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Effect of Brassinolide on the Cultured Rice Root Growth as Modified by Figaron and Gibberellic Acid*

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Abstract : The excised rice root was cultured by using two separate media. The root base was supplied with 12.5% of sucrose via scutellum, while the root tip was immersed in the inorganic nutrients of MS medium diluted 4 times. Different concentrations of brassinolide were supplied via either root tip (root medium) or root base via scutellum (scutellum medium). Brassinolide supplied to the scutellum medium stimulated the excised root growth at a concentration of 10^{-8} M, while application of the same concentration to the root medium was found to be drastically inhibitory. Although the single application of either brassinolide (10^{-8} M) or figaron (10^{-8} M) to the scutellum medium detectably stimulated root growth, a mixture of them applied to the scutellum medium was shown to be inhibitory for root growth, especially for primary root length while the lateral root length was stimulated. On the other hand when GA_3 was supplied to the scutellum medium the primary root length showed a recovery from the slight inhibition observed when brassinolide was applied alone. Results revealed that dispite the observed superiority of the mixture of brassinolide and GA_3 in stimulating root growth and the antagonistic effect in the case of brassinolide and figaron, their regulatory effects seemed to be through a different mode of actions.
Key words : Brassinolide, Figaron, Gibberellic acid, Hormonal regulation, *Oryza sativa* L., Root growth, Root medium, Scutellum medium.

ブラシノライドによるイネ分離根の生長反応とフィガロン及びジベレリン酸の関係：ラディ・サイド・ハッサン・前田英三（名古屋大学農学部）

要　旨：二培地法により，イネ分離根を培養した．胚盤培地に12.5%の蔗糖を，根培地に MS 無機塩の1/4濃度を入れ，それぞれの培地にブラシノライドを添加した．ブラシノライド（10^{-8} M）を胚盤培地に加えたとき分離根の生長が促進されたが，根培地に加えたときには阻害作用が見られた．ブラシノライドとフィガロンを共に胚盤培地に加えた場合，種子根の生長は阻害されたが，側根の生長は促進された．ブラシノライドとジベレリン酸を胚盤培地に混用したときには，フィガロンと混用した場合の如き阻害作用は観察されなかった．
キーワード：イネ，ジベレリン酸，植物ホルモンによる制御，根の生長，根培地，胚盤培地，フィガロン，ブラシノライド．

Brassinolide is a new growth regulator isolated from the pollen of *Brassica napus* L.[12]. It was found to have physiological functions in plant stem growth by both cell expansion and cell division[9,18,21]. It was recently isolated from different plant sources[7]. Its activity was described in about 17 bioassays of auxins, gibberellins and cytokinins[9,19–21], and was found to be dependent on the nature of the bioassay, making it difficult to determine the nature of its activity and also to which hormonal group it belongs. It was observable that most of the interest about the effect of brassinolide on the growth and different physiological activities was restricted to the shoot system and not extended to its effect on the root system. Although brassinolide was shown to be active in most of the auxin bioassays, it showed no activity in the bioassay concerning the inhibition of elongation of intact cress roots[21].

The effect of different hormones on the growth and development of excised rice root cultured by using two separate media was studied in our previous paper[13]. In addition the survivability and activity of the scutellar tissue in absorption of sugar from the scutellar medium were also confirmed in our previous papers[14,15], reflecting a culture method more related to the intact case as compared to the single medium culture method. Therefore this paper is devoted to study the effect of brassinolide applied separately or in combination

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with either figaron or GA_3 in regulating excised rice root growth, as an additional endeavour to detect the nature of the regulatory effect of brassinolide on the rice root system. Because the presence of brassinolide in the shoot system of rice plant was confirmed[1)], the possibility of a regulatory effect of brassinolide coming from the shoot in regulating rice root growth is worth investigation.

Materials and Methods

The excised rice (*Oryza sativa* L., cv. Aichi-asahi) root was cultured under aseptic conditions using two separate media as already described in details in our previous work[13)]. The root tip was immersed in 1/4 strength of the inorganic constituents of MS medium, while the root base with attached scutellum was supplied with 1 ml of 12.5% of sucrose. This sucrose concentration was found from our previous work to be the optimal for root growth[13)]. Different concentrations of brassinolide (2α, 3α, 22R, 23R-tetrahydroxy-24S-methyl-B-homo-7-oxo-5α-cholestan-6-one) were supplied to either root or scutellum media. Mixtures of brassinolide and figaron (ethyl 5-chloro-1-H-3-indazolyl acetate), and brassinolide and gibberellic acid (GA_3) were supplied to the scutellum medium at a concentration of 10^{-8} M for each of the three hormones. These concentrations of figaron and GA_3 were found to be the most stimulatory for root growth in our previous work[13)]. Also a comparison was made between the effect of a relatively high concentration of figaron (10^{-5}M) supplied to the scutellum medium and the effect of the above mentioned mixture of a relatively low concentrations of figaron and brassinolide. The cultures were incubated in darkness at 27 ± 0.5°C. Fresh weight and primary root length were measured after 3 weeks of incubation. The shadowgraphs of the representative samples were prepared and the morphological characteristics of growth were described. The results in Figs.1, 3 and 4 are the mean of 10 replicates.

Experimental Results

The excised rice roots were detectably regulated by brassinolide (Bra) supplied to the scutellum medium (Fig. 1). In this figure 10^{-8}M of brassinolide is shown to be the most

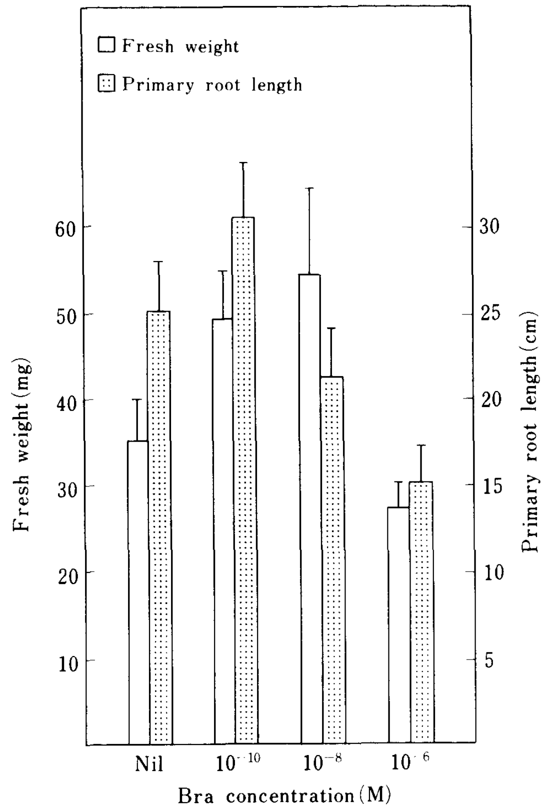


Fig. 1. Effect of different brassinolide concentrations supplied to the scutellum medium on the growth of excised rice root after 3 weeks of culturing.

stimulatory concentration to fresh weight among the three applied concentrations. The fresh weight was remarkably increased to reach the level of 157% of the control when 10^{-8}M of brassinolde was supplied to the scutellum medium, while 10^{-6}M of brassinolide led to a sharp decrease in the fresh weight, which became as low as 50% of its value when 10^{-8}M of brassinolide was supplied to the scutellum medium. Despite that fresh weight was detectably stimulated at 10^{-8} M of brassinolide, the primary root elongation was slightly inhibited at the same concentration, while the lateral branches became more abundant, longer and thicker than that of the control (Fig. 2). On the other hand a moderate stimulation on the primary root elongation was observed at a concentration as low as 10^{-10} M of brassinolide as compared to that of the control, and the relatively high concentration (10^{-6} M) was shown to be drastically inhibitory for all the estimated aspects of root growth (Fig. 1).

Contrary to the above stimulatory effect of

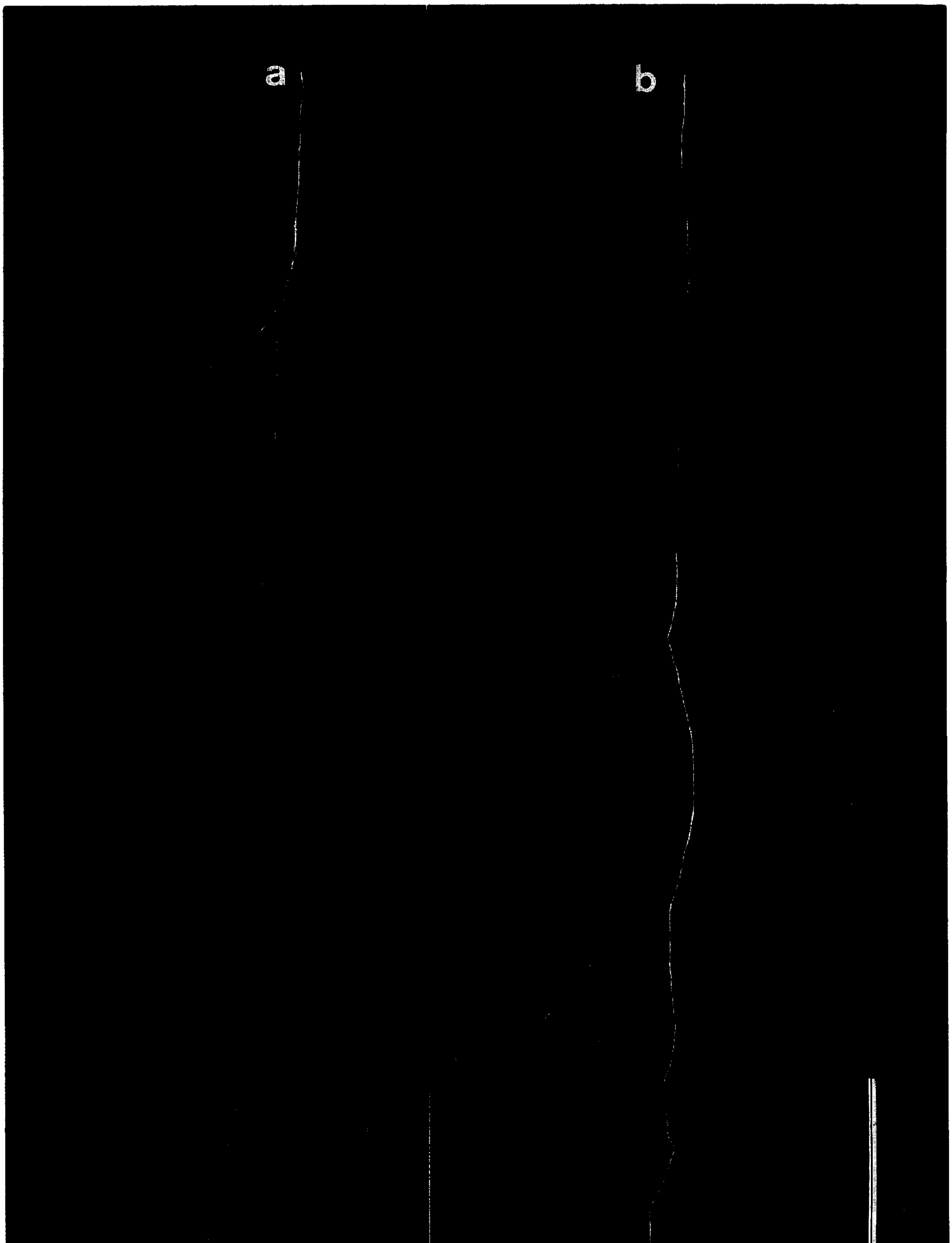


Fig. 2. A shadowgraph showing the effect of brassinolide (10^{-8} M) supplied to the scutellum medium on the growth of excised rice root (b) as compared to control (a) after 3 weeks of culturing. (bar 3 cm).

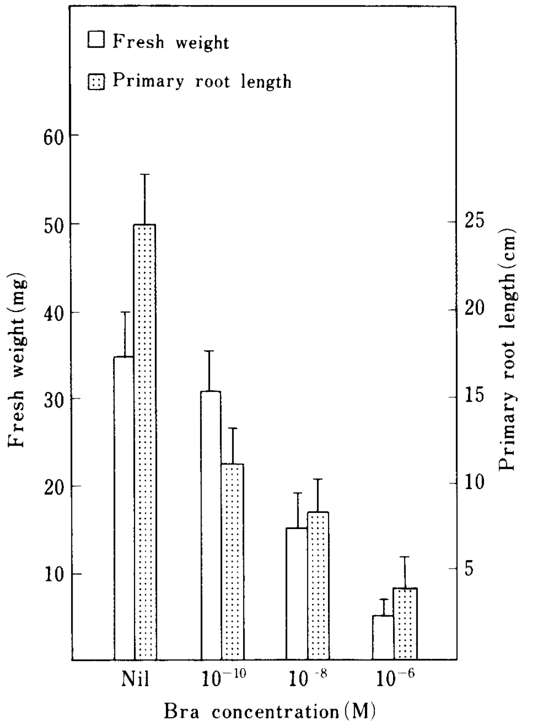


Fig. 3. Effect of different brassinolide concentrations supplied to the root medium on the growth of excised rice root after 3 weeks of culturing.

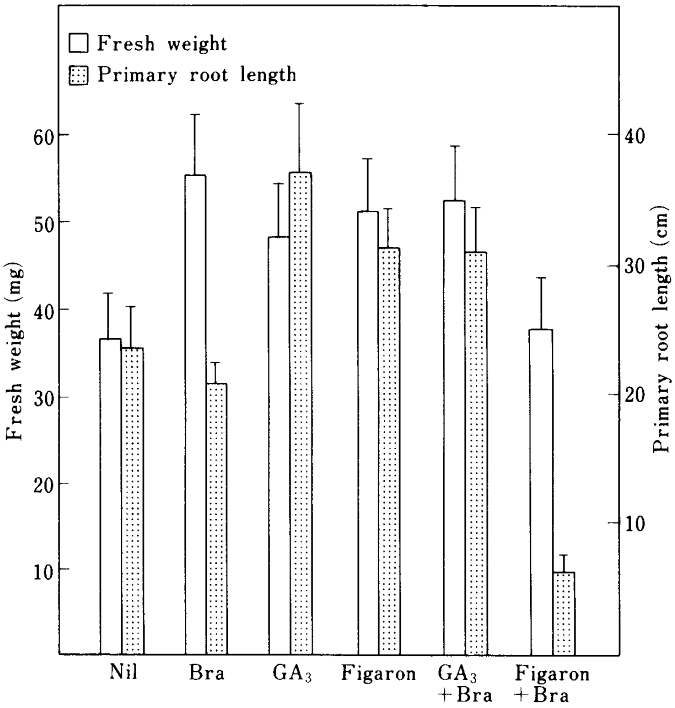


Fig. 4. Effect of a concentration (10^{-8} M) of brassinolide, figaron and GA_3, supplied separately or in combinations to the scutellum medium on the growth of excised rice root after 3 weeks of culturing.

brassinolide applied at a concentration of 10^{-8} M to the scutellum medium on the fresh weight and the number of lateral roots as shown in Figs. 1 and 2, application of brassinolide to the root medium (Fig. 3) was found to be drastically inhibitory for all the estimated growth aspects of the root at all the applied concentrations. The low concentration (10^{-10} M) was shown to be less inhibitory. On the other hand the inhibitory effect of brassinolide at a concentration of 10^{-6} M was so drastic that the fresh weight and primary root length reached the levels of 18% and 26% of its corresponding values, respectively, when the same concentration was applied to the scutellum medium.

Brassinolide, GA_3 and figaron supplied separately at the same concentration (10^{-8} M) to the scutellum medium (Fig. 4) stimulated the root growth as shown in the observed increase in fresh weight and the stimulatory effect of brassinolide was slightly higher than that of both figaron and GA_3. Contrary to brassinolide, figaron and GA_3 showed a detectable stimulatory effect on the primary root length, which was slightly inhibited in case of brassinolide. On the other hand the inhibitory effect of brassinolide on the primary root length was not extended to the lateral branches, which were detectably increased in number and length as compared to control (Fig. 2).

By applying a mixture of brassinolide and figaron at the above concentration to the scutelum medium (Fig. 5a), the root growth was drastically inhibited as compared to the single application of both hormones as shown in Fig. 4. Despite that fresh weight was still more or less in the level of the control (Fig. 4), the primary root elongation was drastically inhibited to reach the levels of about 24% of control and 28 and 19% of single application of brassinolide and figaron, respectively. In contrast, the lateral branches were considerably increased in number and length (Fig. 5a) as compared with control (Fig. 2a). Contrary to the above mentioned inhibitory effect of a mixture of brassinolide and figaron, a stimulating effect was observed when brassinolide was supplied with GA_3 to the scutellum medium (Figs. 4 and 6). Although fresh weight seemed to be more or less in the same

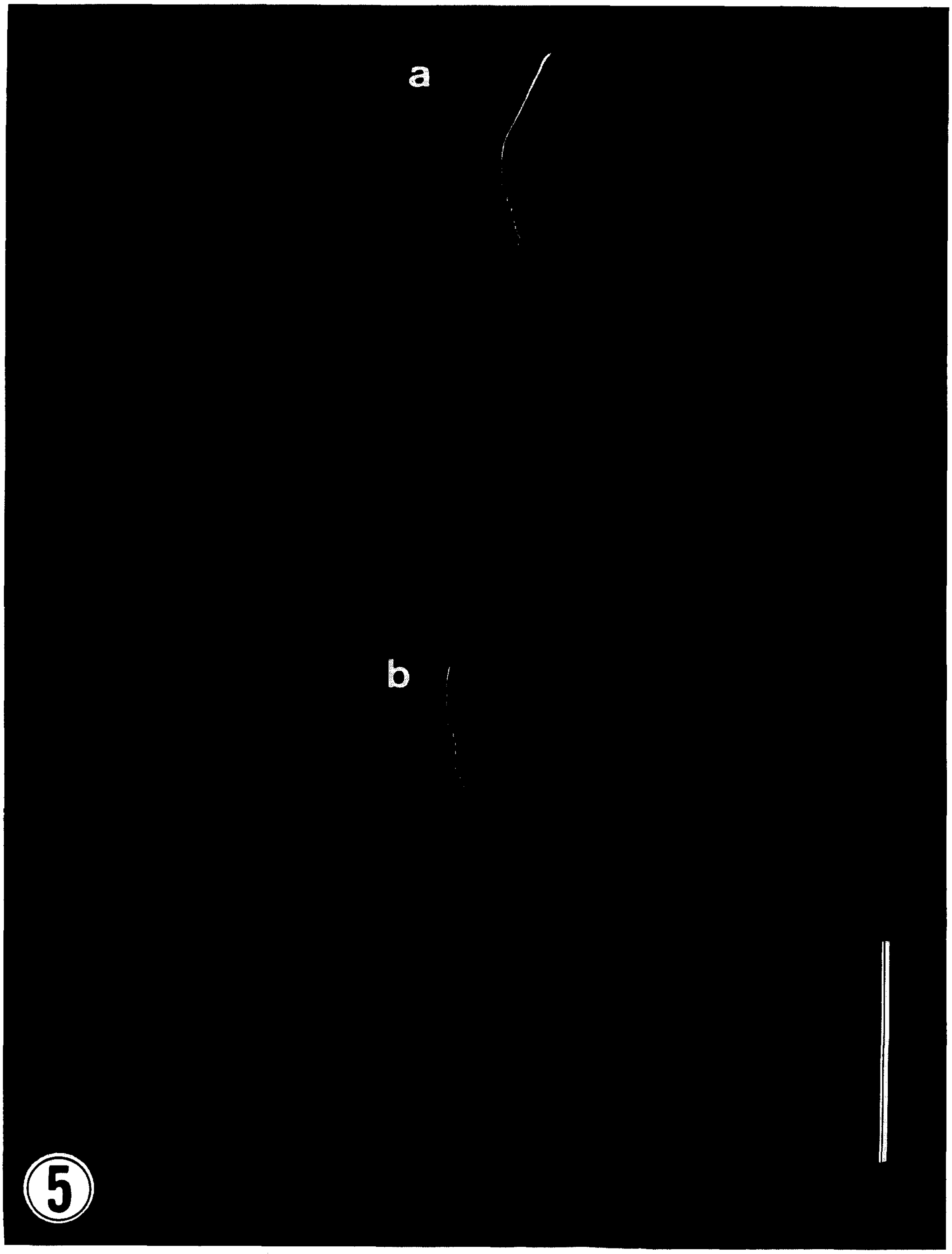
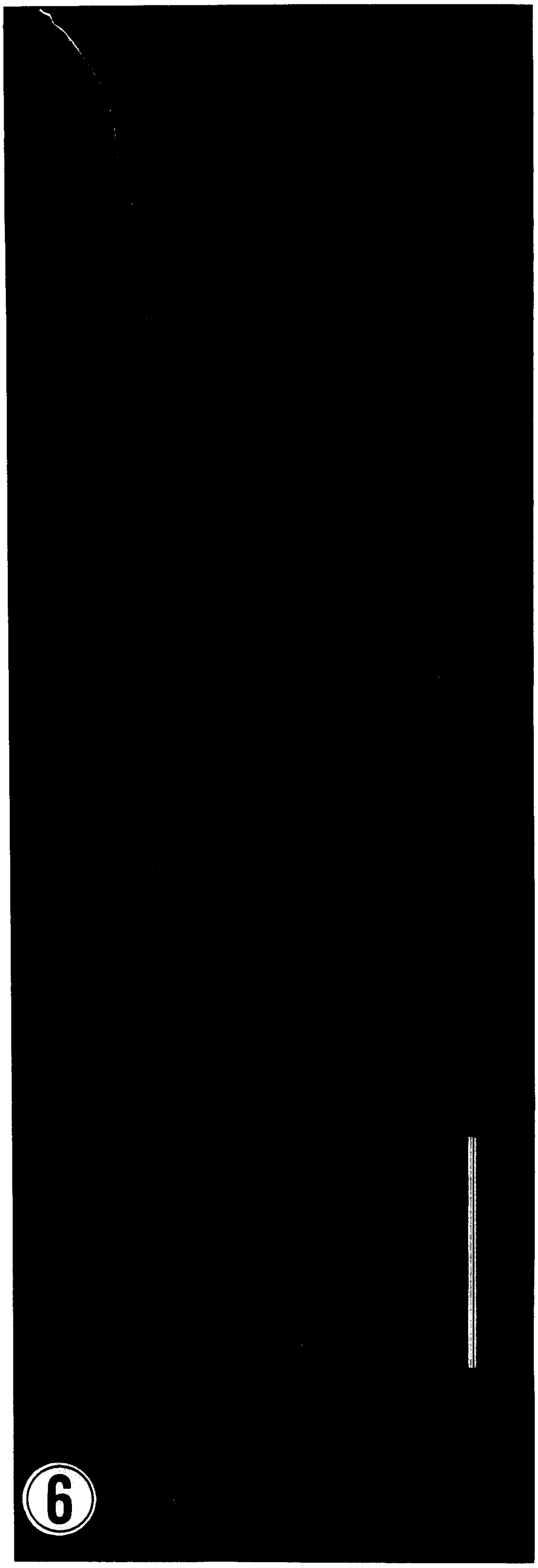


Fig. 5. A shadowgraph showing the effect of a mixture of brassinolide (10^{-8} M) and figaron (10^{-8} M) supplied to the scutellum medium on the growth of excised rice root after 3 weeks of culturing (a), as compared to the effect of a relatively high concentration (10^{-5} M) of figaron supplied separately to the scutellum medium (b). (bar 3 cm).

level as in the single application of brassinolide, it showed a marked stimulation as compared to control and also showed a slight stimulation as compared to GA_3 supplied alone (Fig. 4). On the other hand the primary root length was recovered from the slight inhibition observed when brassinolide was supplied alone, by supplementation of GA_3 to the scutellum medium, and a detectable stimulating effect on the primary root elongation



was observed as compared to control. Yet it was still shorter than its length when GA_3 was supplied separately to the scutellum medium.

Discussion

The above mentioned results revealed that brassinolide has a regulatory effect on the different growth aspects of excised rice root. The regulatory effect was shown to be dependent on brassinolide concentration and the site of application. It was shown that the application of brassinolide to the root base via scutellum detectably stimulated root growth in the favorable concentrations, and the application of the same concentrations to the root medium drastically inhibited root growth. This observation is reflecting the importance of the direction of the hormonal supply on the regulatory effect of brassinolide to excised rice root. Also the superiority of brassinolide applied to the scutellum medium as compared to that applied to the root medium in stimulating root growth may refer to a regulatory effect of brassinolide coming from the shoot in regulating root growth. This view is reasonable because the production of brassinolide was confirmed in the shoot system of rice plant[1], and also it was suggested that rice lamina inclination may be by the independent action of endogenous brassinolide itself in rice plant, or in combination with other phytohormones. Therefore the possibility of a regulatory effect of brassinolide coming from the rice shoot on the root growth in the intact case is worth mentioning and in the light of our data, it is not excluded from expectation.

A synergistic relationship between auxin and brassinolide was detected, and they appeared to act synergistically in most of the tested biological systems[21]. Therefore we tried to throw light on such synergistic effect in regulating excised rice root. When a mixture of figaron and brassinolide was supplied via scutellum medium at the concentrations which were found to be the most stimulatory for root growth in the case of separate application to

Fig. 6. Effect of a mixture of brassinolide (10^{-8} M) and GA_3 (10^{-8} M) supplied to the scutellum medium on the growth of excised rice root after 3 weeks of culturing. (bar 3 cm).

the scutellum medium, contrary to our expectation the root growth was inhibited and the most affected growth aspect was the primary root, indicating that each of them not only masked the stimulatory effect of the other, but also the final response of the root growth to the mixture was inhibitory. On the other hand the results is different in most of the bioassays in which brassinolide exhibits an auxin synergism[21] and also brassinolide elicits strong growth promoting effect in a certain auxin bioassays[6,8,12], inducing cell enlargement and cell division[18].

As shown in Fig. 5, there was a detectable similarity between the root growth characteristics of the mixture treated roots and that treated with a high concentration of figaron (10^{-5} M) which showed an inhibitory effect as previously reported[13]. The primary root elongation was drastically inhibited in both cases, while the lateral branches were still long, and the only detectable difference was the presence of abundant tertiary roots in the mixture treated roots, whose growth was inhibited by the treatment of high figaron concentration (10^{-5} M). Such similarity in the action of the mixture of brassinolide (10^{-8} M) and figaron (10^{-8} M) and the single application of a relatively high concentration (10^{-5} M) of figaron may suggest that brassinolide may affect the concentration of the endogenous auxin. However, this hypothesis is excluded because Cohen and Werner were able to show that brassinolide did not affect neither metabolism nor transportation or even absorption of auxins[3]. Therefore the root growth in both cases seems to be regulated through two different modes of action leading to the same morphological characterisics, and the only recorded similarity in the mode of action of both auxins and brassinolide is the extrusion of proton (H^+) and acidification of the medium[2,4,10,11], which was suggested to be a main characteristic linked to cell enlargement. The inhibitory effect of their mixture on root growth was therefore out of expectation.

Also the fact that the inhibitory effect of a mixture of brassinolide and figaron comes in a disagreement with the apparently observed synergism between auxin and brassinolide in many bioassays using a shoot parts may indicate that the regulatory effect of brassinolide is dependent on the nature of the tissue and the other hormones. This phenomenon was described by Marrè[10] and Sasse[17]. They suggested that brassinolide is a tissue specific hormone in contrast to IAA which lacks target tissue specificity. Also Romani et al was able to show that brassinolide differs from IAA in its opposite effect on proton efflux and growth of root tissue, suggesting that its effect is not mediated by auxin in this case[16]. In the light of their suggestion, the inhibitory effect of a mixture of brassinolide and figaron on the excised rice root growth in our work is explainable.

On the other hand the observed inhibitory effect of a mixture of brassinolide and figaron was not observed in the case of a mixture of brassinolide and GA_3. The root growth in this case seemed to be more or less the mean of its value when the two hormones were applied separately, indicating that the effect of the two hormones in this case seemed to be additive or complementary, rather than to be synergistic. This supposition was supported by the observation that although ancymidol inhibited GA_3 elongation, it did not interfere with brassinolide response[5].

In the light of this view the apparently observed synergism in many bioassays must be taken with some caution and it may be regarded as an additive effect and not as a result of an actual synergism.

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