Endogenous gibberellin relationships in internode elongation of floating rice: A genetic study

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\textbf{ABSTRACT}

Application of an inhibitor of gibberellin biosynthesis, S-327D, inhibited elongation of internode in floating rice under submergence. The amount of endogenous gibberellin decreased corresponding with the increasing dose of the inhibitor. However, no direct correlation was detected between the total length of elongated internode under submergence and the endogenous gibberellin content among \textit{F}_2 plants, obtained from a cross between non floating rice and floating rice, which had different total lengths of elongated internodes.

\textbf{1. INTRODUCTION}

In tropical Asia, rice plants are sometimes subjected to deep water stress for periods of up to 10 months. The rice varieties, called floating or deep water rice, grown in these areas respond to deep water stress mainly by extension of plant height through internodal growth and increased number of elongated internodes. Floating rice plants elongate beyond the water depth reaching up to 6 m, with maximum internodal elongation of up to 25 cm/day (Vergara et al., 1976).

The role of ethylene and gibberellin (GA) in the internode elongation of floating rice under submergence has recently been studied extensively (Métraux and Kende, 1983, 1984; Raskin and Kende, 1984a; Suge, 1985,1987; Bleecker at al., 1986, 1987; Choen and Kende, 1986,1987).

Application of GA biosynthesis inhibitor restrain elongation of floating rice under submergence (Raskin and Kende, 1984b; Suge, 1985), while submergence treatment brought about increase of endogenous GAs (Yamaguchi, 1974; Suge, 1985).

Genetically, plant's ability to elongate internode under submergence can be explained in terms of complementary genes; one controls GA production and the other controls ability to responde ethylene. Thus, the plants having dominant alleles at the loci can elongate their internodes under submergence even in vegetative stage. However, elongation could not be explained by these Mendelian factors alone, since the distribution of total internodal length of elongated internodes was rather continuous (Suge, 1987).
In this study, effects of different dosages of an inhibitor of GA biosynthesis on internodal elongation and content of endogenous GAs of floating rice were examined. And as the second experiment, content of endogenous GAs in F₂ segregants obtained from a cross between non floating rice and floating rice was studied.

2. MATERIALS AND METHODS

Plant materials

Floating rice variety Aswina from Bangladesh and the F₂ segregants from a cross between Tan-ginbozu, a non floating rice that is deficient in GAs (Suget Murakami, 1968), and a floating rice Aswina were used as previously (Suget, 1987).

Seeds of Aswina were directly sown in plastic pots (1/10,000 are), 20 seeds per pot. Each pot was filled with commercial soil mixture containing 0.5 g N, 1.5 g P and 0.5 g K per kg of the soil, Kureha Engei Baido, (Kureha Chemicals, Tokyo). When plants reached the 3 leaf stage, 5 different concentrations of S-327D (E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol-1-yl)-1-penten-3-ol, an inhibitor of GA biosynthesis (Izumi et al., 1984), were applied to the soil. Two sets of pots were prepared for each concentration of the chemical. Twenty days after the chemical application, one set of pots was subjected to submergence treatment. Plants in pots were put into water so that 70% of plants was under water for 20 days and then the total length of elongated internode was measured.

The treatment was conducted in a green house under 24 h daylength which was obtained by natural daylight complemented with fluorescent lamps from 6 p.m. to 7 a.m.

Seeds of the F₂ segregants were sown directly in plastic cups (8.5×13.5 cm) filled with the commercial soil mixture mentioned above in the rate of one seed per cup and grown for 20 days in the green house under 24 h daylength. When F₂ seedlings produced more than 10 tillers each F₂ plant was divided into 2 parts and each part was transplanted to a new cup. Thus, 2 groups of F₂ segregants, one group was a duplicate of the other was obtained. After then, they were grown for 2 more weeks in the green house and one group was subjected to the submergence treatment to measure stem elongation. The plants which did not have elongated internode were omitted from the experiment since they might be recessive homogenates at either one or two complementary genes (Suget, 1987).

GA extraction and bioassay

Plants of floating rice variety Aswina, which received different amounts of S-327D, were used for GA extraction 20 days after the chemical application. F₂ plants used for GA extraction were sampled 2 weeks after the plants were divided into 2 parts of their tillers. Whole plants except roots were used for GA extraction.
The materials were homogenized with 80% acetone. The homogenate was stood for one night, then it was filtered through cheese cloth and filter paper. Acetone was removed under reduced pressure and aqueous solution was adjusted to pH 2.3 with phosphoric acid. The aqueous fraction was then extracted 3 times with equal volume of ethyl acetate. The ethyl acetate fractions, thus obtained 3 times, were combined and further extracted with phosphate buffer at pH 7.0. The buffer was then adjusted to pH 2.3 with phosphoric acid and extracted 4 times with equal volume of ethyl acetate. The ethyl acetate fractions, thus obtained 4 times, were combined and were dehydrated overnight with anhydrous sulfate and concentrated under reduced pressure.

Thin-layer chromatography was adopted for separating GAs from the extracts. Each concentrated extract was dissolved in a small volume of acetone and applied as a 0.4 cm band on 20×20 cm, 0.5 mm thick silica gel thin-layer plates. The plates were developed 10 cm in isopropyl ether:acetic acid (95:5, v/v). After drying chromatograms each of them was divided into 10 equal zones (the first zone was re-divided into 2 zones), and each silica gel zone was placed into 10 ml beaker. About 3 ml of 50% acetone was added to each beaker. The eluate was transferred to 5 ml beaker and evaporated to dry. The residue was redissolved in 100 μl of 50% acetone and 5 μl was used for bioassay by microdrop application, 1 μl each to 5 plants of the dwarf line Tan-ginbozu. The length of the second leaf sheath was measured 3 days after the application. The test plants were grown at 32°C under continuous light of about 4,000 lux. The amount of GAs (the sum of different Rf activities) was expressed as GA<sub>3</sub> equivalent in ng per 100 g fresh weight.

3. RESULTS

The effect of different doses of S-327D on internode elongation with or without the submergence treatment was examined. When plants were treated with S-327D, plant height was greatly reduced (Fig. 1) but width of leaf and the number of tillers increased. S-327D treated plants resembled Tan-ginbozu, a GA deficient mutant (Suge and Murakami, 1968) especially when the dose of S-327D was increased (Fig. 2). Endogenous GA activity was reduced with the increasing dosage of S-327D (Fig. 3).

As shown in Fig. 1, plant height and endogenous GA content showed a similar response curve to the different doses of S-327D, hence they seem to be correlated. Internode elongation was not detected before the submergence treatment. However, 20 days after submergence the plants which had received S-327D less than 5 mg/m<sup>2</sup> gave elongation even in those plants that did not submerge. Almost no elongation was detected on the plants to which S-327D was applied more than 5 mg/m<sup>2</sup>. The submergence treatment increased the rate of elongation. However, the higher S-327D concentration than 20 mg/m<sup>2</sup> inhibited elonga-
Fig. 1. Effect of S-327D, a GA biosynthesis inhibitor, on the elongation of internodes and endogenous GA content in floating rice variety Aswina. S-327D was applied 20 days after sowing and 20 days after the chemical application, one group of plants was submerged. Length of elongated internodes was measured 20 days after the submergence as well as non submerged plants. The GA extraction was done at the time of start of submergence.

Fig. 2. Effect of S-327D on the growth of floating rice variety Aswina. From right to left; dosages of S-327D were 0, 0.1, 0.5, 1, 5, 10, 20 and 50 mg/m². The photograph was taken 20 days after the chemical application.
Fig. 3. Effect of S-327D, an inhibitor of GA biosynthesis, on endogenous GA activities of acidic ethyl acetate fraction from floating rice variety Aswina. The extraction was done 20 days after the chemical application with non submerged plants. Numerals in the upper right hand of each histogram indicate the dosage of S-327D in mg/m². The extracts were prepared from 100 g fresh weight materials.

ation even under submergence (Fig. 1).

Twenty-one F₂ plants with different total lengths of elongated internodes under submergence were selected for the extraction of endogenous GAs (Table 1). The internode length was evaluated with submerged plants, but the GA extraction was done using plants that were not submerged, since elongation was the consequence of the GA action and real floating rice varieties have a tendency to elongate without submergence, while non floating rice do not elongate its internode before entering generative growth.

Histograms in Fig. 4 show GA activities obtained by bioassay for the F₂ segregants and 3 plants of floating rice Aswina. Amounts of GAs were calculated using the standard response curve of Tan-ginbozu dwarf rice to different amount of GA₃ and expressed as GA₃ equivalent in ng per 100 g fresh weight. Amounts of GAs thus obtained and the total length of elongated internodes under submergence are plotted in Fig. 5. No significant correlation was detected between these 2 items.

4. DISCUSSION

Ethylene which accumulates in the air spaces of stem internode of intact plants or stem sections of floating rice is involved in regulating the growth response caused by submergence (Métraux and Kende, 1983; Suge, 1985). The level of O₂
Table 1. Data on F$_2$ plants used for endogenous GA extraction

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant height (cm)</th>
<th>Total length of elongated internode (cm)</th>
<th>Fresh weight (g)</th>
<th>Endogenous GA content (GA$_3$ equivalent in ng per 100 g fresh weight)</th>
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<tr>
<td>F$_2$-1</td>
<td>95</td>
<td>1.0</td>
<td>89</td>
<td>121.3</td>
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<td>2</td>
<td>120</td>
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<td>3</td>
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<tr>
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<td>133</td>
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<td>78</td>
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<td>6</td>
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in tissue is greatly reduced as a result of submergence, and lowered O$_2$ concentrations stimulate ethylene synthesis and ethylene thus accumulated promotes rapid internode elongation by enhancing the activity of endogenous GAs (Raskin and Kende, 1984b).

Treatment by GA biosynthesis inhibitor inhibited internode elongation brought about by ethylene in air (Suge, 1985) and also by submergence (Suge, 1987). Submergence treatment increased endogenous GA level not only in very young seedling stage (Yamaguchi, 1974) but also more advanced stage at which internodes had already elongated in floating rice (Suge, 1985).

These results indicate that endogenous GAs may play an important role in floating rice subjected to submergence. However, it has not been shown critically whether a GA biosynthesis inhibitor reduces endogenous GA level or not in floating rice plants. The present results (Fig. 1) clearly show that the amounts of
Fig. 4. Histograms showing endogenous GA activities of acidic ethyl acetate fraction from F₂ segregants obtained from a cross between non floating rice Tan-ginbozu and floating rice Aswina. The extraction was done at the time of submergence using non submerged plants. The fresh weight of materials is shown in Table 1 with other data. Peaks of active zone in histograms are little different in their Rf values within segregants. This might be due to the differences of laboratory temperature when thin-layer plates were developed in a solvent system.

Fig. 5. Relationships between GA content of non submerged plants and total internode length of submerged plants in F₂ segregants obtained in a cross between non floating rice Tan-ginbozu and floating rice Aswina. Open circles and circles indicate Aswina and F₂, respectively.
endogenous GAs decreased with the increasing dose of the inhibitor. Furthermore not only plant height but also total length of elongated internodes after submergence also decreased after the treatment by S-327D. Hence the level of endogenous GAs may be expected to have high correlation with the quantity of stem elongation under submergence.

However, as shown in Fig. 5, no correlation was detected between them when F2 segregants were examined. The extraction of endogenous GAs from F2 segregants was done from non submerged plants, since it was considered that elongation may occur as a consequence of the action of GAs and actually true floating rice has tendency to elongate its internode without submergence (Inouye and Hagiwara, 1981; Fig. 1), although non floating rice usually do not elongate unless they become generative.

The discrepancy between the inhibitor experiment and the F2 experiment may suggest that localization of GAs in active site such as intercalary meristem rather than the total GA contents may be important. In the present experiments, GAs were extracted from the whole organs above the ground level.

By the method used here, all kinds of GAs including pool GAs such as GA19 (Murakami, 1971; Kuroguchi et al., 1975) were included but different kinds of GA must be carefully analyzed separately for investigating the role of GAs in internode elongation of floating rice under submergence. Since non submerged plants were used here for GA extraction, however, possibility that submerged plants produce endogenous GAs differently from non submerged plants is not neglected. This is a topic for future studies.

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