Effects of Plant Growth Regulators on the Appearance of “MC Type” Rice Seedlings*

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Abstract : Rice seedling with the mesocotyl and foliage leaves enclosed in the coleoptile are here referred to as “MC type” seedlings and are considered to be suitable for deep sowing. We investigated the effects of seven plant growth regulators (PGRs), including gibberellic acid (GA_3), indole-3-acetic acid (IAA), abscisic acid (ABA), kinetin (KIN), ethylene (ET), brassinolide (BR) and fluridone (Flu) on the appearance of MC or non-MC type rice seedlings. The seedlings were grown on 0.8% agar medium in the presence or absence of PGRs under aseptic conditions at 30°C in the dark for 14 days. Among the PGRs tested, both ABA and BR promoted the appearance of MC type rice seedlings. Flu, an inhibitor of carotenoid synthesis, and indirectly, of ABA biosynthesis, reduced the percentage of MC type rice seedlings and promoted the growth of leaves. The response of mesocotyl was different from that of the leaves. Mesocotyl elongation growth was stimulated by ABA but reduced by Flu. Furthermore, when applied simultaneously with Flu, the effect of ABA was eliminated. These results suggest that the appearance of MC type rice seedlings is under the control of endogenous ABA and, that Flu affects not only ABA biosynthesis but also its action.

Key words : Absciscic acid, Brassinolide, Coleoptile, Deep sowing, Fluridone, Mesocotyl, Rice-seedling.

イネの「MC 個体」の出現に及ぼす各種生長調節物質の影響：渡邊 肇・高橋 清（東北大学農学部）

要 旨：「MC 個体」とはその苗条が鞘葉と中茎からなり第1葉以降の葉が鞘葉内に包まれて出葉せず、その後の発育が外見上停止する生育型である。MC 個体と非MC 個体の出現に及ぼす各種植物生長調節剤の影響を調査した。使用した生長調節剤は、ジベレリン A_3 (GA_3)、オーキシシン (IAA)、アブシジン酸 (ABA)、エチレン (ET)、カイネチン (KIN)、ブラシノリド (BR)、フルリドン (Flu) の7種である。種子を消毒後、生長調節剤を含む0.8%寒天培地に置床し、30°C、完全暗条件下で14日間無菌培養した。使用した生長調節剤の中で、ABAとBRがMC 個体の出現を促した。一方、カロチノイドの生合成を阻害することによってABAの生合成を阻害するFluはMC 個体の出現を顕著に抑制した。Flu処理により、葉の伸長が促進され、逆に中茎の伸長は阻害された。さらに、ABAとFluを併用処理した場合、ABAの作用が打ち消された。以上の結果、MC 個体の出現は内生ABAレベルによって調節されと考えられ、さらにFluはABAの生合成のみならずその作用に影響を及ぼしてMC 個体の出現を抑制するものと考えられる。

キーワード：アブシジン酸、イネ幼植物、鞘葉、中茎、深播き、ブラシノリド、フルリドン。

We have recently investigated varietal differences and geographical distribution with respect to the growth of mesocotyls and internodes of etiolated rice (*Oryza sativa* L.) seedlings¹⁸⁾. Two hundred and sixty varieties of conserved germplasm received from the International Rice Research Institute (IRRI) were used in these studies. In a series of experiments, we discovered unique seedlings consisting only of mesocotyl and coleoptile (the leaves were enclosed by the coleoptile). We identified these seedlings as “MC type” seedlings^{18,20)}. “MC” is derived from the capital letter of mesocotyl (M) and coleoptile (C), respectively. Usually under dark conditions,

the first, the second and the third leaves grew in a spindly manner. Hoshikawa⁶⁾ reported that seedlings of rice are able to develop the 4th leaf even in the dark. In contrast, the first and the second leaves did not develop in the MC type seedling. It is likely that the growth of the successive leaves and the internodes in MC type seedling was inhibited.

In our previous experiments, we reported that cultivars originating from the Indian subcontinent, especially the foothills of the Himalayas, had high percentage of MC type seedlings²⁰⁾. In addition, we could identify that two ancestral species of *O. sativa*; *O. rufipogon* and *O. nivara*, contained a high frequency of MC type seedlings as compared with those of cultivated species. Furthermore, we found that the traditional cultivars used in shifting cultivation system in Panama, “a chuzo”, had many MC type seedlings as

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compared with those of improved cultivars²⁰). In “a chuzo”, rice is deep seeded by dibbling¹), therefore, we considered that MC type seedling is suitable for deep sowing²⁰). In addition, the morphological and anatomical characteristics of MC and non-MC type seedlings are also discussed elsewhere²¹).

It is well known that plant growth regulators (PGRs) affect the growth of rice seedling organs, including mesocotyl, coleoptile and internode^{7,14,16,17}). The objective of this study is to investigate the effects of the PGRs on the appearance of MC type rice seedlings in order to clarify the physiological conditions which may be related to their appearance.

Materials and Methods

1. Plant materials

We used two Indica rice (*Oryza sativa* L.) cultivars, namely, cv. ‘Basmati 217’ (“MC type” cultivar) and cv. ‘JC 91’ (“Non-MC type” cultivar). MC type cultivar refers to a cultivar which has a high frequency of the occurrence of MC type seedlings among the investigated plants. Non-MC type cultivar refers to a cultivar which has low frequency of the occurrence of MC type seedlings^{18,20}).

2. Culture conditions

Dehulled uniform rice seeds were selected and sterilized in 70% (v/v) ethanol for 30 s and then in 1% (v/v) sodium hypochlorite for 20 min. Sterilized seeds were promptly washed with sterilized water. Six seeds were placed in a test tube (3 × 30 cm) which contained 30 mL of culture medium. The medium consisted of 8 g L⁻¹ agar. Plant growth regulators were added after autoclaving by filter sterilization. The concentrations used were 10⁻⁸~10⁻⁵ M for ABA (abscisic acid), IAA (indole-3-acetic acid), GA₃ (gibberellic acid), ET (ethephon), KIN (kinetin) and 10⁻⁹~10⁻⁶ M for BR (brassinolide). In the case of combinations of ABA and Flu (fluridone), 1.5 × 10⁻⁶ M ABA and 10⁻⁸~10⁻⁵ M Flu, were used. The test tubes were sealed with Milli-Wrap™ (Millipore Japan, Tokyo, Japan) except in the case of ET treatment. For ET treatment, aluminum foil was used to seal the test tubes so as to retain the evolved ethylene in the test tube. The cultures were incubated in the dark at 30 °C for 14 days. The experiments were performed with 3 to 5 replications.

3. Measurements

Fourteen days after incubation, plant height and the length of the each seedling organs were measured using the stereoscopic microscope. From these data the frequency of the occurrence of MC type seedlings among the investigated plants was calculated.

4. Plant growth regulators

All of the PGRs except Flu were obtained from Sigma Chemical Company (St. Louis, MO, USA). Flu was purchased from Wako Pure Chemical Industries, LTD. (Osaka, Japan). Absciscic acid (mixed isomers) consisted of 40% of trans, trans abscisic acid and 60% of cis, trans abscisic acid (This information was obtained from Sigma Chemical Company).

Results

1. Effects of PGRs on the appearance of “MC type” rice seedlings of “MC type” rice cultivar and “non-MC type” rice cultivar

Fig. 1 shows the effects of PGRs on the frequency of occurrence of MC type seedlings grown from MC and non-MC type rice cultivar. In the case of MC type cultivar (Fig. 1A), all PGRs failed to cause a significant diminution in the percentage of MC type seedlings. Only IAA (10⁻⁸ M and 10⁻⁶ M) and ET (10⁻⁸ M and 10⁻⁷ M) caused a slight reduction in the percentage of MC type seedlings as compared to that of control. These two substances tend to stimulate the elongation of the coleoptile. In the case of the non-MC type cultivar (Fig. 1B), ABA (10⁻⁵ M) and BR (10⁻⁷ M and 10⁻⁶ M) significantly increased the percentage of MC type rice seedlings. All PGRs, except for ABA and BR, did not appear to show significant effects on the percentage of MC type seedlings, except for the fact that the percentage of MC type seedlings tended to increase slightly with increasing concentration of KIN, and decreased slightly with increasing concentration of GA₃.

2. Effects of ABA and Flu on the appearance of MC type rice seedlings

According to the results described above, ABA strongly stimulates the appearance of MC type rice seedlings. It has also been reported that Flu, inhibitor of carotenoid synthesis, inhibits ABA biosynthesis^{2,4}). For the next step in the study, therefore, we examined the effect

Fig. 1. Effects of plant growth regulators on the percent occurrence of MC type seedlings using a MC type rice cultivar (cv. 'Basmati 217') (Fig. 1A) and a non-MC type rice cultivar (cv. 'JC91') (Fig. 1B). The seedlings were cultured at 30°C in the dark for 14 days. Asterisks in the figures mean significant differences between the control (without PRGs) and each treatment at the 1% level according to Fisher's exact probability test. KIN treatment (10⁻⁵M) brought about necrotic brown spots in the seedlings, therefore, the data of 0~10⁻⁶M KIN treatments were described.

of ABA, in the presence or absence of Flu, on the frequency of occurrence of MC type rice seedlings.

(1) The case of MC type rice cultivar

The typical MC type rice seedlings is shown in Fig. 2 (left). This seedling consisted only of mesocotyl and coleoptile. The coleoptile showed a cylindrical sheath-like form, a conical tip, and it enclosed the undeveloped leaves (the first, second, and third leaves). Flu caused the elongation and expansion of the first and second leaves from the coleoptile. Sometimes the third leaf emerged from the coleoptile. Also, elongation of the first and second internodes occurred (Fig. 2, center). Combined treatment of Flu + ABA promoted the elongation of foliage leaves from coleoptile. The length of mesocotyl of Flu + ABA treatment was relatively similar to that of control (Fig. 2, right). Fig. 3 shows the effects of ABA, in the presence or absence of Flu, on the frequency of occurrence of MC type rice seedlings. A concentration of ABA of 1.5×10^{-6} M was adequate for the appearance of

MC type seedlings and for the elongation of the mesocotyl. With increasing concentrations of Flu, the percentage of MC type seedlings was reduced. At Flu concentrations ranging from 10⁻⁷M to 10⁻⁵M, the percentage of MC type seedlings was significantly reduced ($P < 0.01$). In fact, at 10⁻⁶M and 10⁻⁵M Flu, the percentage of MC type seedlings was 0%. Fig. 4 shows the effect of Flu on the growth of seedling organs of MC type rice cultivar. With increasing concentrations of Flu in the culture medium, the growth of coleoptiles and of foliage leaves were stimulated. Flu (10⁻⁵M) promoted the growth of coleoptiles, the first leaves and second leaves by 70%, 120%, 660% above those of the control, respectively. In contrast, the response of the mesocotyl was different from that of the control, respectively. In contrast, the response of the mesocotyl was different from that of the leaves; i.e., 10⁻⁵M Flu reduced the growth of mesocotyl by 82%.

(2) The case of non-MC type cultivar

The typical non-MC type rice seedling is shown in Fig. 5 (left). This seedling consisted

Fig. 2. Typical effects of Flu in the presence or absence of ABA on the morphology of MC type seedlings using a MC type rice cultivar (cv. 'Basmati 217'). The seedlings were cultured at 30°C in the dark for 14 days. Left to right; control, 10^{-6} M Flu, and 10^{-6} M Flu + 1.5×10^{-6} M ABA. Arrows indicate the coleoptilar node (the node between the mesocotyl and coleoptile). The part below the arrow is the mesocotyl. Bar = 10mm.

of greatly elongated leaves and internodes. When ABA was added to the culture medium, the phenocopy of MC type seedling was obtained as shown in Fig. 5 (center); this seedling consisted of only mesocotyl and coleoptile. ABA together with Flu promoted the elongation of foliage leaves from coleoptile. However, the length of mesocotyl was longer than that of the control (Fig. 5, right). Fig. 6 shows the effects of ABA, in the presence or absence of Flu, on the frequency of the occurrence of MC type seedlings using a non-MC type rice cultivar (cv. 'JC 91') for treatments. Single applications of ABA significantly ($P < 0.01$) increased the percentage of MC type seedlings. With this treatment, growth of mesocotyl was markedly enhanced (Fig. 5). The effects of Flu on the extension growth of organs of non-MC type rice seedlings are shown in Fig. 7. As compared with the control, Flu promoted the growth of coleoptiles, first

Fig. 3. Effect of different concentration of Flu in the presence ($\square$) or absence ($\blacksquare$) of 1.5×10^{-6} M ABA on the percent occurrence of MC type seedlings using a MC type rice cultivar (cv. 'Basmati 217'). The seedlings were cultured at 30°C in dark for 14 days. Asterisks in the figures mean significant differences between the control (0M Flu in the absence of ABA) and each treatment at the 1% level according to Fisher's exact probability test.

Fig. 4. Effect of different concentration of Flu on the growth of seedling organs for a MC type rice cultivar (cv. 'Basmati217'). Seedlings were cultured at 30°C in the dark for 14 days. Vertical bars indicate standard errors. ■, plant height; ●, mesocotyls; ▲, coleoptiles; ◆, first leaves; □, second leaves.

leaves and second leaves in a manner similar to that mentioned for case of the MC type rice cultivar. In contrast, Flu greatly inhibited the growth of mesocotyls and first internodes. This is especially evident for 10^{-5} M Flu, which inhibited the growth of mesocotyls and first internodes by 76% and 99.9%, respectively.

Fig. 5. Typical effects of ABA in the presence or absence of Flu on the morphology of non-MC type seedlings using a non-MC type rice cultivar (cv. 'JC 91'). The seedlings were cultured at 30°C in the dark for 14 days. Left to right; control, 1.5×10^{-6} M ABA, and 1.5×10^{-6} M ABA + 10^{-6} M Flu. White arrows indicate the coleoptilar node (the node between the mesocotyl and coleoptile). The part below the arrow is the mesocotyl. Bar = 10mm.

Discussion

In the present studies, we investigated the physiological conditions related to the appearance of the "MC type" rice seedlings. ABA and BR, added *in vitro* to the culture medium, increased the frequency of occurrence of MC type rice seedlings (Fig. 1). This result suggests the possibility that BR increases the levels of endogenous ABA in such seedlings. Kuraishi *et al.* reported that BR-treated segments of squash hypocotyls showed a tendency towards decrease in levels of ABA⁸⁾. Furthermore, BR treatment did not produce consistent results concerning levels of ABA in rice panicles³⁾. Much more information is needed; therefore, to establish an unequivocal relationship between changes in ABA levels brought on by BR treatment and the appearance of MC type rice seedlings. In rice seedlings,

Fig. 6. Effect of different concentration of Flu in the presence (□) or absence (■) of 1.5×10^{-6} M ABA on the percent occurrence of MC type seedlings using a non-MC type rice cultivar (cv. 'JC 91'). The seedlings were cultured at 30°C in the dark for 14 days. Asterisks in the figures mean significant differences between the 0M Flu + 1.5×10^{-6} M ABA and each treatment at the 1% level according to Fisher's exact probability test.

Fig. 7. Effect of different concentration Flu on the growth of seedling organs for a non-MC type rice cultivar (cv. 'Basmati 217'). Seedlings were cultured at 30°C in dark for 14 days. Vertical bars indicate standard errors. ■, plant height; ●, mesocotyls; ▲, coleoptiles; ◆, first leaves; □, second leaves; ○, first internodes; △, second internodes.

BR¹⁷⁾ and ABA^{5,15)} both increased the growth of rice seedling mesocotyls. However, the effect of ABA is more pronounced than that of BR by about 2.9 times at optimum ABA concentrations (data not shown). Furthermore, BR induced both curvature and swelling of mesocotyls which are distinguishing character-

istics for BR action. With ABA treatment, we did not observe these morphological changes. These results suggest that the modes of action of ABA and of BR are probably different in terms of the appearance of MC type rice seedling. Further investigation of the interaction of ABA and BR, as related to the appearance of MC type rice seedling, is needed.

Flu, an inhibitor of carotenoid biosynthesis^{2,4)}, reduced the percent occurrence of MC type rice seedlings. There is strong evidence that ABA is synthesized via an indirect pathway from carotenoids^{9,11)}. In addition, inhibition of ABA biosynthesis by Flu has been reported previously^{4,12)}. This evidence suggests that ABA plays a role in regulating the appearance of MC type seedlings via control of leaf and internode growth. When Flu is added in combination with the 1.5×10^{-6} M ABA, only a relatively high concentration of Flu (10^{-6} M and 10^{-5} M) significantly reduces the percentage of MC type seedlings (Fig. 5, right and Fig. 6). It suggests that Flu apparently affects the appearance of MC type seedlings by blocking ABA biosynthesis and not otherwise. ABA inhibits the development of K^+ uptake that is coupled to H^+ extrusion (the so-called proton pump system) at the cell membrane^{10,13,19)}. We have suggested that appearance of MC type rice seedling may be regulated by this H^+/K^+ ion exchange system²²⁾. It is possible that Flu may act directly on this system. However, more information is needed to confirm this possibility. We are now investigating the relationship between the endogenous ABA levels and the appearance of MC type rice seedlings.

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