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Effects of Sequential Application of Plant Growth Regulators on the Internodal Elongation of Rice Seedlings Grown in Light

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Summary

The role of plant hormones in the internode elongation of light grown seedlings was investigated using two rice cultivars (*Oryza sativa* L.): a japonica rice, Sasanishiki and a deepwater rice, Habiganj Aman VIII. Six growth regulators i. e, abscisic acid (ABA), benzyl adenine (BA), brassinolide (BR), ethrel (ET), gibberellin A_3 (GA_3) and indole-3-acetic acid (IAA) were applied at two different growth stages of the second internode; the stage before that visible elongation starts (phase I) and the stage after that extension growth starts (phase II). The latter was further subdivided into two phases; dominant cell division (IIA) and dominant cell elongation (IIB) phases. Application of GA_3 in phase I produced the most evident effect on internode elongation. The combined application of GA_3 with ABA or ET was also effective when the concentration of GA_3 was low. BA also caused an additive effect when applied with GA_3 in phase I. Each of ABA, BA, BR, ET and IAA when applied alone in phase I was not effective. GA_3 caused marked promotion of internode growth when applied in phase IIA or IIB, especially in the former. GA_3 seemed to act as a major promoter of internode elongation whereas ABA and BA inhibited elongation growth when applied with GA_3 in the phase IIA and IIB, although they acceralated the stimulative effects of GA_3 in phase I. The role of those hormones seems to be stage dependent.

Rice internodes do not usually elongate under ordinal culture at seedling stage, but they do elongate when seeds are placed deeply in the soil or are germinated in darkness and allowed to develop in darkness (1). The light-induced inhibition of internode elongation of dark grown seedlings was recovered completely by high concentrations of gibberellin A_3 (GA_3) (1, 2). These responses of the second internode (2IN) were dependent on the elongation stage of the internode. There were two major phases sensitive to GA_3 or light. The first phase can be seen within few days after germination and it corresponds to the

formation of the meristematic tissues in the internodes, while the second one is at the rapid internode elongation stage which occurs just after the beginning of the elongation of 2IN (1, 3). These are described as phase I and phase II, respectively in the present paper.

Under the condition of 12 hrs day-length, a significant 2IN elongation was induced by GA_3(10^{-3} M) in japonica rice, and in deepwater rice by GA_3 (10^{-5} $\sim 10^{-6}$ M) (4). However, significant elongation of 2IN was induced with relatively low concentration of GA_3 in japonica rice in the presence of both abscisic acid (ABA) and ET (Ethrel) (4, 6). Interestingly enough, ABA and ET act as a stimulator of 2IN in the presence of GA_3 although in some cases, ABA alone produced a growth inhibition of the 2IN. On the other hand, brassinolide (BR) or benzyladenine (BA) inhibited the internode growth induced by the combination of ABA, ET and GA_3, however, indole-3-acetic acid (IAA) had little effects on 2IN elongation (4).

In all these experiments mentioned above using light grown seedlings, various plant growth regulators (PGRs) were applied during whole period of the 2IN growth, which is seen through 14 days after seed immersion.

In this study we examined the effects of sequential application of PGRs on the second internode elongation in order to clarify the role of the plant hormones in the two major growth phase : phase I and phase II.

Materials and Methods

Plant materials and growth conditions

A japonica rice, cultivar "Sasanishiki" (Sasa) and a indica deepwater rice, cultivar "Habiganj Aman VIII" (HA-8) were used as materials. The seeds were immersed in a 1% sodium hypochlorite solution for 20 minutes, washed throughly with several changes of distilled water and allowed to germinate under darkness at 30°C for 48 hrs. Germinated seeds were selected for the uniformity and the selected 6 seeds were placed in a test tube (26 mm in diameter) containing 2 ml of test solutions. The test tubes were placed in a flexiglass box and grown for 14 days in 12 hrs light (white fluorecent lamps 1,600 lx), under controlled temperature (day/night temperature=29/21°C) and humidity (RH=70%) conditions. The length of the 2IN, which connected the first leaf and the second leaf of the seedlings, was measured at 14 days after seedling.

Application of plant growth regulators

Fig. 1 indicates the growth curve which is estimated from the beginning of elongation (more than 1 mm) to the completion of elongation in different plant parts of the GA_3-treated plants using HA-8. No visible growth of both mesocotyl and first internode was observed in the control plants and the GA_3-treated plants.

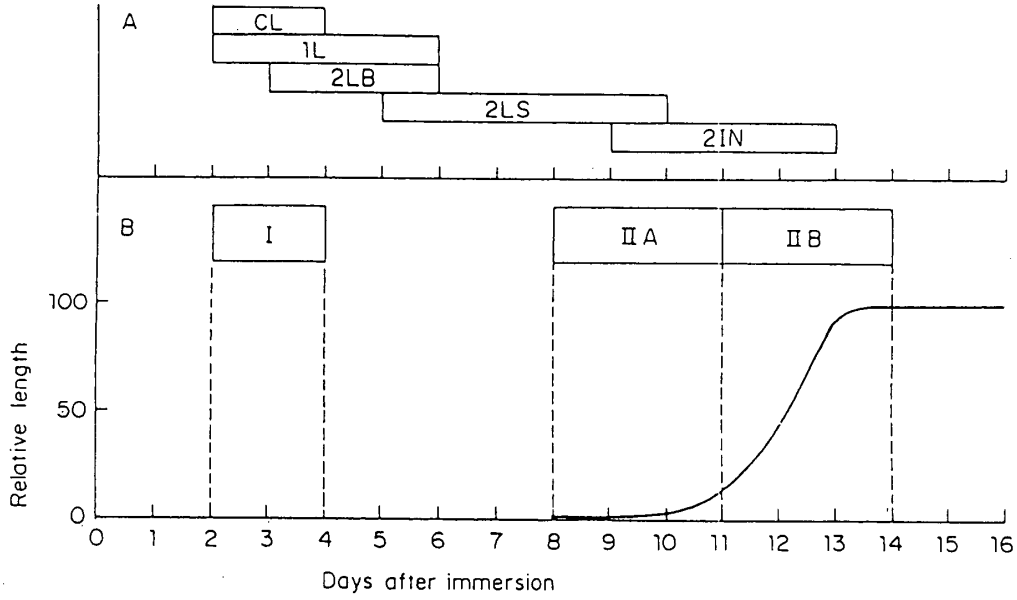

FIG. 1A. Growth period of each plant part from the beginning of elongation (more than 1 mm) to completion of elongation.
CL: coleoptile, 1L: first leaf, 2LB: second leaf blade, 2LS: second leaf sheath, 2IN: second internode.

FIG. 1B. Growth curve of the second internode elongation of Habiganj Aman VIII with GA_3 treatment (2×10^{-5}M) and the designated period of the treatment (I, IIA and IIB). Relative length is calculated as follows: measured length/final length $\times$ 100.

The 2IN started to elongate at around 9 days after seeding and was completed at 4 days after that. It was shown in the preliminary experiments that the highest growth promotion of the internodes by GA_3 and inhibition by light took place when applied at 3 days after seeding. This first sensitive phase is called by phase I in the present paper.

Plants also responded to GA_3 or light even after the visible elongation started. This second sensitive phase is called by phase II. Phase II can be divided into two phases: namely, the dominant cell division phase (IIA) and the dominant cell elongation phase (IIB) because the cell multiplication of the second internode ceased when the internode reached about 1/8 of total length as a result of experiments using Sasa although the experiment was done in darkness (5).

On the basis of these results, six PGRs, ABA, BA, BR, ET, GA_3, and IAA were applied at two or three different growth stages (I, IIA and IIB) of the second internode (2IN) in the following experiments.

Experiment 1. Single and combined treatments of ABA and ET with or without GA_3, in phase I and II

For the treatment in phase I, at 3 days after seeding, 2 ml of distilled water

was replaced with 2 ml of PGRs solutions in which the germinated seeds were treated for 2 days. Then the seeds were washed several times with distilled water at 5 days after seeding and back to distilled water until 8 days after seeding.

Then, they were washed several times with distilled water and they were replaced with 2 ml of single or combined solutions of PGRs as shown in Table 1-3. Finally, the length of 2IN was measured at 15 days after seeding. The concentrations of PGRs were as follows ; 4×10^{-6} M ABA, 4×10^{-6} M ET and 2×10^{-4} M GA_3 (Sasa) or 2×10^{-5} M GA_3 (HA-8).

Experiment 2. Single and combined treatment of ABA and ET with or without GA_3 in phase IIA and IIB in the presense of GA_3 in phase I

Two ml of GA_3 solution was applied at 3 days after seeding in all treatments and replaced at 5 days after seeding with 2 ml of distilled water after washing several times with distilled water. At 8 days after seeding they were divided into several groups and treated with ABA, ET, GA_3, ABA+GA_3, ET+GA_3, ABA+ET, or water until 11 days after seeding (phase IIA treatment). They were washed several times with distilled water and replaced with 2 ml of single or combined solutions mentioned above until 15 days after seeding (phase IIB treatment). Finally, the length of 2IN was measured at 15 days after seeding.

The concentrations of PGRs were as follows ; 4×10^{-6} M ABA, 4×10^{-6} M ET and 1×10^{-5} M GA_3.

Experiment 3. Single and combined treatments of BA, BR and IAA with or without GA_3 in the phase I, IIA and IIB

At 3 days after seeding, 2 ml of BA, BR, GA_3, IAA or water were applied and replaced with 2 ml of distilled water after washing several times with distilled water at 5 days after seeding (phase I). After washing several times with distilled water at 8 days after seeding, they were replaced with the combined solutions of water, BA, BR, IAA with or without GA_3 (phase IIA treatment), then washed several times with distilled water at 11 days after seeding, then replaced again with the combined solutions mentioned above (phase IIB treatment). The concentration of each PGR were the same as those in Experiment 2. The length of 2IN was measured at 15 days after seeding.

Results

Experiment 1. Single and combined treatments of ABA and ET with or without GA_3, in phase I and II

The results were shown in Table 1-3.

In japonica rice, the internode elongation took place only when treated with the combination of ABA, ET and GA_3 in phase I and with GA_3 in phase II (Table

TABLE 1. *Growth response of the second internode as affected by ABA, ET or GA_3 alone applied before elongation (phase I) and during elongation (phase II).*

Treatment phase		Length (mm)	
I	II	Sasa	HA-8
Water	Water	−a	−a
Water	ABA	−a	−a
Water	ET	−a	−a
Water	GA_3	−a	−a
ABA	Water	−a	−a
ABA	ABA	−a	−a
ABA	ET	−a	−a
ABA	GA_3	−a	−a
ET	Water	−a	−a
ET	ABA	−a	−a
ET	ET	−a	−a
ET	GA_3	−a	−a
GA_3	Water	−a	11.4c
GA_3	ABA	−a	6.5b
GA_3	ET	−a	14.5c
GA_3	GA_3	−a	58.4d

− : invisible (less than 1 mm). Treatment in phase I: PGRs were applied at 3 days after seeding to rice seedlings which were treated for 2 days after that. II: PGRs were applied at 8 days after seeding for 6 days treatment. Sasa: Sasanishiki, HA-8: Habiganj Aman VIII. Means followed by different letters are significantly different at 5% level through Table 3.

3). In HA-8, however, GA_3 alone induced the internode elongation even by single application in phase I, but no elongation occured by the single application in phase II (Table 1).

This difference in varieties was basically the same as those obtained in previously (4), although the PGRs were given in the whole growth period from seedling emergence through the completion of the 2IN in the previous experiment (4).

The present results gave more detailed information as follows. GA_3 plays major role in inducing the meristematic activities of the second internode along with other plant hormones such as ABA and ET in japonica rice. The presence of GA_3 in phase I is essential to induce meristematic activity of 2IN whereas GA_3 applied in phase II stimulates the elongation. ABA alone applied in phase II

TABLE 2. *Growth response of the second internode as affected by treatments of ABA, ET or GA_3 alone before elongation (phase I) and followed by the combined treatment of ABA, ET and GA_3 during elongation (phase II).*

Treatment phase		Length (mm)	
I	II	Sasa	HA-8
Water	GA_3+ABA	−a	−a
Water	GA_3+ET	−a	−a
Water	ABA+ET	−a	−a
Water	GA_3+ABA+ET	−a	−a
ABA	GA_3+ABA	−a	−a
ABA	GA_3+ET	−a	−a
ABA	ABA+ET	−a	−a
ABA	GA_3+ABA+ET	−a	−a
ET	GA_3+ABA	−a	−a
ET	GA_3+ET	−a	−a
ET	ABA+ET	−a	−a
ET	GA_3+ABA+ET	−a	−a
GA_3	GA_3+ABA	−a	37.9c
GA_3	GA_3+ET	−a	38.5c
GA_3	ABA+ET	−a	5.3b
GA_3	GA_3+ABA+ET	−a	34.0c

See the footnotes in Table 1.

produced an inhibition (Table 1) although ABA act as stimulator in combination with GA_3 as shows in Sasa (Table 3). ET slightly inhibited the GA_3 induced growth when applied with GA_3 in phase I or II in HA-8 (Table 2 and 3). In conclusion, the presence of GA_3 in phase I is prerequisite for induction of elongation of 2IN.

Experiment 2. Single and combined treatment of ABA and ET with or without GA_3 in phase IIA and IIB in the presence of GA_3 in phase I

No elongation was observed in the single treatment of GA_3 applied in phase I (Table 4), although GA_3 induced the elongation when applied in phase I in the experiment 1 (Table 1). This is caused by the lower concentration of GA_3 used in the experiment 2 than that of experiment 1. GA_3 application in phase IIA and/or IIB caused the prominent elongation when GA_3 is applied in phase I as shown in Table 4.

The application of GA_3 was more effective when applied in phase IIA than that in phase IIB. However ABA inhibited the internode growth when applied together with GA_3 in phase IIA and/or IIB. ET affected little the growth when

TABLE 3. *Growth response of the second internode as affected by combined treatments of ABA, ET and GA_3 before elongation (phase I) and followed by treatments of ABA, ET or GA_3 alone during elongation (phase II).*

Treatment phase		Length (mm)	
I	II	Sasa	HA-8
ABA+ET	Water	−a	−a
ABA+ET	ABA	−a	−a
ABA+ET	ET	−a	−a
ABA+ET	GA_3	−a	−a
GA_3+ABA	Water	−a	5.1bc
GA_3+ABA	ABA	−a	3.8bc
GA_3+ABA	ET	−a	6.2c
GA_3+ABA	GA_3	−a	40.0e
GA_3+ET	Water	−a	6.0c
GA_3+ET	ABA	−a	6.4c
GA_3+ET	ET	−a	9.2cd
GA_3+ET	GA_3	−a	55.7e
GA_3+ABA+ET	Water	−a	2.7b
GA_3+ABA+ET	ABA	−a	2.3b
GA_3+ABA+ET	ET	−a	7.8bcd
GA_3+ABA+ET	GA_3	17.0cd	43.3e

See the footnotes in Table 1.

applied with GA_3 in phase IIA or IIB.

In the previous experiment, the internode elongation in HA-8 was induced by the combination of ABA and ET even in the absence of GA_3. However, the combined treatment of ABA and ET in phase IIA and IIB did not produce any stimulative effect in the present experiment (Table 4).

Thus, ABA and ET seemed to stimulate the meristematic activity in phase I and not the extension growth in phase II. ABA may act as an inhibitor of gibberellin action in extension growth phase because the combined treatment of ABA and GA_3 in IIA and IIB resulted shorter internode than that of only GA_3 was applied in IIA and IIB.

Experiment 3. Single and combined treatments of BA, BR and IAA with or without GA_3 in the phase I, IIA and IIB

The presence of GA_3 in phase I was a prerequisite for inducing internode elongation in this experiment 3, too (Table 5). GA_3 in phase IIA and IIB was also effective in stimulating elongation.

All of BA, BR and IAA were not able to induce internode elongation when

TABLE 4. *The effects of ABA, ET, GA_3 and combined treatments applied at different stages of growth on the second internode elongation in the presence of GA_3 in phase I.*

Treatment phase			Length	Treatment phase			Length
I	IIA	IIB	(mm)	I	IIA	IIB	(mm)
GA_3	water	water	−a	GA_3	GA_3+ABA	GA_3	40.6cd
GA_3	GA_3	water	56.4d	GA_3	GA_3	GA_3+ABA	40.8cd
GA_3	water	GA_3	15.1b	GA_3	GA_3+ABA	GA_3+ABA	29.6c
GA_3	GA_3	GA_3	55.4d	GA_3	ET+ABA	GA_3	23.6c
GA_3	ET	water	−a	GA_3	GA_3	ABA+ET	32.8c
GA_3	water	ET	−a	GA_3	ABA+ET	ABA+ET	−a
GA_3	ET	ET	−a	GA_3	GA_3+ET	GA_3	57.1d
GA_3	ABA	water	−a	GA_3	GA_3	GA_3+ET	51.9d
GA_3	water	ABA	−a	GA_3	GA_3+ET	GA_3+ET	60.7d
GA_3	ABA	ABA	−a	GA_3	GA_3	GA_3	55.4d

− : invisible (less than 1 mm).
Means followed by different letters are significantly different at 5% level. I : PGR applied at 3 days after seeding for 2 days treatment. IIA : PGR was applied at 8 days after seeding for 3 days treatment. IIB : PGR was applied at 11 days after seeding for 3 days treatment.

TABLE 5. *Growth response of the second internode as affected by the BA, BR and IAA in the presence or absence of GA_3. Left : PGRs except GA_3 were applied befor elongation phase (phase I). Right : PGRs except GA_3 were applied during elongation phase (phase IIA and IIB).*

Treatment phase			Length	Treatment phase			Length
I	IIA	IIB	(mm)	I	IIA	IIB	(mm)
Water	GA_3	GA_3	−a	GA_3	Water	Water	−a
BA	GA_3	GA_3	−a	GA_3	BA	BA	−a
BR	GA_3	GA_3	−a	GA_3	BR	BR	1.7a
IAA	GA_3	GA_3	−a	GA_3	IAA	IAA	1.2a
GA_3	GA_3	GA_3	44.4d	GA_3	GA_3	GA_3	44.4d
GA_3+BA	GA_3	GA_3	57.8de	GA_3	GA_3+BA	GA_3+BA	16.5bc
GA_3+BR	GA_3	GA_3	44.6d	GA_3	GA_3+BR	GA_3+BR	38.8d
GA_3+IAA	GA_3	GA_3	46.8d	GA_3	GA_3+IAA	GA_3+IAA	36.7cd

− : invisible (less than 1 mm). Means followed by different letters are significantly different at 5% level through Table 6. I : PGR applied at 3 days after seeding for 2 days treatment. IIA : PGR was applied at 8 days after seeding for 3 days treatment. IIB : PGR applied at 11 days after seeding for 3 days treatment.

TABLE 6. *Growth response of the second internode as affected by the BA, BR and IAA in the presence or absence of GA_3. Left : PGRs except GA_3 were applied during early elongation phase (phase IIA). Right : PGRs except GA_3 were applied during late elongation phase (phase IIB).*

Treatment phase			Length	Treatment phase			Length
I	IIA	IIB	(mm)	I	IIA	IIB	(mm)
GA_3	Water	GA_3	20.3bc	GA_3	GA_3	Water	40.2d
GA_3	BA	GA_3	12.9bc	GA_3	GA_3	BA	31.8cd
GA_3	BR	GA_3	9.7b	GA_3	GA_3	BR	29.5cd
GA_3	IAA	GA_3	11.7b	GA_3	GA_3	IAA	36.3cde
GA_3	GA_3	GA_3	44.4d	GA_3	GA_3	GA_3	44.4d
GA_3	GA_3+BA	GA_3	34.2dc	GA_3	GA_3	GA_3+BA	29.0c
GA_3	GA_3+BR	GA_3	43.5d	GA_3	GA_3	GA_3+BR	37.7d
GA_3	GA_3+IAA	GA_3	41.8d	GA_3	GA_3	GA_3+IAA	48.4d

See the footnotes in Table 5.

applied alone in phase I (Table 5, left) or phase II (Table 5, right). BA applied with GA_3 in phase I (Table 5, left) promoted the internode growth, however it inhibited the elongation when applied, with or without GA_3, in phase IIA or IIB (Table 6) especially when applied both of them in IIA and IIB (Table 5, right). BR or IAA with GA_3, applied in phase I, had little effect whereas slight decrease was found when applied, with or without GA_3, in phase IIA and IIB (Table 5, right).

BA may play some role in the induction of the meristematic activity of the 2IN, since cytokinins are effective to enhance the activity of cell division (5). Cytokinins are also known to promote the leaf growth (6).

Discussion

There are few reports concerning the hormonal control of elongation in the 2nd internodes of rice seedlings since the internodes of the seedlings usually do not elongate under ordinal culture. However they do elongate when seeds are sown deeply in soil or artificialy incubated in darkness.

Takahashi *et al.* (1) found that GA_3 was the most effective stimulator among several PGRs in the elongation of the 2IN in dark grown rice seedlings. Then the light inhibition of the dark grown rice seedlings was recovered by the application of GA_3 (1). And only very high concentrations of GA_3 produced visible elongation of the 2IN in light grown seedlings in japonica rice (4, 2). Because of the very high concentration which is thought to be abnormal as a endogenous level of gibberellin in rice seedling, this mechanism is not likely so simple in the seedlings

grown in the light.

On the other hand, in deepwater rice, the low concentrations of GA_3 induced the elongation. This result suggests that deepwater rice has higher ability to respond to endogenous GA or it can produce higher concentrations of endogenous GA than that of japonica rice. And it may be one of the reason why deepwater rice can elongate their internodes easily even during vegetative stage of growth of rice although japonica rice elongated their internodes during reproductive stage.

Then Takahashi and Kaufman (4) checked if the low concentration of GA_3 at physiological level can incduce the elongation of 2IN of light grown seedings of japonica rice. They found in the previous report (2) that low concentrations of GA_3 induced the internode elongation in japonica rice seedlings in some cases as follows. 1. When GA_3 was applied with ABA and Ethylene. 2. When applied with triazinone (gibberellin synergist). 3. When GA_3 was applied under water stress condition by using the polyethlene glycol which is considered to stimulate endogenous ABA synthesis.

In light grown seedlings of rice, it is suggested that GA_3 is working as a major stimulator of internode elongation in cooperation with ABA and ethylene (4, 2). In the present paper, we checked the role of GA and other PGRs along the process of internode elongation. Internode elongation was divided into two major phases, namely, the meristem formation and extension growth phases. The latter was subdivided into two phases: the dominant cell division and dominant cell elongation phase as indicated in Fig. 1.

As a result of sequential application of GA_3 and other PGRs, the role of plant hormones seemed to be summarized as follows.

1. GA_3 was the most effective in the stimulation of internode elongation when applied in phase I. Therefore, GA is most likely to play a major role in the stimulation of meristematic activity. ABA, and ET, in combination with GA_3 was also effective. Then ABA has some role in the promotion of meristematic activity in the internode. ET may enhance the tissue sensitity to GA and ABA as previously reported[7]. BA, BR and IAA had only little effects on the elongation of the 2IN.

2. GA_3 was also most effective in the stimulation of extension growth of the 2IN when applied in the phase II especially in the early stage (phase IIA). Accordingly, GA is a major stimulator for cell multiplication in the extension growth of the rice internode. ABA works as an inhibitor in the extension growth phase and ABA antagonizes with GA_3 action. BA and ET also antagonize with GA_3 action since BA and ET inhibited GA_3-stimulated growth when applied with GA_3 in the phase IIA and IIB. BR or IAA affected little on the internode elongation.

For obtaining more precise informations, more detailed experiments controlling the level of endogenous hormones such as gibbereellin using the proper

inhibitors of hormone biosynthesis must be needed. And the anatomy of the 2IN is also essential to confirm the role of GA as descrived above. This will be the topic of our next paper.

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