Effects of the Combined Application of Ethephon and Gibberellin on Growth of Rice (*Oryza sativa* L.) Seedlings

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Abstract: Improvement of early seedling growth, such as seedling emergence and vigor is one of the most important agronomic traits in direct seeding rice cultivation. The effects of two plant growth regulators (PGRs), gibberellic acid (GA₃) and ethephon (ET), on seedling growth under flooded soil conditions at different temperatures and water depths were investigated. The PGRs were applied during the seed soaking process. A single treatment with GA₃ or ET increased seedling growth. However, combined application of GA₃ and ET was more effective than that of GA₃ or ET alone in many cases at both growing temperatures (15 and 20ºC). The growth of different organs in the rice seedlings, such as the coleoptiles, first leaves, and second leaves was also increased by PGR treatment. The nitrogen concentration of the shoot and the ratio of shoot dry weight to shoot length did not differ significantly among the treatments. The results of our study show that rice seedling growth in direct seeding cultivation may be improved by treatment with GA₃ and ET in combination.

Key words: Coleoptile, Direct seeding, Ethylene, Gibberellin, Mesocotyl, Plant growth regulator, Rice, Seedling.

Early seedling growth, including seedling emergence and vigor is one of the most crucial agronomic issues in direct seeding rice cultivation. Plant hormones, such as gibberellin (GA), ethylene, and abscisic acid (ABA), are known to affect the elongation of rice seedling organs (Suge, 1974; Raskin and Kende, 1983; Takahashi and Kaufman, 1983, 1992; Furukawa et al., 1997; Watanabe and Takahashi, 1997; Watanabe et al., 2001). Suge (1974) noted that the combined application of ethylene and GA, a naturally active gibberellin in rice plant, synergistically stimulated the growth of coleoptiles and the first leaves of rice seedlings.

Using dose-response and feeding experiments of 14C-GA Furukawa et al. (1997) discovered that ethylene may stimulate leaf elongation by increasing responsiveness to and turnover of GA. An additional GA-ethylene interaction can be observed in internode elongation in deepwater rice. Ethylene promotes the growth of internodal tissue in deepwater rice, which responds to flooding by rapid elongation (Metraux and Kende, 1983). Ethylene promotes growth in part by increasing the responsiveness of the internodal tissue to GA in deepwater rice (Kende et al., 1998). This suggests the possibility of improving early seedling growth of rice grown under direct seeding culture through growth regulation of target organs by combined application of ethylene and GA. Indeed, synergistic interaction of plant hormone can be found in several plant growth systems (Davies, 1995).

In direct seedling cultivation, rice seeds are faced with various environmental factors, such as variable temperature and flooding conditions. Therefore, we investigated the effects of single and combined application of ethylene and GA on rice seedling growth at different temperatures and flooding depth. The potential for practical use of PGRs in improving early growth of seedlings grown under direct seeding cultivation are also discussed.

Materials and Methods

The cultivar used in the study was Kokoromachi (*Oryza sativa* L.), a japonica type. The seeds were sterilized with a thiuram and benomyl solution for 24 h according to instructions then immersed in water for 24 h and subsequently soaked in a test solution for 48 h. Ethephon (2-chloroethylphosphonic acid, Ishihara Sangyo Kaisha, LTD, Osaka, Japan) was used as an ethylene-releasing agent (Arteca, 1996) and GA₃ (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) 100 ppm GA₃ (GA₃), 4) 50 ppm ET + 100 ppm GA₃ (ET+GA₃). After treatment with the plant hormone solution, the seeds were again immersed in water for 48 h to remove any excess test solution. Seed sterilization and water and test solution soaking were performed at 5 February 2007. Accepted 2 May 2007. Corresponding author: H. Watanabe (watanabe@bios.tohoku.ac.jp, fax +81-229-84-6490, *present address; Toyohashi University of Technology, 1-1 Hibarigaoka, Toyohashi, Aichi 441-8580, Japan).
15°C. The imbibed seeds were germinated in 30°C water in the dark. The germinated seeds were then sown at a seeding depth of 1 cm in seedling pots with small compartments containing fertilized granulated soils (Kureha Chemical Co., LTD, Tokyo, Japan). Twenty-one uniform seeds were used for each treatment and the experiments were performed with 4 replicates. The seeds were allowed to grow at 15 or 20°C in continuous light conditions. Fluorescent lamps supplied a photosynthetic photon flux of 120 μmol m⁻² s⁻¹. The flooding depths (FD) were 2 and 5 cm in each experiment. Seedlings were sampled at 17 and 25 days after seeding (DAS) at 20°C and 15°C, respectively. After sampling the seedlings, plant height and the length of each organ was measured. The seedling shoots were then separated into each organ and dried for 48 h at 70°C to measure dry weight, and analyzed for the nitrogen concentration. The nitrogen concentration of the shoots was determined using a NC-analyzer (Sumigraph NC-80S, Sumica Chem. Anal. Service). A randomized complete block design with four replicates was used. All experiments were repeated two or more times with similar results; and one set of data is presented. Treatment means were separated by Fisher’s Protected LSD Test (P<0.05).

**Results**

1. **Growth at 15°C**

   Plant height significantly increased under all tested PGR treatments when compared with the control at 2 cm of FD (2FD) (Fig. 1A). Effects of ET+GA₃ on plant height was more pronounced than either ET or GA₃ alone at 5 cm of FD (5FD) (Fig. 1B). ET or GA₃ alone at 2FD did not stimulate mesocotyl growth. However, the combination of the ET and GA₃ treatment markedly increased mesocotyl growth at 2FD (Fig. 1A). At 5FD, all PGR treatments increased the mesocotyl length with the maximum elongation caused by the pairing of ET and GA₃ (Fig. 1B). For coleoptiles, the trend of the effects of PGR treatments on elongation was similar to those for mesocotyl at both flooding depths (Fig. 1). Interestingly, the synergistic effect of the combined application of ET and GA₃ was also observed in the case of mesocotyl growth at both flooding depths (Fig. 1). In the first leaf, which mainly consists of a leaf sheath, ET alone and ET+GA₃ treatments showed prominent increases in the growth at both flooding depths (Fig. 1). In the second leaf, significant growth-promoting effects were observed by GA₃ alone and ET+GA₃ applications over the control at 2FD (Fig. 1).
In addition to these treatments, GA₃ alone increased second leaf growth at 5FD (Fig. 1B). No difference in the ratio of shoot dry weight to shoot length (RWL) was observed among the PGR treatments at either FD (Table 1). This indicates that PGR promoted not only elongation but also dry-matter production in the shoot. The difference in total nitrogen content of seedling shoot was not obvious among the treatments irrespective of flooding depth (Fig. 3).

### 2. Growth at 20°C

All PGR treatments significantly increased plant height in comparison to the control, with maximum elongation induced by ET + GA₃ treatment at 2FD (Fig. 2).

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**Table 1. Effects of ethephon (ET) and gibberellic acid (GA₃) on DW and RWL of rice seedlings grown at 15°C.**

| Treatment | DW (mg plant⁻¹) 2 cm | DW (mg plant⁻¹) 5 cm | RWL (mg cm⁻¹) 2 cm | RWL (mg cm⁻¹) 5 cm |
|-----------|-----------------------|-----------------------|---------------------|---------------------|
| Control   | 5.9a                  | 4.2a                  | 0.57a               | 0.56a               |
| ET        | 6.2a                  | 5.4b                  | 0.54a               | 0.58a               |
| GA₃       | 6.8b                  | 4.8b                  | 0.56a               | 0.52a               |
| ET+GA₃    | 6.7b                  | 5.6b                  | 0.55a               | 0.54a               |

Means followed by the same letter within same column do not differ at P < 0.05, according to Fisher’s LSD test. DW, Dry weight; RWL, Ratio of shoot dry weight to shoot length; FD, Flooding depth.
Table 2. Effects of ethephon (ET) and gibberellic acid (GA3) on DW and RWL of rice seedlings grown at 20 °C.

| Treatment | DW (mg plant⁻¹) | RWL (mg cm⁻¹) |
|-----------|----------------|----------------|
|           | 2 cm | 5 cm | 2 cm | 5 cm |
| Control   | 5.9a  | 1.8a | 0.50a | 0.30a |
| ET        | 6.3a  | 2.4a | 0.50a | 0.30a |
| GA3       | 6.6ab | 4.1b | 0.49a | 0.31a |
| ET + GA3  | 7.0b  | 4.8b | 0.47a | 0.31a |

Means followed by the same letter within same column do not differ at P < 0.05, according to Fisher’s LSD test.

DW, Dry weight; RWL, Ratio of shoot dry weight to shoot length; FD, Flooding depth.

No obvious growth-promoting effect with respect to plant height was observed with ET treatment at 5FD (Fig. 2B). For mesocotyls, only the ET+GA3 treatment showed a growth-promoting effect in comparison to the control at 2FD which is similar to response at 15°C (Fig. 2A). However, both GA3 and ET + GA3 treatments significantly increased mesocotyl elongation at 5FD (Fig. 2B). For coleoptiles, marked elongation occurred from ET alone and ET+GA3 treatments at 2FD (Fig. 2A), whereas, growth-promoting effect was not obvious in any treatment at 5FD (Fig. 2B). In the first leaf, only the ET + GA3 treatment had significant stimulating-effects on elongation at 2FD (Fig. 2A). However, all PGR treatments showed an increase in elongation at 5FD compared to that of the control (Fig. 2B). In the second leaf, both GA3 and ET + GA3 treatments showed prominent effect on growth at both 2 and 5 FDs with the maximum elongation (Fig. 2). No significant difference in the RWL was observed among the PGR treatments at either flooding depths, as was the case at 15°C (Table 2).

### Discussion

In this study, the growth of rice seedlings under direct-seeding condition was clearly promoted by the treatment with GA3, ET and ET + GA3. However, the growth-promoting effects of these treatments were diverse and varied with the target organ and with the environmental condition such as temperature and flooding depth. Furthermore, the effect of ET + GA3 on the growth of rice seedlings was more stable than the single treatment with GA3 and ET, irrespective of the environmental conditions. An additive or synergistic action of ET and GA3 on seedling growth was observed in this experiment, except in coleoptile growth at 5FD at 20°C. The coleoptile growth might have reached a maximum by the sampling date. In our series of experiments, coleoptile growth was promoted by ET + GA3 treatment earlier than any other PGR treatments (data not shown).

Suge (1974) and Takahashi and Kaufman (1992) observed the synergistic action of ethylene and GA in the growth of rice seedlings. However, PGRs were applied continuously in the culture medium and growth temperature was relatively high (30°C) in their experimental system while direct seeding cultivation is usually conducted in cold regions. This study focused on enhancing the early growth of rice seedlings in direct seeding cultivation in cold regions, including the Tohoku district in Japan, using various PGRs. The physiologically critical temperature for germination and early seedling growth is approximately 17°C (Nishiyama, 1978; Maruyama and Tajima, 1986; Hagiwara and Imura, 1993). Early seedling growth, including seedling establishment is one of the most crucial agronomic issues in direct seeding rice cultivation. In this study, the growing temperatures (15 and 20°C) were selected considering these factors and the actual direct seeding cultivation was conducted in the Tohoku district of Japan. Furthermore, rice seeds were pre-soaked for uniformity of germination as in the case of most direct seeding cultivation conducted in the same region. We applied PGRs during the seed soaking, which is a relatively simple method and may be easily integrated into a practical direct seeding system. From an agronomical point of view, our experimental system approximates more closely the practical direct seeding cultivation method than seen in previous experiments, especially in terms of an experimental design.

The findings suggest that early seedling growth in direct seeding cultivation is promoted by applying proper combinations of plant growth regulators (ex. ET and GA).

### Acknowledgements

This work is partly supported by the Deans Fund for Frontier Research on Agricultural Sciences, Tohoku University, and a Grant-in-Aid for Science Research (No. 18780010) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Japan Society of the Promotion of Science (JSPS).

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