Localization of High Concentration of Gibberellins in Elongating Internodes of Floating Rice

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Extremely high concentration of pool type of gibberellins (GAs) which was detected by the Tan-ginbozu dwarf rice assay was found to be localized in the uppermost internodes of submerged and non-submerged floating rice plants. However, the level of the biologically active type of GAs which was detected by the Waite-C dwarf rice assay was higher in the internodes of the submerged plants than in those of the non-submerged ones. Amounts of GAs in leaves were also found to be higher in the submerged plants than in the non-submerged ones.

KEY WORDS: Oryza sativa, floating rice, gibberellins, internode elongation, localization

Introduction

In tropical Asia, rice plants are sometimes subjected to submergence for a period of up to ten months. The rice varieties, called floating rice or deep water rice, grown in these areas respond to deep water stress mainly by the increase of plant height through internode elongation and increase in the number of elongated internodes. Elongation of floating rice plant can occur at a water depth up to 6 m, and a maximum internodal elongation up to 25 cm/day (Vergara et al. 1976) can be recorded.

Although floating rice plants are characterized by the rapid elongation of internodes after submergence, their internodes are able to elongate in the absence of submergence before the onset of reproductive growth, while in non-floating rice varieties the internodes do not elongate during the vegetative growth period (Inouye and Mogami 1980, Inouye and Hagiwara 1981).

Takahashi and Wada (1972) showed that a large amount of exogenously applied GA₃ can induce internode elongation in seedlings of non-floating rice varieties. The role of GAs in the internode elongation of floating rice has been emphasized in previous studies (Raskin and Kende 1984, Suge 1985).

On the other hand, we could not find a direct correlation between the total internodal length after submergence and endogenous GA (pool type) content in shoots in an F₂ population between non-floating rice Tan-ginbozu and floating rice Aswina. Thus, we suggested the importance of localization of GAs during the elongation of internodes (Suge and Türkân 1990), and we analyzed the localization of endogenous GAs in non-submerged and submerged floating rice plants.

Materials and Methods

Plant materials: A floating rice variety Aswina from Bangladesh was used. Seeds were

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directly sown, after 48 hr immersion in water, in plastic cups (8.5 \times 13.0 \text{ cm}) at the rate of 3 seeds per cup. Each cup was filled with a commercial soil mixture (Kureha Engei Bai-do, Kureha Chemicals, Tokyo) containing 0.5 g N, 1.5 g P and 0.5 g K per kg of soil.

Seedlings were grown for 35 days in a greenhouse under 24 hr daylength, to prevent the onset of reproductive growth. When the plants reached the 7–8 leaf stage, they were divided into two groups: One group subjected to the submergence treatment and the other group as non-submerged control. Plants were partially submerged until about 70% of the plant height stood under water for 20 days. The water level was adjusted every 5 days. The final water depth was 70 cm.

Twenty days after submergence, the plants were separated into different organs (internodes, leaves including leaf sheaths and folded leaves), and subjected to the GA extraction. Non-submerged plants in which internode elongation started at this time, were separated into different organs for the GA extraction. When the plants had reached the 8th leaf emerging stage, the 7th internode (between 6th and 7th leaf nodes) began to elongate and was designated as 1st elongated internode. Plants reached to 10 leaf stage at the time of sampling.

**GA extraction:** The materials were homogenized with 80% acetone. The homogenate was allowed to stand for one night, then it was filtered through cheese cloth and filter paper. Acetone was removed under reduced pressure and the aqueous solution was adjusted to pH 2.3 with phosphoric acid. The aqueous fraction was then extracted 3 times with an equal volume of ethyl acetate. The ethyl acetate fractions thus obtained were combined and further extracted with phosphate buffer at 7.0. The buffer was then adjusted to pH 2.3 with phosphoric acid and extracted 4 times with an equal volume of ethyl acetate. The ethyl acetate fractions thus obtained were combined, dehydrated overnight with anhydrous sodium sulfate and concentrated under reduced pressure.

**Thin-layer chromatography and bioassay:** Thin-layer chromatography was used for separating GAs from the extracts. Each concentrated extract was dissolved in a small volume of acetone and applied to a 0.4 cm band on 20 \times 20 \text{ cm}, 0.5 mm thick silica gel thin-layer plates. The plates were developed over a distance of 10 cm in isopropylether: acetic acid (95:5, v/v). After drying, each chromatogram was divided into 10 equal zones (the first zone was subdivided into two zones), and each silica gel zone was placed into a 10 ml beaker. About 3 ml of 50% acetone was added to each beaker and the eluate was transferred to 5 ml beaker and evaporated to dryness. The residue was redissolved in 100 \mu l of 50% acetone and 5 \mu l was used for the bioassay by microdrop application, 1 \mu l each to 5 plants of the dwarf lines Tan-ginbozu and Waito-C. The length of the second leaf sheath was measured 3 days after the application. The test plants were grown at 32°C under continuous illumination of about 4,000 lux. The amount of GA (the sum of different Rf activities) was expressed as GA$_3$ equivalent in ng per 100 g fresh weight.

Dwarf line Waito-C shows a much reduced or almost no response to non-3 \beta-hydroxylated GAs such as GA$_{19}$ and GA$_{20}$ to which the dwarf line Tan-ginboz is sensitive (Murakami 1972). These results may be helpful in the characterization of the GAs involved. GAs which were detected by the Tan-ginbozu bioassay and not by the Waito-C one were characterized
as pool type of GAs, whereas GAs to which Waito-C responded were characterized as biologically active type of GAs, such as GA$_4$.

Results

Histograms showing the endogenous GA activities of the acidic ethyl acetate fractions from different organs obtained from plants of both the non-submerged and submerged floating rice variety Aswina are presented in Fig. 1. Table 1 shows the GA content calculated as GA$_3$ equivalent in ng per 100 g on a fresh weight.

Fig. 1. Histograms showing endogenous gibberellin activities of acidic ethyl acetate fraction from different organs of non-submerged and submerged plants of the floating rice variety, Aswina. The fresh weight of the materials is 24.7 g (folded leaves), 50.0 g (leaves and leaf sheaths), 49.6 g (first elongated internodes), 43.1 g (second elongated internodes), 22.9 g third elongated internodes and 2.2 g (fourth elongated internodes) in the submerged plants and 22.7 g (folded leaves), 50.0 g (leaves and leaf sheaths), 5.0 g (first elongated internodes), 8.3 g (second elongated internodes) and 2.6 g (third elongated internodes) in the non-submerged plants. T and W refer to the Tan-ginbozu dwarf rice assay and Waito-C dwarf rice assay, respectively.
basis as well as the average length of the internodes used for the extraction.

In the submerged plants only 2.2 g fresh weight materials of the uppermost internodes, 3.1 ± 1.8 cm in average length, showed an extremely high GA activity in the Tan-ginbozu dwarf rice bioassay as indicated in Fig. 1 and Table 1. This finding suggested that an extremely high concentration of pool type of GAs was localized in the uppermost internodes of the submerged plants. In the non-submerged plants, almost the same or a much higher activity of pool type of GAs was detected in the uppermost internodes and a comparatively high activity was also detected in other lower elongating internodes as shown in Fig. 1 and Table 1, whereas the pool GA activity in the lower elongating internodes was low in the submerged plants.

However, much larger amounts of GAs which were detected by the Waito-C dwarf rice bioassay as biologically active GAs were observed in the submerged plants than in the non-submerged ones. The activity was detected only in the uppermost internodes in the submerged plants. In the non-submerged plants, although the activity was also detected in the 2nd internode, the activity amounted only one-sixth of that of the submerged plants.

In submerged plants, the length of 4 elongated internodes decreased from the base toward the elongating uppermost internode, whereas the pool GA activity increased from the base toward the top (Table 1). In the non-submerged plants, 3 elongated internodes were identified although the length of the internodes was short, and the pool GA activity also increased from the base toward the top as in the case of the submerged plants.

The level of GAs in the leaves, both in the pool and active types, was higher in the submerged plants than in the non-submerged ones (Table 1).

Contents in each organ, both of pool and active types of GAs, are illustrated diagrammatically in Fig. 2.

**Discussion**

Involvement of GAs in the rapid elongation of internodes of floating rice under submer-

<table>
<thead>
<tr>
<th>Plant organs</th>
<th>Length (cm)</th>
<th>GA₃ equivalent in ng per 100 g fresh weight</th>
<th>Non submerged</th>
<th></th>
<th>Submerged</th>
<th></th>
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<tr>
<td></td>
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<td></td>
<td>T W</td>
<td>T W</td>
<td>T W</td>
<td></td>
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<tr>
<td>Folded leaves</td>
<td></td>
<td></td>
<td>408 4</td>
<td>615 43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves and leaf sheaths</td>
<td></td>
<td></td>
<td>314 8</td>
<td>520 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th internode</td>
<td>3.1 ± 1.8</td>
<td></td>
<td></td>
<td>2045 136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd internode</td>
<td>2.0 ± 1.7</td>
<td>9.8 ± 4.1</td>
<td>2092 0</td>
<td>210 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd internode</td>
<td>5.0 ± 1.5</td>
<td>18.6 ± 3.7</td>
<td>1000 24</td>
<td>210 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st internode</td>
<td>3.5 ± 1.7</td>
<td>20.5 ± 5.5</td>
<td>660 0</td>
<td>195 0</td>
<td></td>
<td></td>
</tr>
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</table>

T and W refer to Tan-ginbozu assay and Waito-C assay, respectively.

1) Elongated internode counted from the base toward the top.
2) Mean ± SD
gence has been emphasized (YAMAGUCHI 1974, RASKIN and KENDE 1984, SUGE 1985, 1987). However, no direct correlation was detected between total length of elongated internodes and the endogenous GA content of pool type GAs in the segregants of the F₂ in a cross between non-floating rice Tan-ginbozu and floating rice Aswina, when shoots (except roots) were used for GA extraction. This may indicate that the localization of GAs in certain target sites must be important for the role played by GAs in the rapid internode elongation of floating rice under submergence.

In rice shoots, GA₉ and GA₁₆, are known to be present and the former is considered to act as a pool GA in the biosynthetic pathway of biologically active GA, presumably GA₁₆ (SUZUKI et al. 1981). Thus, the GA activity detected by the Waito-C dwarf rice assay is considered to correspond to that of GA₁₆. Among the high GA activities detected by the Tan-ginbozu dwarf rice assay in the uppermost elongating internode of submerged floating

![Diagram](image)

**Fig. 2.** Diagrammatic representation of endogenous gibberellin content in different organs of non-submerged and submerged plants of the floating rice variety, Aswina. Plant organs are indicated as follows. A: folded leaves, B: leaves and leaf sheaths, C: first elongated internodes, D: second elongated internodes, E: third elongated internodes, F: fourth elongated internodes. Numerals in the denominator and in the numerator indicate amounts of gibberellins, GA₉ equivalent in ng per 100 g fresh weight, detected by Tan-ginbozu dwarf rice assay and those detected by Waito-C dwarf rice assay, respectively.
rice, on amount of about 7% in the calculation of GA₃ equivalent was also detected by the Waito-C dwarf rice assay. The existence of this avtive type of GAs may contribute to the rapid elongation under submergence.

In the non-submerged plants, although the activity of pool GAs was also high in the uppermost internode, that of biologically active GAs in Waito-C dwarf rice, was low compared with that in the submerged plants. This finding indicates that submergence may increase the conversion of pool GAs to biologically active GAs.

The amount of pool GAs was higher in the non-submerged plants than in the submerged plants especially in the lower elongating internodes. This phenomenon may be ascribed to the fact that the internodes of non-submerged plants are short and still are able to elongate when they are exposed to submergence. On the other hand the lower internodes of the submerged plants were long the their elongation was almost completed at the time of GA extraction in this experiment.

It is interesting to note that the GA concentration in the leaves especially in folded unexpanded leaves was about 50% higher in the submerged plants than in the non-submerged ones. Since apical regions of elongating shoots become the site of GA production in plants (Sponser 1987), the increase of the GA activity in immature folded leaves due to submergence may act as a source of GAs to promote the rapid elongation of internodes.

**Literature Cited**


浮稲の伸長中の中間における高濃度のジペリンの局在

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浮稲は水浸漬に反応して、その茎間を著しく伸長する。浮稲の節間伸長は、気中においても、エチレンとジペリンに依存して進展する。水浸漬は内生ジペリンの活性を増加させることが明らかにされているが、水に浸漬したときの節間伸長は、植物体全体を対象にして抽出したときの、総内生ジペリン含量とは関係を示さないのでき、節間伸長と内生ジペリンの関係をさらに明らかにするためには、特定の器官におけるジペリンの局在、あるいは種類の異なるジペリンの分布を調べる必要がある。

バングラデシュの浮稲 Aswina を供試して、水に浸漬したものと、しないものについて植物体各器官のジペリン含量を、有機溶媒抽出、薄層クロマトグラフィーによる分離、イネ苗バイオアッセイ法によりその局在について調査した。抽出は不水アセトンを用い、酸性塩酸エチル芳水をシリカゲル薄層クロマトグラフィーを用いて分離精製した。バイオアッセイは、矮性イネの矮性苗と矮性C-5を用いた。前者は炭素数20個のプール型ジペリン（イネの場合茎葉では主としてGA₃）にも反応するが、後者は炭素数19個の生物活性に活性型と思われるジペリン（イネの場合茎葉ではGA₃）にしか反応しないので、ある程度類似の推定ができる。

生産生長に入るのを妨げるため、24時間日長で35日間育て、7〜8株期に達した植物を水浸漬処理し、20日後に採取してジペリン分析に供した。水浸漬期間中は、植物体の約70%が水中に保持されるように、5日おきに水位を補正した。比較のため、水浸漬しないものを同時に供試した。

水浸漬したものでも、しないものでも伸長中の最上部節間には極めて高いプール型ジペリンの存在が認められた。しかし、活性型ジペリンの含量は、水浸漬したものの方が、しないものに比べて著しく高いことが分かった。最上部節間における活性型ジペリンの含量は、水浸漬したものではプール型ジペリンの約70%に達したが、水浸漬しないものでは、活性型ジペリンは最上部節間には認められず、水位の範囲に弱い活性性が認められた。プール型ジペリン含量は、水浸漬しないものでも、特に下位の節間において、水浸漬したものよりも多かった。これにより、水浸漬しないものでは、伸長節間は短く、また水浸漬などにより急速に節間を伸長する能力を保持しているのに、したがって浸漬したものでは、節間は長く、すでに伸長をほとんど終了しており、伸長の能力を失ってしまっているためと思われる。

浮稲は水浸漬しないものでも、栄養生長期に節間伸長を開始する特徴を有しているが、それを水に浸漬すると、急速に節間長を増大する。このときに、水浸漬がプール型から活性型への、ジペリンの代謝経路を引き起こすものと思われる。

また、水浸漬したものでは、しないものに比べて展開葉（葉軸を含む）、未展開葉共にそのジペリン含量は著しく高いことが判明した。特に未展開葉におけるジペリン含量は、水浸漬したものでは、しないものに比較してプール型ジペリンは約50%増加したが、活性型ジペリンは約10倍に増加した。若し未展開葉は一般に、ジペリンの生成源と考えられているので、これらのジペリンは水に浸漬されたときの葉先の急速な伸長に寄与すると共に、その一部はここから伸長節に移動して急速な伸長に寄与するものである。すでに展開した葉（葉軸を含む）においても、活性型ジペリンの濃度は水浸漬したものでは、しないものに比べて約2倍の増加を示した。

このように、浮稲の伸長中の最も節間におけるジペリンの局在、水浸漬した植物における活性型ジペリンの増加は、水浸漬に遭遇した浮稲における急速な節間伸長現象をある程度説明するものであろう。