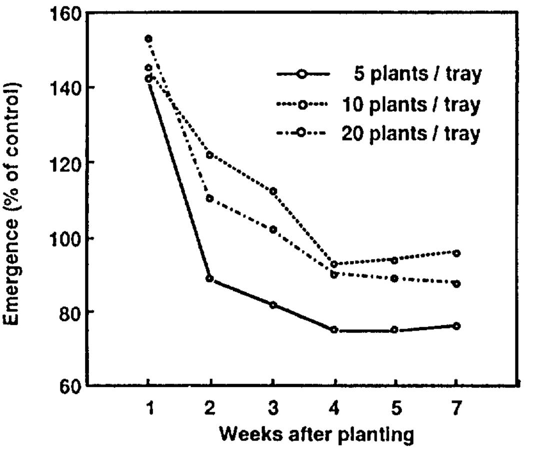

Fig. 7. Effect of 30 day-old rice seedlings(4.2-4.5 leaf stage) on emergence of *M. vaginalis.*

Table 8. Effects of rice plants on emergence and growth of *Monochoria vaginalis.*

Rice planting system	Number of weed (per m^2)	Dryweight of weed	
		(g/m^2)	(mg/plant)
No rice plant(control)	116.0±16.6	10.1±4.7	86
Dry seed			
2.5 g/m^2	204.7±37.5	21.0±6.2	102
5.0 g/m^2	166.7±15.5	17.3±3.8	104
Pre-germinated seed			
2.5 g/m^2	155.7±19.7	12.9±2.6	83
5.0 g/m^2	187.0±25.8	17.1±2.0	91
Infant seedling*			
80 plants/m^2	215.3±27.7	19.3±2.1	90
Young seedling**			
80 plants/m^2	152.3±19.3	10.2±1.4	67

* 2.0 leaf-stage, ** 3.2 leaf-stage

vaginalis. Fig. 6 shows the effects of 20 day-old rice seedlings on the germination of the weed. The number of emerged weeds increased in the presence of 20 day-old rice seedlings at the densities of 5 and 10 plants per plot, but decreased at 20 plants per plot. The effects of 30 day-old rice seedlings on the emergence of the weeds were shown in Fig. 7. During the first 3 weeks of the incubation period, the emergence of the weeds was promoted in the presence of such rice seedlings, whereas after 4 weeks the emergence was inhibited. The number of the emerged weeds then decreased by 4~25% after 7 weeks of incubation.

A field experiment was conducted to evaluate the effects of rice plants at different growth stages on the emergence and early-growth of *M. vaginalis* in early summer. The emergence of the weed was promoted in all the plots where rice plants were growing. In addition, the dry weight of the weeds per plot increased in the presence of rice plants, except for the plots where young seedlings(3.2 leaf-stage) had been transplanted. The growth of the weed in terms of dry weight per plant was stimulated only in the plots where dry or pre-germinated rice seeds had been sown.

By contrast, in the plots where infant seedlings (2.0 leaf-stage) had been transplanted, the dry weight per plant was almost equal to that of the control plots. Furthermore, the growth of the weed was inhibited in the plots where young seedlings(3.2 leaf-stage) had been transplanted (Table 8). These results indicate that rice seeds and infant seedlings at the 2 leaf-stage promote the germination and growth of *M. vaginalis*. It has been reported that the germination of *M. vaginalis* was stimulated by ethylene and carbon dioxide under low-oxygen conditions. Furthermore, we found some water-soluble substances released from rice seeds and young seedlings induced germination of *M. vaginalis*. Water extracts from rice seeds were subjected to solvent partitioning, ODS, gel filtration and ion-exchange column chromatographies. The stimulant(s) appeared to be highly water soluble cationic compound(s) of relatively low molecular weights.

In paddy fields, rice seeds and seedlings release ethylene and carbon dioxide and consume oxygen in the irrigation water and soil. In paticular, more ethylene was released from germinating rice seeds and younger seedlings than from older plants. Therefore all of these may play an important role in inducing the germination of the weed[4,7].

REFERENCES

1. Anonymous 1995. Annual report of studies of plant growth regulators in summer crops(Japan Association for Advancement and Phyto-regulators) p.1-134.(in Japanese)
2. Ho, T.D., J. Abramsand, J.E. Varner 1982. Effect of ethylene on the release of α-amylase through cell wall of barley aleuron layers. Plant Physiol., 69 : 1128
3. Katsuta, M. and Y. Ota. 1983. Improvement of seed germination of rice under low temperature conditions using gibberellins. Japanese Journal of Crop Science 52(Suppl.2), 186~187.(in Japanese)
4. Kawaguchi, S., Y. Takeuchi, M. Ogasawara, K. Yoneyama, T, Maeda and M. Konnai 1995. Effects of rice(*Oryza sativa* L.) on the seasonal variation in emergence and growth of *Monochoria vaginalis* var. *plantaginea*. Proc. of 15th Asian-pacific Weed Science Society Conference p.725~730
5. Komoto, H., Y. Takeuchi, M. Ogasawara, K. Yoneyama and M. Konnai 1995. Application of ethephon for direct-sowing rice in flooded paddy soil. Proc. of 30th annual meeting of Society for chemical Regulation of Plants p.80~81.(in Japanese)
6. Ku, H.S., H. Suge, K. Lappaport and H.K. Pratt. 1970. Stimulation of rice coleoptile growth by ethylene. Planta 90 : 333~339.
7. Momoki, Y. 1992. Effect of ethylene and carbone dioxide on seed germination of *Monochoria vaginalis* var. *plantaginea*. Weed Res. Japan. 40 : 221~224.
8. Takeuchi, Y., H. Komoto, M. Ogasawara, K. Yoneyama and M. Konnai 1996. Proc. of 21th Annual Meeting of Japan Pesticide Science Society p.115~116.
9. Takeuchi, Y., T. Sassa, S. Kawaguchi, M. Ogasawara, K. Yoneyama and M. Konnai 1995. Stimulation of germination of *Monochoria vaginalis* seeds by seed coat pancture and cotylenins. Weed Res. Japan. 40 : 221~224.