Internodal Elongation and Ethylene Concentration of Floating Rice Stem Sections Submerged at Different Water Depths

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Abstract Internodal elongation and endogenous ethylene concentration were analysed in floating or deep water rice stem sections submerged at different water depths. The elongation rate of the internodes was independent of the water depth. Elongation rate and ethylene concentration of the internodes were high in the stem sections submerged completely in water, but they decreased when parts of the stem sections were above the water surface. In the stem sections previously submerged, in which the ethylene levels were high, internodal elongation continued after parts of the sections were exposed to ambient air even though the ethylene levels decreased. These results suggest that internodal elongation is independent of the water pressure and that the different concentrations of internodal ethylene partially reflect the difference in the diffusion rate of ethylene in water. They also suggest that ethylene acts as a trigger of internodal elongation.

Key words Deepwater rice, Ethylene, Floating rice, Internodal elongation, Oryza sativa, Water depth

Introduction

Plant organ response to ethylene has been studied in a large number of plant species and in many plant species ethylene showed an inhibitory effect on tissue elongation, known as the so-called triple response. However, in certain organs of semi-aquatic plants like rice exogenous ethylene was found to promote elongation.

The ethylene level in the internodes was higher in floating or deep water rice grown under submerged conditions than in those of rice grown under ordinary conditions. Also, the application of exogenous ethylene promoted internodal elongation in rice plants grown under non-submerged conditions. Based on these reports, it can be assumed that the submergence treatment of rice plants increases the endogenous ethylene level of internodes and
thereby promotes internodal elongation\(^1\).

To explain the reason for the increase in the ethylene level of submerged internodes, RASKIN and KENDE\(^3\) showed that a hypoxic condition (low O\(_2\) level) in water was one of the most important factors. In general, ethylene production in some plant species is induced by physical stresses like contact stimulation\(^7\). Thus, it is possible that the water pressure under submerged conditions acts as a physical stress, thereby inducing ethylene production in plant tissues. Also, it is assumed that a high level of internodal ethylene concentration in the submerged plants is caused by a large amount of ethylene accumulation in the internodes resulting from the low diffusion rate of ethylene in water (ca. 1/1000 of the value in air).

In this study, attempts were made to examine the internodal elongation and determine the endogenous ethylene concentration of floating rice stem sections submerged at different water depths and to analyse the possible functions of ethylene in the control of internodal elongation.

**Materials and Methods**

**Plant material**

Habiganj Aman II, a floating rice variety from Bangladesh which exhibits internodal elongation at a relatively early growth stage (7th or 8th leaf emergence stage)\(^6\), was used in this study. Seeds were sown in seedling boxes. Seedlings were transplanted in an experimental paddy field at the Faculty of Agriculture, Kobe University when they reached the 3rd or 4th leaf emergence stage. Fertilizer was applied at rates of 5 g each of N, P\(_2\)O\(_5\) and K\(_2\)O per m\(^2\). Plants were harvested at the age of 2 to 3 months (vegetative growth stage) for preparing the stem sections.

Two types of stem sections were prepared from the main stems and tillers according to the method of RASKIN and KENDE\(^3\) with slight modifications. One type of stem section, 20 cm long and containing the highest two nodes and the topmost internodes, was excised with a razor blade in water so that the lower node was located at 2 cm above the basal cut end. All the leaf sheaths arising from nodes other than those in the stem sections were peeled from the sections. The stem sections were prepared from the culms in which the youngest internode was 2 to 6 cm long. The other type of stem section was cut off from culms in the same manner as described above except that the stem sections were 25 cm long and the lower node was located at 5 cm above the basal cut end. The initial length of each internode was measured by holding the sections in front of a strong light which allowed the localization of the two nodes included in each section. At the end of the experiment, the stem sections were dissected to measure the final length of each internode.

**Submergence treatment**

Experiment I: Ten 25 cm-long stem sections were horizontally submerged at 7 different water depths of 0 to 100 cm in a concrete pool (1 m × 2 m, 1.2 m depth) placed outdoors. Stem sections floating on the water surface were used as controls. The submergence treatment was carried out under natural light conditions. The water temperature ranged from 20–27°C. The length of the internodes and leaves of the stem sections was measured after 2 days.

Experiment II: Fifteen 20 cm-long stem sections were placed upright in a 50 ml beaker and were fixed with glass beads to prevent them from floating up. Each beaker containing stem sections was placed at the bottom of a plastic cylinder (55 cm high, 5 cm in diam.). The cylinders were filled with deionized water to obtain 10 different water levels of 50 to 500 mm. The stem sections were incubated at a light intensity of 60 \(\mu\)mol m\(^{-2}\) sec\(^{-1}\) and at 28°C in a growth cabinet. The length of the internodes and leaves and ethylene concentration in the internodes were measured after 3 days.

Experiment III: A beaker containing fifteen stem sections 25 cm long was placed at the bottom of a cylinder in the same way as described above, and then the cylinder was filled with deionized water to obtain 3 water levels of 50, 300 and 500 mm. The stem sections submerged at a 50 mm water depth were used as controls. The submergence treatments were carried out under the same incubation conditions as those in Experiment II. The length of the internodes and leaves and ethylene con-
centration in the internodes were measured routinely for 5 days.

**Measurement of ethylene concentration in internodes**

The Internodes of the stem sections were opened longitudinally with a razor blade in water. Air vesicles from the internodes were trapped using a funnel whose stem hole was tightly plugged with a silicon cap (Fig. 1). One ml of the collected gas was sampled through the cap of the funnel with a gas-tight syringe. Ethylene was assayed on a gas chromatograph (Simadzu, GC-6A) equipped with a 3 mm × 2 m alumina column and a flame ionization detector.

**Results**

**Effect of water depth on the elongation of stem sections**

Table 1 depicts the elongation of internodes and leaves in the stem sections submerged horizontally at different water depths of 0 to 100 cm for 2 days (Exp. I). There was no difference in the internodal elongation among all the water depth plots in which the stem sections were incubated in water, except for the 0 cm plot in which the stem sections were floating on water and were partially exposed to air. Internodal elongation rate in the 2 to 100 cm water depth plots was 4 to 5 times higher than that of the 0 cm plot. Leaf elongation was slightly larger in the stem sections incubated in water than in those floated on the water surface.

**Effects of water levels on internodal elongation and ethylene concentration of stem sections**

Internodal elongation and ethylene concentration in the internodes of the stem sections submerged at 10 water levels are shown in Table 2 (Exp. II). The internodal length increased at water levels higher than 250 mm and leaf elongation was inhibited at water levels higher than 200 mm. Ethylene concentration in the internodes was obviously higher in the stem sections submerged at the water level of 350 mm or above than in those submerged at 300 mm or below. The stem sections submerged at 250 and 300 mm water levels sank completely under water at the start of the treatment but the upper portions of the stem sections appeared above the water surface due to internodal elongation during the incubation period.

**Changes with time in internodal elongation and ethylene concentration of stem sections**

A time-course comparison was made of the effects of the water levels of 50 (control), 300 and 500 mm on internodal elongation, leaf elongation and internodal ethylene concentration of stem sections during an incubation period of 5 days (Exp. III).
Table 2 Effect of water levels on internodal elongation and internodal ethylene concentration (Experiment II)

<table>
<thead>
<tr>
<th>Water level (mm)</th>
<th>Elongation (mm)</th>
<th>Total length (mm)</th>
<th>Ethylene concentration in internode (µl/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internode</td>
<td>Leaf</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>2.4±0.2</td>
<td>53.8±3.7</td>
<td>256.2±3.8</td>
</tr>
<tr>
<td>100</td>
<td>3.0±0.5</td>
<td>64.4±3.9</td>
<td>267.4±4.0</td>
</tr>
<tr>
<td>150</td>
<td>4.9±1.0</td>
<td>54.7±2.9</td>
<td>259.6±3.6</td>
</tr>
<tr>
<td>200</td>
<td>10.9±4.7</td>
<td>63.0±3.9</td>
<td>273.9±6.9</td>
</tr>
<tr>
<td>250</td>
<td>84.7±3.8</td>
<td>51.7±2.8</td>
<td>336.3±4.6</td>
</tr>
<tr>
<td>300</td>
<td>101.0±3.2</td>
<td>41.8±6.4</td>
<td>342.8±6.7</td>
</tr>
<tr>
<td>350</td>
<td>116.0±3.6</td>
<td>33.3±4.1</td>
<td>349.3±4.2</td>
</tr>
<tr>
<td>400</td>
<td>111.7±3.4</td>
<td>44.6±6.7</td>
<td>356.3±6.8</td>
</tr>
<tr>
<td>450</td>
<td>104.7±3.6</td>
<td>41.4±5.0</td>
<td>346.1±7.1</td>
</tr>
<tr>
<td>500</td>
<td>109.8±2.8</td>
<td>37.5±4.5</td>
<td>347.3±5.1</td>
</tr>
</tbody>
</table>

Stem sections (20 cm long) were submerged vertically in a plastic cylinder. They were incubated under continuous light for three days. Water level shows the depth from the water surface to the bottom of the cylinder. Each value is the average of 15 stem sections±SE.

Fig. 2 Changes with time in the total length of the stem sections submerged at different water levels.

![Graph showing changes in stem section length over time at different water levels.](image)

The changing pattern of ethylene concentration in the internodes differed among the water level treatments (Fig. 4). Ethylene concentration in the internodes was very low and almost constant throughout the incubation period in the 50 mm (control) plot. However, in the 500 mm plot the values were higher than about 1.5 µl/l on the 3rd day of incubation and tended to level off thereafter. On the other hand, the internodal ethylene level of the 300 mm plot increased on the 1st day, then decreased until the 3rd day and remained at al-
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Fig. 3 Changes with time in the elongation of internodes and leaves of stem sections submerged at different water levels: 50 mm (control), 300 mm, 500 mm water level.

Discussion

METRAUX and KENDE reported that ethylene which accumulated in the submerged internodes is, at least in part, responsible for the growth stimulation of floating rice under water. In fact, the internodal ethylene level increased in floating rice plants when they were submerged in water. On the other hand, exogenous ethylene stimulated internodal elongation in non-submerged floating rice plants. RASKIN and KENDE reported that a reduction in the O₂ level in water was one of the factors responsible for the increase of the ethylene level of the submerged internodes. Further studies should be carried out to determine which factors control the ethylene level and whether the ethylene action is qualitative or quantitative and/or instantaneous or continuous. In the current study, the changes in the internodal elongation and ethylene concentration were investigated in floating rice stem sections submerged at different water depths and the physiological implications of water pressure and gas diffusion were evaluated.

Physical stresses stimulate ethylene synthesis in a great variety of plant species. In the germination of dicotyledonous seeds, soil pressure, which takes the form of physical stress, stimulates ethylene synthesis and ethylene regulates the growth of plants. Although it can be assumed that the water pressure caused by the water depth acts as a physical stress which regulates the growth of floating rice, the results in Table 1 indicate that the internodal elongation of the stem sections was independent of the water depth, suggesting that the water pressure does not regulate the internodal elongation of floating rice plants.

It was shown in Exp. III that the internodal ethylene concentration and internodal elongation were greater in the stem sections submerged completely at the start of incubation than in the partially submerged ones (Table 2). Also, the stem sections for which the growth conditions changed from entire submergence
to partial submergence in air during the incubation period (submergence at 250 and 300 mm) exhibited a more rapid elongation than the stem sections whose upper portions remained above the water surface at the start of incubation. However, in these sections the levels of ethylene concentration were lower than in the stem sections which were completely submerged during the incubation period. It is considered that in the stem sections in the 250 and 300 mm water level plots the internodal ethylene level increased by complete submergence at the start of incubation and decreased by exposure to air, due to the partial exposure of the sections above the water surface. The low rate of ethylene diffusion in water is considered to be one of the most important factors for the high level of internodal ethylene concentration in the submerged stem sections of floating rice plants.

As shown in Fig. 2 to Fig. 4, the internodal elongation rate in the stem sections submerged at the 300 mm water level did not decrease even though the ethylene level was lowered after the tips of the sections appeared above the water surface owing to elongation. This finding suggests that ethylene acts as a trigger for rapid internodal elongation in submerged floating rice.

References