Korean J Crop Sci 49(5) 363~367(2004)

Ethylene Production and Accumulation in Leaf Sheath and Its Relation to Tillering Suppression of Deep-Irrigated Rice Plants

Eul-Jae Myung*, Yong-Woong Kwon, and Byun-Woo Lee†**

*Dongbu Fine Chemicals Co. Ltd, Hwasung gun 441-960, Korea
**Department of Plant Science, Seoul National University, Seoul 151-921, Korea.

ABSTRACT: The deep irrigation of rice plants brings about some beneficial effects such as reduced tiller production which results in the formation of bigger panicles, prevention of chilling injury, reduced weed growth, etc. The present study was carried out to examine the involvement of ethylene in the suppression of tiller production due to deep water irrigation in rice (cv. Dongjinbyeo). The ethylene production was induced in leaf sheath within 24 hours after the deep water irrigation and has increased even until 30 days after the treatment, recording 4.5-fold increase as compared to the shallow-irrigated rice plants. In the deep water irrigated rice plants, ethylene was accumulated to a high concentration in the air space of submerged leaf sheath as the irrigated water deterred the diffusion of ethylene out of the leaf sheath and ethylene biosynthesis was accelerated by the deep irrigation as well. The ethylene concentration recorded 35-fold increase in the deep-irrigated rice plants for 35 days. The tiller production was reduced significantly by the deep irrigation with water, the tiller bud, especially tertiary tiller bud differentiation being suppressed by the deepwater irrigation treatment, whereas the rice plants deep-irrigated with solutions containing 10^{-5} M or 10^{-6} M silver thiosulfate (STS), an action inhibitor of ethylene, showed the same or even higher production of tillers than those irrigated shallowly with water. This implies that the ethylene is closely linked with the suppression of tiller production due to deep water irrigation. In conclusion, ethylene, which was induced by hypoxic stress and accumulated in the leaf sheath due to submergence, played a key role in suppressing the tiller production of the deepwater irrigated rice.

***Keywords*:** rice, deep irrigation, tiller, ethylene, silver thiosulfate

Deep water irrigation has been a practice of long standing for the weed control and the prevention of chilling injury during the panicle formation stage. Recently, the deep water irrigation has been investigated as a technical system for improving the yield by suppressing the overgrowth of rice, particularly, non-productive tillers (Ohe *et al*, 1994).

†Corresponding author (Phone) +82-02-880-4544 (E-mail) leebw@snu.ac.kr <Received August 6, 2004>

When the leaf sheath portion is submerged by the deep water irrigation, rice makes the morphological, anatomical, and biochemical changes in order to get out of the inferior environments. In case of floating rice, the internode elongation becomes vigorous to avoid stress from insufficient oxygen due to the submergence, and the rice grows continuously by letting its leaf out above the water surface (Abeles *et al.*, 1992; Vartapetian, 1993). Kende and others (Raskin & Kende, 1984a; Satler & Kende, 1985; Stunzi & Kende, 1989) clarified the internode elongation mechanism of floating rice through a series of studies. As submergence creates hypoxic conditions and increases the partial pressure of carbon dioxide in the internode of floating rice, the production of ethylene is accelerated greatly in the internodes of rice plants (Raskin & Kende, 1984b). Moreover, ethylene accumulates to very high levels in the submerged plant tissues because its diffusion rate in water is 10,000-fold lower than that in the air (Jackson, 1985; Kawase, 1976). This ethylene enhances GA activity in intercalary meristem of internode at least in part by a reduction in ABA content (Raskin & Kende, 1984b) and also raises endogenous GA (Hoffman & Kende, 1992). The rapid internode elongation would be regulated by the altered balance between the counteracting hormones of GA and ABA.

Deepwater irrigation in rice cultivation generally suppresses tiller production, the number of tiller being greatly reduced as the water depth of irrigation becomes greater (Sato, 1951; Hanada, 1983; Myung, 1997). The growth of tiller buds which are subtended within the leaf sheath completely submerged up to leaf collar, are suppressed. However, the elongation of the main culm and the leaf blade and sheath of tiller already protruded out of the leaf sheath is accelerated (Hanada, 1983). Tiller bud differentiation is not hindered, but its growth is retarded due to the leaf sheath submerge (Hanada & Kagawa, 1985). It is reported that the production of tillers is suppressed but the size of panicles born at the tillers produced becomes greater in the deep water irrigation (Ohe *et al.*, 1994; Myung, 1997). As described in the above, most studies on the deep water irrigation have been mainly focused on the phenomenal studies, but few studies have been conducted to clarify the cause of the tillering suppression phenomenon which is most characteristic in the deep-irrigated rice. Accordingly, the present study was carried out to clarify the cause of the suppression of tiller production due to deepwater irrigation. We examined the production and accumulation of ethylene in leaf sheath of rice deep-irrigated with water and the tiller production of rice plants deep-irrigated with water and silver thiosulfate solution which is an action inhibitor of ethylene.

MATERIALS AND METHODS

Plant material, culture condition and treatments

The following experiments were carried out by employing a japonica rice variety (Dongjinbyeo) in glasshouse in 1997. Two presoaked seeds were sown in a plastic pot (10 cm in diameter and 15 cm deep) filled with commercial nursery soil for rice seedling rearing. Seedlings were thinned to one per pot after seedling establishment Pots were placed in a box filled with water up to 3 cm above pot soil surface and grown in this condition until deep irrigation treatments. In deep irrigation treatments, the rice plants were submerged up to the leaf collar of the leaf sheath subtending the currently-emerging leaf on the main culm. In shallow irrigation treatment, water level was maintained at 3 cm above soil surface.

Experiment 1: To investigate the induction and production of ethylene due to deep water irrigation, rice plants which have been grown in shallow-irrigated condition were subjected to deep water irrigation of different durations. Deep irrigation treatments were started at 0, 24, 48, 72, and 720 hours before plant sampling. Plant samples were taken on the same date at which rice plants have 9 leaves on the main culm. Four plants were sampled and the leaf sheath was chopped into pieces of about 5 mm. The chopped samples of 10 g were transferred to a 50-ml airtight container, and incubated at 25°C. Before incubation air inside the container was removed with syringe. After 20 hours,. 1ml of gas was withdrawn with a 1-ml syringe to analyze the ethylene.

Experiment 2: To investigate the ethylene accumulation in the leaf sheath due to deep water irrigation, deep water treatments were imposed for 0, 36, 60, 84, and 732 hours before plant sampling at 9 leaf stage of the main culm. Four plants for each treatment were sampled and leaf blades were removed under water. The air locked in the leaf sheath part was squeezed out by hand and roller, and trapped in a beaker completely submerged upside down so that no external air can be allowed according to Michiyama & Saka (1988). The air trapped was withdrawn with a syringe and used for the measurement of air space volume and ethylene concentration in leaf sheath.

Experiment 3: Deep irrigation treatments with water and STS solution that is known as an action inhibitor of ethylene were made to compare the tiller bud differentiation and growth The STS solutions were prepared by mixing silver nitrate and sodium thiosulfate of the same mole concentration at the ratio of 1 : 4 (Abeles *et al.*, 1992). The STS solutions of 1×10^{-4} M, 1×10^{-5} M, and 1×10^{-6} M were used for the treatments. Deep irrigation treatments with water and STS solutions were started from 18 days after seeding and lasted 41 days. The entire STS solutions were exchanged every 7-day. The experiments were done with 6 replications. The number of tiller and the length of tiller buds were investigated periodically. Only the tillers that protruded out of the sheath wrapping them were included in the tiller number counting.

Ethylene measurement

Ethylene was analyzed by gas chromatograph (Chrompack, CP9001) equipped with flame ionization detector. A stainless steel column (0.216 cm in inner diameter and 1.8 m in length) filled with Porapak-Q (80/100 mesh) and helium as carrier gas were used. The temperature of injector and detector were maintained at 120 °C and that of oven at 80 °C in the measurement. 1 ml of air sample was injected by using a 1 ml disposable syringe.

RESULTS

Ethylene induction and production of deep-irrigated rice plant

The leaf sheath of rice plants subjected to deep irrigation for different durations was incubated for 20 hours at 25 °C and ethylene was measured to investigate the timing of ethylene induction due to submergence and its production rate. Ethylene production rate was 11.4 nl $g^{-1}FW\ hr^{-1}$ in shallow-irrigated rice plant. Ethylene production rate was enhanced significantly by deep irrigation, increasing as the duration of deep irrigation prolonged. As compared to the shallow-irrigated rice plant (0 hour deep irrigation), ethylene production rate was increased by 2.4-, 2.5-, 2.8-, and 4.5-fold in the rice plants deep-irrigated for 24, 36, 72, and 720 hours, respectively. Therefore, it was surmised that the ethylene production is induced within 24 hours at least by the deepwater treatment and maintains at higher rates continuously.

Ethylene accumulation in leaf sheath subjected to deepwater irrigation

The air space volume and the ethylene concentration and content were measured in the leaf sheath of rice plants subjected to different durations of deepwater irrigation treatment (Table 1). At the time of plant harvest for ethylene determination rice plant had 11 tillers and fresh weight of 5.4 g on average. The volume of air squeezed out of the leaf sheath increased as the duration of the deepwater treatment increased. The volume of the air in the leaf sheath with shallow irrigation measured 2.9 ml plant^{-1}. Upon the deep irrigation of 36, 60, and 84 hours, 1.6-, 1.9-, and 2.0-fold increases were found, respectively. The deep-irrigated rice plants for 30 days showed even greater increase of 2.7-fold. This means that the longer the duration of the deepwater treatment was, the greater the air space in the leaf sheath was formed. Hypoxia and ethylene caused by deepwater irrigation might have caused cell death to form greater air space (Jackson & Armstrong, 1999; Drew *et al.*, 2000). The ethylene concentration measured 27 nl l^{-1} in the air of the leaf sheath subjected to continuous shallow irrigation. Ethylene concentrations significantly increased as the duration of the deepwater irrigation increased, recording 1.9-, 6.6-, 13.4- and 35-fold increase in the air of the leaf sheath with deep irrigation treatments for 36, 60, 84, and 732 hours, respectively. The ethylene contents in the leaf sheath that were calculated by multiplying the air volume by the ethylene concentration showed 2.9, 12.0, 26.6, and 92-fold increase, respectively. This implies that ethylene accumulation have been accelerated with the increased duration of deepwater irrigation.

Tiller production in response to deep irrigation with water and STS solution

The deep irrigation treatments with water and STS solution, an ethylene action inhibitor, were imposed to rice plants in their active tillering stage. As in Table 2 and 3, a significant decrease in tiller production has been found in the deep irrigation with water since 11 days after treatment as compared to the shallow irrigation. The reduced number of tillers in the deepwater irrigation was mainly due to the result of the decreased differentiation of secondary tillers (Table 3). This is not in agreement with the report by Hanada & Kagawa (1985) that the differentiation of tiller buds is not hindered but their growth is retarded. However, Rice plants subjected to the deep irrigations with STS solutions of 10^{-5} M and 10^{-6} M produced as many tillers as the shallow-irrigated rice plants with water except that the deep irrigation with STS solution of 10^{-4} M caused severe phytotoxicity to decrease the tiller production. It could be inferred from these results that the suppressed tiller production due to deepwater irrigation is closely related to the physiological actions of

Table 1. Concentration and content of ethylene in the air space of leaf sheath of rice plants imposed to deepwater irrigation for different durations.

Treatment duration (hours)	Fresh weight of sheath (g/plant)	Volume of air space (ml/plant)	Ethylene concentration. (nl/l)	Ethylene content (nl/plant)
0	5.6	2.9e	27 0e	0.08e
36	5.2	4 5d	51 5d	0.23d
60	5.7	5 4c	178.4c	0.96c
84	5.4	5 9b	361.4b	2 13b
732	5 3	7.8a	944 5a	7.37a

Within a column, means followed by the common letters are not significantly different at 5% level by DMRT.

Table 2. Changes in the tiller number of rice plants deep-irrigated with water and silver thiosulfate (STS) solutions of different concentrations.

Treatment	Days after treatment				Tillering rate (tillers/10-day)
	0	6	11	21	
	No. of tiller				
STS 10^{-4} M	2.0a	2 8a	3.0c	4.3c	1.1 (36.7)
STS 10^{-5} M	2 0a	3 0a	5.0a	9.3a	3.5 (116 6)
STS 10^{-6} M	2.0a	3 0a	5.0a	9.5a	3.6 (120 0)
Deep water	2 0a	3 0a	3.5bc	6 3b	2.0 (66 7)
Shallow water	2.0a	3.0a	4 0b	8.3a	3.0 (100)

Within a column, means followed by the common letters are not significantly different at 5% level by DMRT.
Deep irrigation with water and STS solutions were initiated from 18-day old seedling stage and plant were submerged up to the n-2nd leaf collar from the currently-emerging leaf (n) of the main culm.

Table 3. Effects of deep-irrigation with water and silver thiosulfate (STS) solutions of different concentration on the number of tillers and tiller buds at 41 days after treatments.

Treatment	Primary		Secondary		Tertiary		Total	
	Tiller	Bud	Tiller	Bud	Tiller	Bud	Tiller	Tiller + Bud
STS 10^{-4}M	3.5c	3.5a	0.0c	6.5	0.0	0.3c	4.5c	15.8c
STS 10^{-5}M	6.0a	1.0b	9.0a	5.3	0.3	1.3bc	16.3a	23.9a
STS 10^{-6}M	5.8ab	1.0b	9.8a	5.0	1.3	3.0a	17.9a	26.9a
Deep water	5.0b	1.5b	5.0b	4.8	0.3	2.3ab	11.3b	19.9b
Shallow water	6.0a	1.0b	9.3a	3.8	1.0	2.8ab	17.3a	24.9a

Within a column, means followed by the common letters are not significantly different at 5% level by DMRT.
Deep irrigation with water and STS solutions were initiated from 18- day-old seedling stage and plant were submerged up to the n-2th leaf collar from the currently-emerging leaf (n) of the main culm.

ethylene induced and accumulated in the leaf sheath by the deepwater irrigation.

DISCUSSION

The reduced production of tillers due to deepwater irrigation is well documented in rice (Hanada & Kagawa, 1985; Ohe *et al.*, 1994; Myung, 1997). However, the causal factors to suppress the tiller production of deep-irrigated rice plants have not been addressed yet. The present study was to clarify the possible involvement of ethylene in suppressing the tiller production of deep-irrigated rice plants. Ethylene production is reported to be induced in floating rice, deep water rice and lowland rice cultivar by hypoxia caused by flooding (Stunzi & Kende, 1989; Metraux & Kende, 1983), and the induction of ethylene production by hypoxia is caused by the enhanced activity of 1-aminocyclopropane-1-carboxilic acid (ACC) synthase, a key enzyme in the ethylene biosynthetic pathway and encoded by multigene family (Straeten *et al.*, 1997). In agreement with these previous reports, the ethylene production was induced in the submerged leaf sheath within 24 hours after the deepwater irrigation. The rate of ethylene production has increased even until 30 days of deepwater irrigation, showing 4.5-fold increase as compared to the shallow-irrigated rice plants (Fig. 1). The ethylene concentration of the air entrapped in the submerged leaf sheath have almost doubled within 36 hours after deep water irrigation and has showed accelerated increase, recording 35-fold higher concentration as compared to the shallow-irrigated rice plants (Table 1). Stunzi & Kende (1989) also reported that the ethylene concentration of the air in the lacunae of sheath and internode increased rapidly up to 1 $\mu l/l$ at 72 hours after submergence, showing 50-fold increase as compared to non-submerged floating rice. Ethylene accumulated to a very high concentration in the air space of submerged leaf sheath as not only the ethylene production was accelerated by hypoxia caused by the deep water irrigation

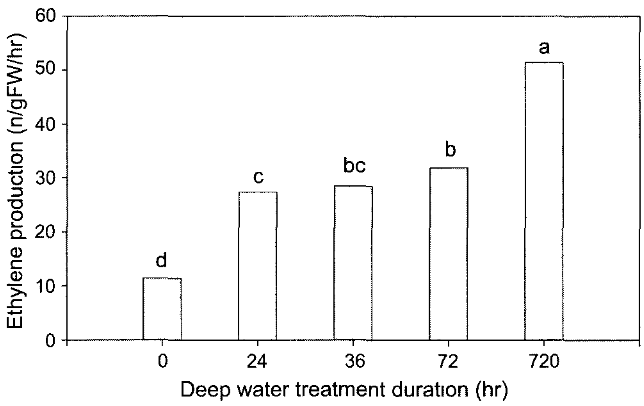

Fig. 1. Ethylene production of leaf sheath of rice plants imposed to deepwater irrigation for different durations before plant harvest for ethylene determination Rice plants were sampled on the same day and incubated for 20 hours at 25 °C before ethylene determination The common letters on the bars do not differ significantly at 5% level by DMRT.

(Fig. 1) but also the deep-irrigated water deterred the diffusion of ethylene out of micro-pores in the leaf sheath. Gases inside the rice plant are discharged through the micro-pores on the leaf sheath, not through stomatal openings (Nouchi, 1994). Thus, the deepwater irrigation treatment in this experiment that submerged the rice plants up to the leaf collar of the leaf sheath subtending the currently-emerging leaf on the main culm might have blocked the micropores with water in which gases such as oxygen and ethylene shows 10,000-fold lower diffusion rate than that in the air (Jackson, 1985; Kawase, 1976), creating hypoxia because the rate of O_2 consumption outpace the supply rate and accumulating ethylene inside the leaf sheath. Tiller production of rice plants deep-irrigated with STS solution, an action inhibitor of ethylene, was examined to know whether the submergence-induced accumulation of ethylene in the leaf sheath is linked with the suppression of tiller production The rice plants deep-irrigated with solutions containing 10^{-5} M or 10^{-6}

M silver thiosulfate (STS) produced the same or even more tillers as/than those irrigated shallowly with water, whereas the rice plants deep-irrigated with water produced significantly less tillers (Table 2 and 3). This implies that the ethylene is closely associated with the suppression of tiller production due to deep water irrigation. In conclusion, ethylene, which was induced by hypoxic stress and accumulated in the leaf sheath due to submergence, played a key role in suppressing the tiller production of the deep-irrigated rice. Further experiments, however, are still needed to clarify the physiological roles of ethylene entrapped and accumulated to a high concentration in the air space of leaf sheath in hindering the differentiation and growth of tiller buds.

REFERENCES

Abeles, F. B., P. W. Morgan, and J. M. E. Saltveit. 1992. Ethylene in plant biology. Academic Press, New York pp 414.

Drew, M. C , C. J He, and P W. Morgan. 2000. Programmed cell death and aerenchyma formation in roots. Trend in Plant Science 5(3) · 123-127.

Hanada, K. 1983. Growth of internodes, leaves and tillers of floating and non-floating rice varieties under deep water conditions Japan, J. Trop. Agri. 27 . 221-236.

Hanada, K. and K Kagawa 1985. Growth of tiller buds of a floating rice variety under deep water with special reference to submergence of the subtending leaf sheaths. Japan J. Trop. Agri 29(1) : 9-16.

Hoffman, S. and H Kende 1992. On the role of abscisic acid and gibberellin in the regulation of growth in rice Plant Physiol 99 : 1156-1161.

Jackson, M. B. 1985. Ethylene and responses of plants to soil waterlogging and submergence Ann Rev Plant Physiol 36 · 145-174

Jackson, M. B and W Armstrong. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biology 1(3) . 274-287.

Kawase, M. 1976. Ethylene accumulation in flooded plant. Physiol. Plant. 36 · 236-241.

Metraux, J. D and H. Kende. 1983 The role of ethylene in the growth response of submerged deepwater rice. Plant Physiol. 72 . 441-446

Michiyama, H. and H. Saka. 1988 Endogenous ethylene levels in leaf sheath and panicle of rice plant. Japan. J. Crop Sci. 57(2) : 366-370.

Myung, E. J. 1997. Suppression of tillering in the deep-irrigated rice plants and its mechanism. Ph.D. dissertations, Graduate School, Seoul National University

Nouchi, I 1994 Mechanisms of methane transport through rice plants. pp. 87-104. In CH_4 and N_2O: Global Emissions and Controls from Rice Fields and Agricultural and Industrial Sources, NIAES, Japan

Ohe, M , Y. Goto, and K. Hoshikawa. 1994. Effects of the deep water treatments on the emergence of tillers of rice plant. Japanese J. Crop Sci. 63(4) 576-581.

Raskin, I. and H. Kende. 1984. Regulation of growth in stem sections of deep-water rice. Planta 160 : 66-72.

Raskin, I and H Kende. 1984 Role of gibberellin in the growth response of submerged deep water rice Plant Physiol. 76 : 947-950.

Satler, S O and H Kende 1985 Ethylene and the growth of rice seedling Plant Physiol.79 194-198.

Sato, K 1951 Studies on the flooding irrigation for rice 2. Effect of continuous submergence on the growth and tillering of rice plants. Proc Crop Sci. Soc. Japan 20 . 41-44.

Straeten, D. V. D., S. Anuntalabhochai, W. V. Caeneghen, Z. Zhou, J. Gielen, and M. V. Montagu. 1997. Expression of three members of the ACC synthase gene family in deepwater rice by submergence, wounding and hormonal treatments. Plant Sci. 124 · 79-87

Stunzi J T and H. Kende 1989. Gas composition in the internal air spaces of deep water rice in relation to growth induced by submergence. Plant Cell Physiol. 26 . 607-614.

Vartapetian, B B 1993 Plant physiological responses to anoxia. *In* Boxton *et al* (ed) International Crop Science I CSSA, Madison, Wisconsin. USA. pp. 721-726.