Effects of combined application of ethephon and gibberellin on growth and nutrient uptake of rice seedlings growing under direct seeding conditions

Hajime Watanabe and Masahiko Saigusa

Field Science Center, Graduate School of Agricultural Science, Naruko, Miyagi 989-6711, Japan. Email watanabe@bios.tohoku.ac.jp

Abstract

Seedling establishment is one of the most important agronomic traits in direct seeding rice cultivation. We investigated the effects of two plant growth regulators (PGRs), gibberellic acid (GA_3) and ethephon (ET), on seedling growth under flooded soil conditions. Seedling growth was increased by a single treatment of GA_3 or ET over that of the control. However, effects of combined applications of GA_3 and ET were more pronounced than that of GA_3 or ET alone at both growing temperatures (15 and 20?C). The growth of different organs of rice seedlings, such as coleoptiles, first leaves and second leaves were also increased by PGR treatment. No significant differences were found in nitrogen concentration and the ratio of shoot dry weight to shoot length of the seedlings among the treatments. Taken together, a high seedling establishment rate in direct seeding cultivation in the cold regions of Japan will be possible by using proper combinations of PGRs.

Media summary

The growth regulation of target organs of rice seedlings will be possible by using proper combinations of plant growth regulators.

Key Words

Direct seeding, ethylene, gibberellin, nutrient uptake, rice, seedling.

Introduction

Seedling establishment is one of the most important agronomic traits in direct seeding rice cultivation. It has been considered that plant hormones, such as gibberellin (GA), ethylene and abscisic acid (ABA), have promotive effects on rice seedling organs. However, the effects of these plant hormones are occasionally diverse due to various environmental factors, including temperature and flooding depth. As well, synergistic (plus) or counteracting (minus) plant hormone interaction can be found in several growth systems of plants (Davies, 1995). A notable case of the former interaction is internode elongation in deep-water rice. Ethylene promotes the growth of internodal tissue of deep-water rice, which responds to flooding by rapid elongation induced by ethylene formation (Metraux and Kende, 1983). Ethylene promotes growth in part by increasing the responsiveness to the internodal tissue to GA, and appears to do so by causing a reduction in the endogenous levels of ABA, so that the growth rate is determined by the relative levels of endogenous GA and ABA, a potent antagonist of GA (Kende et al., 1998). Here, we investigated the effects of single or combined applications of ethylene and GA on the growth of different organs of rice seedlings growing under different temperatures and flooding conditions.

Material and Methods

The cultivar used in the present study was Kokoromachi (*Oryza sativa* L.), a japonica type. The seeds were sterilized with a Benlate T^{TM} solution and then immersed in water, subsequently washed by water, and then soaked in the test solution. Ethephon (2-chloroethylphosphanic acid, Ishihara Sangyo Kaisha, LTD, Osaka, Japan) was used as an ethylene-releasing agent, and GA_3 (Sigma Chemical Co., MO, USA) was used for gibberellin. The components of the test solution were as follows; 1) Water alone (control), 2) 50 ppm ethephon (ET), 3) 100ppm GA_3, 4) 50 ppm ET+100ppm GA_3. After treatment with the plant hormone solution, the seeds were again immersed in water to remove any excess test solution. The imbibed seeds were geminated in water at 30?C in the dark, and the germinated seeds sown at 1 cm of seeding depth in seedling pots with small compartments containing nursery soil (Kureha Chemical Co., LTD. Tokyo, Japan). The seeds were allowed to grow at 15 or 20℃ in continuous light conditions. The flooding depths (FD) were 2 and 5 cm in each experiment. Nutrient uptake of rice seedlings was determined using a NC-analyzer (Sugigraph NC-80). The experiments were done with 4 replications.

Results and discussion

With a growing temperature of 15?C

The plant height was significantly increased by all PGR treatments tested compared with that of control at 2cm of FD (2FD) (Fig. 1A), whereas, the effect of combined application of ET+GA_3 was more pronounced than those of ET and GA_3 alone at 5cm of FD (5FD) (Fig. 1B). ET or GA_3 alone at 2FD did not stimulate mesocotyl growth; however, a combination of ET and GA_3 treatments significantly increased mesocotyl growth at 2FD (Fig. 1A). At 5FD, all PGR treatments increased significantly mesocotyl length, with the maximum elongation caused by the pairing of ET and GA_3 (Fig. 1B). For coleoptiles, the trends of the effects of PGR treatments on elongation were similar to those for mesocotyls at both flooding depths (Fig. 1). Interestingly, the synergistic effect of a combined application of ET and GA_3 was also observed as with the case of mesocotyl growth (Fig. 1A and B). In the first leaf, which mainly consists of a leaf-sheath, ET alone and ET+GA_3 treatments showed significant increases in the growth at both flooding depths (Fig. 1). In the second leaf, significant growth-promoting effects were observed by GA_3 alone and ET+GA_3 applications over that of the control at 2FD (Fig. 1A). In addition to these treatments, GA_3 alone also significantly increased 2nd leaf growth at 5FD (Fig. 1B). No significant difference in the ratio of shoot dry weight to shoot length (RWL) was observed among the PGR treatments at both FDs (Table 1), indicating that the promotive effects of PGR treatments on rice seedlings are not merely a spindly growth; as an increase in shoot growth by PGRs treatments were accompanied by the enhancement of dry weights.

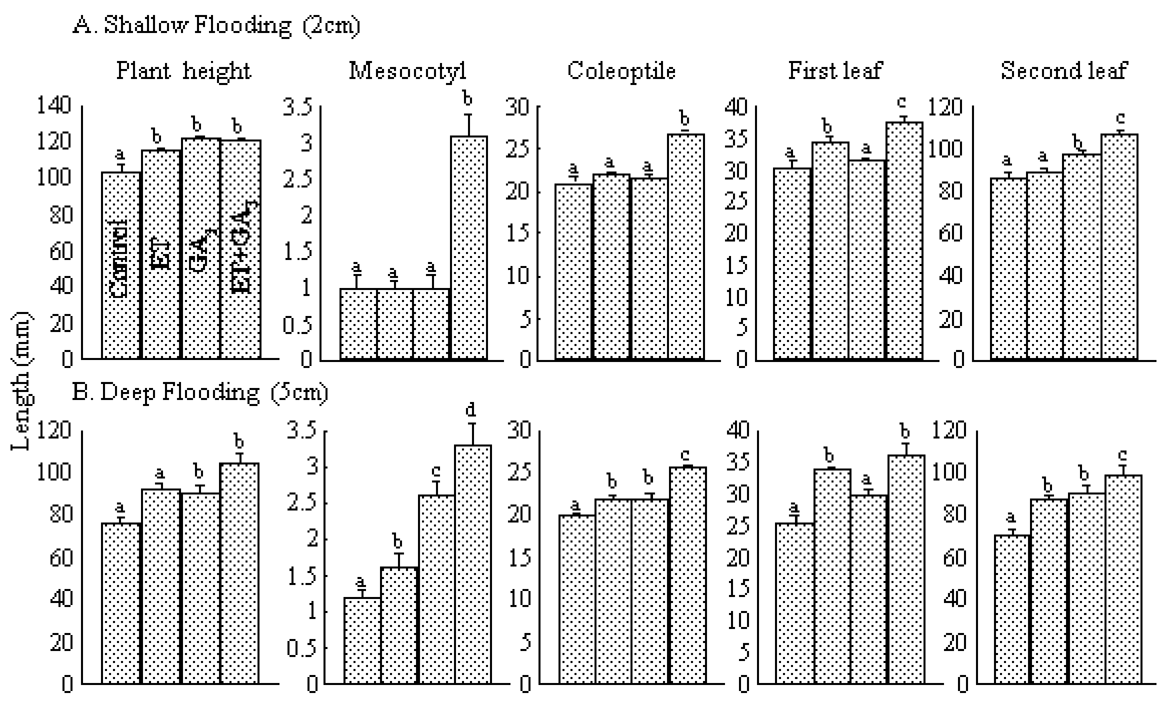

Figure 1. Effects of PGRs on the growth of different organs of rice seedlings (15 ℃ Vertical lines are standard errors of the mean of the four replications. Means followed by the same letter do not differ at P<0.05, according to Fisher's LSD test.

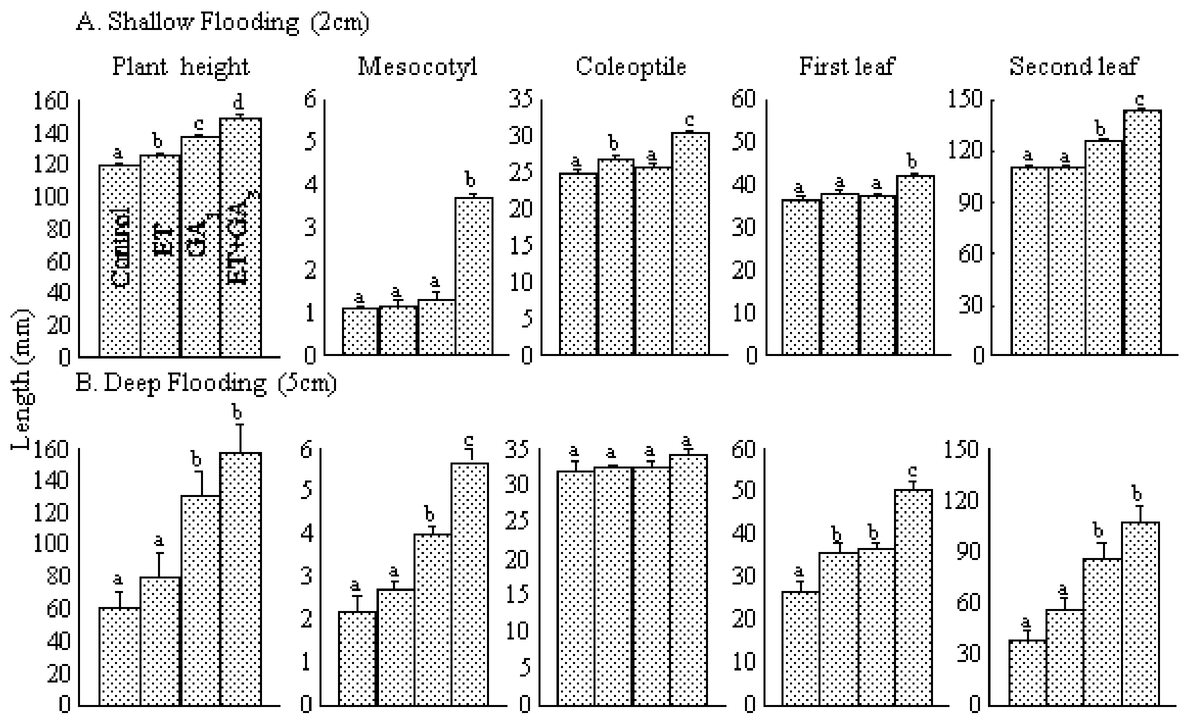

Figure 2. Effects of PGRs on the growth of different organs of rice seedlings (20 ℃)

Vertical lines are standard errors of the mean of the four replications. Means followed by the same letter do not differ at $P<0.05$, according to Fisher's LSD test.

2. With a growing temperature of 20?C

All PGR treatments significantly increased plant height compared with that of the controls, but maximum elongation was induced by ET+GA_3 treatment at 2FD, whereas (Fig. 2A), no significant growth-promoting effect with respect to plant height was observed with GA_3 treatment at 5 FD (Fig. 2B). For mesocotyls, only the ET+GA_3 treatment gave significant growth-promoting effect over that of the control at 2cm FD, as was the case at 15?C (Fig. 2A), however, both GA_3 alone and ET+ GA_3 treatments significantly increased mesocotyl elongation at 5 FD (Fig. 2B). For coleoptiles, significant elongation occurred from ET alone and ET+GA_3 treatments at 2FD (Fig. 2A), whereas, no significant growth-promoting effect was obvious in any treatment at 5FD (Fig. 2B). In the first leaf, only the ET+GA_3 treatment had significant stimulating-effects on elongation at 2FD (Fig. 2A), but all PGR treatments showed the significant increase of the elongation at 5 FD (Fig. 2B). In the second leaf, both GA_3 alone and ET+GA_3 treatments showed the prominent growth stimulating effects at both 2 and 5 FDs, but the maximum elongation was obtained with the combination of ET+ GA_3 at both FDs (Fig. 2). No significant difference in the RWL was observed among the all PGR treatments at both flooding depths as shown in the case of the 15?C growing temperature (Table 2).

Table 1 Effects of PGRs on DW and RWL of rice seedlings grown at 15 ℃

Treatment	DW[2] (mg/plant) FD[1]		RWL[3] (mg/cm) FD	
	2 cm	5 cm	2 cm	5 cm
Control	5.9a	4.2a	0.57a	0.56a
ET	6.2ac	5.4bc	0.54a	0.58a
GA_3	6.8b	4.8ab	0.56a	0.52a
ET+GA	6.7bc	5.6c	0.55a	0.54a

Means followed by the same letter do not differ at P<0.05, according to Fisher's LSD test
1) FD: Flooding depth
2) DW: Dry weight
3) RWL: Ratio of shoot dry weight to shoot length

Table 2 Effects of PGRs on DW and RWL of rice seedlings grown at 20 ℃

Treatment	DW[1] (mg/plant) FD[3]		RWL[2] (mg/cm) FD	
	2 cm	5 cm	2 cm	5 cm
Control	5.9a	1.8a	0.50a	0.30a
ET	6.3a	2.4ac	0.50a	0.30a
GA_3	6.6ab	4.1b	0.49a	0.31a
ET+GA	7.0b	4.8b	0.47a	0.31a

Means followed by the same letter do not differ at P<0.05, according to Fisher's LSD test
FD: Flooding depth
DW: Dry weight
RWL: Ratio of shoot dry weight to shoot length

In present studies, rice seedling growth under direct seeding conditions increased with a single treatment of GA_3 or ET compared to that of the control in some cases. However, the growth- promoting effects were diverse and accorded with the differences in the target organs of rice seedlings; and in environmental conditions, such as temperature and flooding depth. However, the effects of combined applications of GA_3 and ET were more pronounced than those of GA_3 or ET alone; and further, these growth-promoting effects were more stable than single treatments of each PGR in spite of the various environmental conditions. These results suggest that ET and GA_3 acted additively or synergistically. This synergism was observed in almost all cases in this experiment, except for the coleoptiles grown under at 20?C at 5 FD. This might be because coleoptile growth had already reached a maximum at the sampling date. In our series of experiments, the coleoptile growth rate of the ET+GA_3 treatment was observed to be quicker than in other treatments during the early stages of seedling growth (data not shown).

Suge (1974) and Takahashi and Kaufman (1992) pointed out that the synergistic action of ethylene with GA was seen in the growth of rice seedlings. However, PGRs were applied continuously in the culture medium, and growth temperature was relatively high (30℃) in their experimental system for considering direct seeding cultivation in cold regions. The aim of our experiment was to enhance the early growth of rice seedlings in direct seeding cultivations in cold regions, including the Tohoku district in Japan, using various PGRs as chemical controls. It has been considered that the physiologically critical temperature for the germination and early seedling growth is around 17?C (Nishiyama 1978). Early seedling growth, including seedling establishment is one of the most crucial agronomic issues in direct seeding rice cultivations. In fact, we set up the growing temperatures (15 and 20?C) considering these facts and the actual situation where direct seeding cultivation was conducted in the Tohoku district of Japan. Furthermore, rice seeds were pre-soaked for uniformity of germination as in most direct seeding cultivation methods conducted in the Tohoku district of Japan. We applied PGRs during the seed soaking process; a relatively simple method for using PGRs on rice seeds; and one that would be easily integrated into a practical direct seeding system. From an agronomical point of view, our experimental system might be closer to a practical direct seeding cultivation method than seen in previous experiments, especially so in terms of an experimental system.

Conclusion

High seedling establishment rates from direct seeding cultivation in cold regions of Japan will be possible using proper combinations of plant growth regulators.

References

Davies PJ (1995). Plant Hormones: Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, Pages 1-35.

Kende H, Van der Knaap and Cho HT (1998). Deep water rice: A model plant to study stem elongation. Plant Physiol. 118, 1105-1110.

Metraux JP and Kende H (1983). The role of ethylene in the growth response of submerged deep water rice. Plant Physiol. 72, 441-446.

Nishiyama I (1978). Further evidence for the break on the Arrhenius plot of germination activity in rice seeds. Jpn. J. Crop Sci. 47, 557-562.

Suge H (1974). Synergistic action of ethylene with gibberellins in the growth of rice seedlings. Proc. Crop Sci. Soc. Japan 43, 83-87.

Takahashi K and Kaufman PB (1983). Growth regulation of rice seedlings. Proc. Plant Growth Regulator. Soc. America 10, 229-234.