イネ芽ばえの生長におけるエチレンとジベレリンの相乗作用

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Synergistic Action of Ethylene with Gibberellins in the Growth of Rice Seedlings*

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In comparing several types of regulatory functions, Scott and Leopold[4] found that ethylene and gibberellin (GA) had directly opposite effects. I reported previously that ethylene and GA acted additively or synergistically in the growth of rice mesocotyls in darkness[6]. As GA stimulates plant growth more effectively under light than in darkness[1,2], ethylene-GA interaction in the growth of rice coleoptiles and first leaf sheaths was investigated more precisely in this experiment.

The interaction of ethylene and endogenous GA-like substances extracted from several plant materials was also tested in the growth of rice seedlings.

Materials and Methods

Rice seeds were soaked in water for 2 days in darkness at 30°C. Germinated seeds were selected for uniform coleoptile length (0.5 mm). Ten seedlings each were transferred into 50 m*l* Erlenmeyer flasks containing 2 m*l* each of distilled water or various concentrations of GA_3. The flasks were stoppered with vaccine cap. Ethylene was injected through the vaccine cap with a gas-tight syringe. One ppm of ethylene was sufficient to cause maximum elongation of rice coleoptiles under red light[7]. Ethylene was added to each flask to yield a final concentration of 20 ppm as a "saturation" concentration of ethylene for rice coleptile growth. To remove endogenously evolved ethylene, a flask with a center well containing 0.5 m*l* of mercuric perchlorate solution (0.25 M) was used.

Treated seedlings were grown at 30°C under continuous irradiation of red light from four 20 watt fluorescent tubes (*Mitsubishi* colored fluorescent lamp, red, REL 20-RT), providing a radiant energy of 7150 ergs $cm^{-2}sec^{-1}$ at the plant surface. Measurement of coleoptile and first leaf sheath length was made 96 hrs after the start of irradiation. Transfer of seedlings was made as quickly as possible under dim green light.

GA extraction and fractionation were performed as previously described[5]. GA-like substances were separated by thin-layer chromatography using isopropylether-acetic acid (95:5, v/v) as the developing solvent. Chromatograms were divided into 10 equal zones (first zone was subdivided into 2 zones) and eluted with 50% acetone. After drying, eluates were dissolved in 100 μl of 50% acetone and 5 μl were used for bioassay (micro drop method); 1 μl each to five plants[3]. Zones showing GA-like activity were combined and used for studying interaction with ethylene. Half of the combined eluates was placed in each of two 50 m*l* Erlenmeyer flasks and evaporated to dryness before 2 m*l* of water and 10 germinating rice seeds (Tan-ginbozu) were added to each flask. One flask was used for ethylene treatment (20 ppm of ethylene was added) and the other was used for removing endogenously evolved ethylene. In the control 2 m*l* of water was added instead of the eluates.

Results

The results of dwarf rice variety, Tan-ginbozu, presented in fig. 1 show that ethylene acted synergistically with GA_3 in the growth of coleoptiles under red light. The time course of growth in length of coleoptiles under red light in the presence or absence of ethylene and GA_3 is shown in fig. 2. Coleoptiles grown in the presence of both ethylene and GA_3 continued to elongate for about 4 days after the start of the experiment. The final length was about

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24 mm. Coleoptilar growth ceased after two days in the control and in coleoptiles grown in either GA_3 or ethylene alone. When endogenously evolved ethylene was removed, a single application of GA_3 gave a small growth promotion in coleoptiles (fig. 2). However, growth of first leaf sheaths was evidently promoted by GA_3 even when endogenously evolved ethylene was removed (fig. 3). Thus, GA_3 stimulated the growth of first leaf sheaths more than that of coleoptiles, whereas, ethylene stimulated the growth of coleoptiles more than that of the first leaf sheaths or other upper organs. The combined application of ethylene and GA_3 showed a synergistic effect in the growth of coleoptiles and first leaf sheaths (fig. 4).

Since the variety Tan-ginbozu is a dwarf mutant known to be deficient in GA-like substances[8], several normal varieties were also tested for comparison As shown in table 1, the combined application of ethylene and GA_3 showed a synergistic effect on the growth of coleoptiles and first leaf sheaths in several normal varieties.

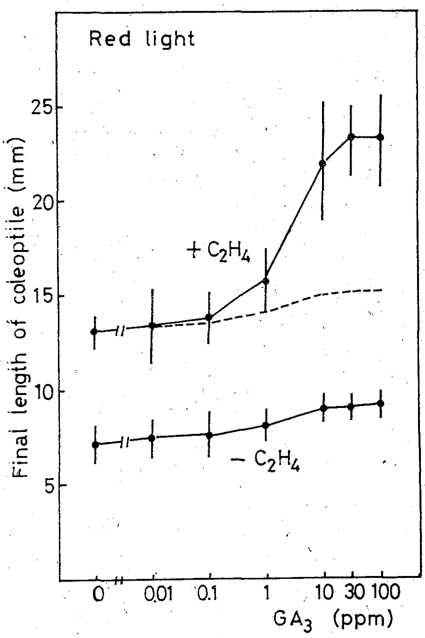

Fig. 1 Ethylene-GA_3 interaction in the growth of rice coleoptiles (cv. Tan-ginbozu) under red light. Values in the figure indicate average length and standard deviation. Dotted line indicates the limit of additive effect. Actual response curve ($+C_2H_4$, 20 ppm ethylene) exceeded this line, indicating that the effect was synergistic rather than additive. For removing endogenously evolved ethylene ($-C_2H_4$) mercuric perchlorate was used.

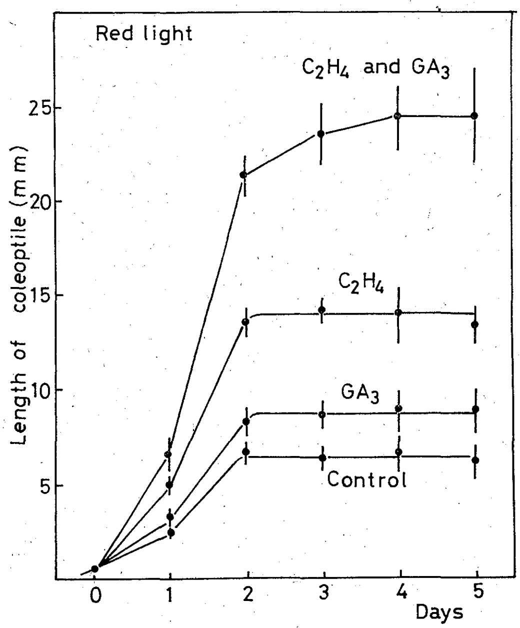

Fig. 2 The time course of the growth of coleoptiles under red light in the presence or absence of ethylene and GA_3 (cv. Tan-ginbozu). Values in the figure indicate the average length and standard deviation.

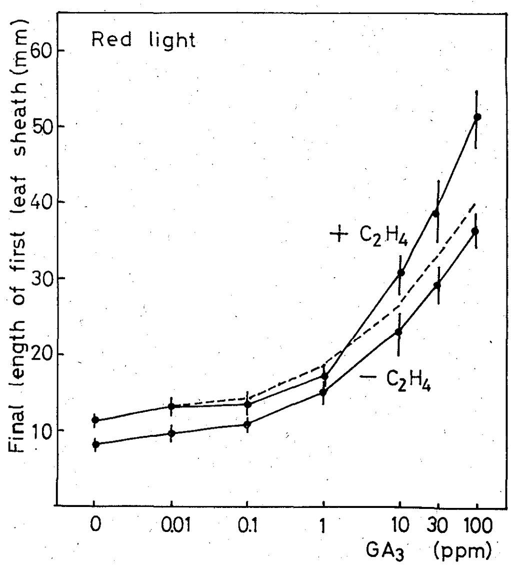

Fig. 3 Ethylene-GA_3 interaction in the grwoth of first leaf sheaths (cv. Tan-ginbozu) under red light. Otherwise as in fig. 1

Table 1. Ethylene-GA_3 interaction in growth of rice coleoptiles and first leaf sheaths under red-light

Variety	Type	Plant part	Final length(mm) with standard deviation			
			$-GA_3$ $-C_2H_4$	$+GA_3$ $-C_2H_4$	$-GA_3$ $+C_2H_4$	$+GA_3$ $+C_2H_4$
Norin No. 25	*Japonica*	Coleoptile	7.2±1.00	9.4±0.93	14.3±1.13	20.2±1.11
		First leaf sheath	13.9±0.81	36.6±3.64	17.9±1.58	50.9±3.18
Sasanishiki	*Japonica*	Coleoptile	7.4±0.78	8.7±0.94	17.6±0.67	26.2±0.43
		First leaf sheath	14.4±1.31	18.4±2.94	22.7±2.11	51.6±2.83
Nato	*Indica*	Coleoptile	6.6±0.60	9.0±1.28	13.9±0.82	23.5±0.92
		First leaf sheath	14.2±1.10	41.4±3.61	25.6±1.86	51.1±4.16
T-136	*Indica*	Coleoptile	6.0±0.48	8.7±0.74	11.7±0.62	17.9±0.94
		First leaf sheath	16.2±0.96	35.1±3.94	21.5±1.57	45.8±2.79

Concentration of GA_3 and C_2H_4 used here was 30 and 20 ppm respectively. For removing endogenously evolved ethylene mercuric perchlorate was used.

GA-like substances were obtained from rice shoots and pea seedlings. Histograms showing GA-like activities in the plant extracts are presented in Fig. 5. It is evident that Waito-C dwarf rice showed no response to GA-like substances extracted from both plant sources. However, both Tan-ginbozu and Waito-C dwarf rice responded strongly to GA_3. For testing interaction between ethylene and GA-like substances, active zones in Fig. 5 were used. As shown in Table 2, GA-like substances extracted from plant materials also exerted synergistic action with ethylene in the growth of rice coleoptiles and first leaf sheaths.

GA_7 was also tested in this experiment and the results from GA_7 were similar to that of GA_3.

Discussion

The results in this paper provide new evidence that not only GA_3 but also GAs extracted from plant materials act synergistically with ethylene in the growth of rice seedlings under red light. It is also important to note that the action of ethylene

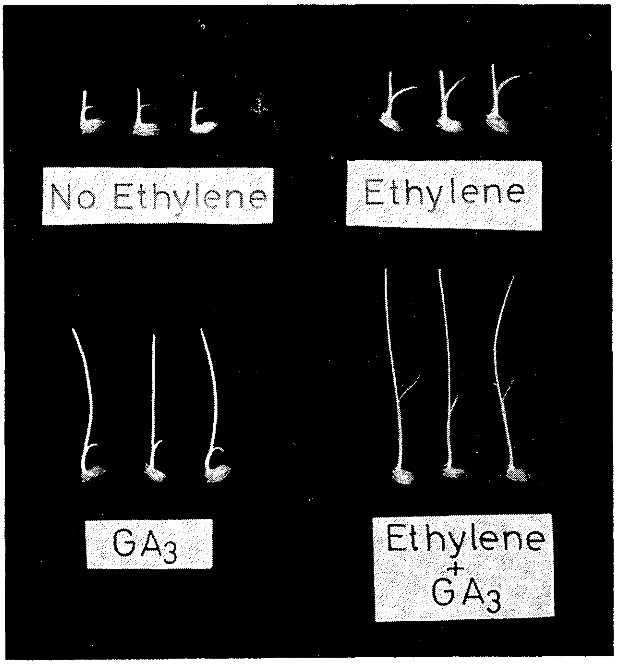

Fig. 4 Effect of ethylene (20 ppm) and GA_3 (30 ppm) on the growth of coleoptiles and first leaf sheaths of rice (cv. Tan-ginbozu) under red light. Second leaf was cut off at the position of the top of the first leaf sheath to show the difference of ethylene- and GA-effects more effectively. In rice plants, first leaf is called as "incomplete leaf" since no leaf blade is formed as shown. Roots were removed for convenience to take the photograph.

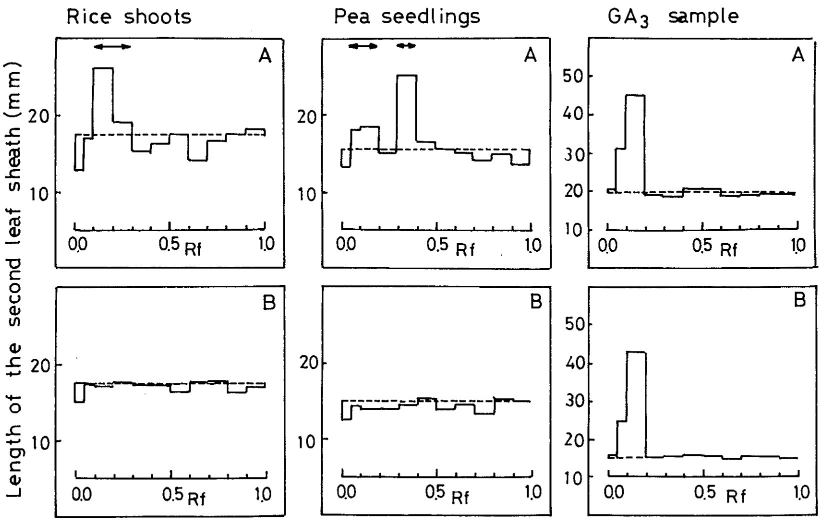

Fig. 5 Histograms showing GA activities of plant extracts from rice shoots (95 gram fresh weight) and from pea seedlings (50 gram fresh weight) and of GA_3 sample (1 μg) separated with thin-layer chromatography. Eleven eluates were tested on Tan-ginbozu dwarf rice (A) or Waito-C dwarf rice (B) seedlings. Broken line indicates the water control. Arrows in the upper histograms indicate the zones used for testing interaction with ethylene in the growth of rice seedlings.

Table 2 Interaction of ethylene with GA-like substances extracted from plant materials in the growth of rice coleoptiles and first leaf sheaths (c.v. Tan-ginbozu) under red-light

GA-like substances extracted from	Final length (mm) with standard deviation			
	Coleoptile		First leaf sheath	
	$-C_2H_4$	$+C_2H_4$	$-C_2H_4$	$+C_2H_4$
Rice shoots	7.4±0.64	15.5±1.07	10.4±0.40	12.5±1.28
Pea seedlings	11.3±2.60	18.4±4.33	12.1±1.28	16.0±2.14
Water control	5.7±0.74	11.7±0.93	8.2±0.57	10.5±1.34

Zones indicating GA-like activities in Fig. 5 were used in this experiment
$-C_2H_4$: endogenously evolved ethylene was removed using mercuric perchlorate
$+C_2H_4$: ethylene was added to yield a final concentration of 20 ppm

in stimulating rice seedling growth may be separated from that of GAs. In the rice seedling test for GA bioassay, the length of 2nd leaf sheath is measured for evaluating GA activity (Fig. 5). This organ was most sensitive to exogenously supplied GAs. Ethylene affected the growth of coleoptiles more than that of the first leaf sheaths or other upper organs. Thus, the mechanism of action may be different between ethylene and GA in rice seedling growth although a combined application of ethylene and GA exerted a synergistic effect on both sensitive organs. Ethylene acted synergistically not only with GA_3 but also with endogenous GA-like substances extracted from plant materials, indicating that ethylene may play an important regulatory role in the growth of rice seedings even under natural conditions. As shown in Fig. 5, Waito-C dwarf did not respond to GA-like substances extracted from rice shoots and pea seedlings although its response was strong toward GA_3. Murakami reported that both Waito-C and Tan-ginbozu dwarf rice were highly responsive to GAs with OH-group in the C-2 position of the gibbane ring. However, little or no response was observed toward GAs with no OH-group in the C-2 position. Both GA_3

and GA_7 have an OH-group in the C-2 position. Results in Table 2 suggested that ethylene acts synergistically not only with highly active GAs as GA_3 or GA_7 but also with GAs that have no OH-group in the C-2 position of the gibbane ring.

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SUMMARY

Both ethylene and GAs (GA_3 and GA-like substances extracted from plant materials) stimulated the growth of coleoptiles in rice seedlings under red light. A combined application was shown to be synergistic. Similar synergism was observed also in the growth of the first leaf sheath.

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〔和 文 摘 要〕

イネ芽ばえの生長におけるエチレンとジベレリンの相乗作用

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赤色光に下における，イネ子葉鞘および第1葉鞘の生長に対してエチレンとジベレリン (GA) は著しい相乗効果を示すことが見出された．エチレン単独では子葉鞘の伸長に対して著しい効果を示すが第1葉鞘の伸長促進効果は小さい．一方, GA は内生エチレンを除去した気中では，子葉鞘伸長に対する効果は小さいが，第1葉鞘伸長に対しては著しい促進効果を示す．このようにエチレンと GA はイネ芽ばえの伸長に対して作用する時期がずれており，メソコチル (Suge, 1971) や子葉鞘のように発芽初期に発生する器官は，エチレンによりよく反応し，第1葉鞘や第2葉鞘のようにそれよりおくれて発生してくる器官は GA によりよく反応する．しかし，エチレンと GA を同時に与えると，この両方の器官の伸長に対して著しい相乗効果をあらわした．

エチレンは GA_3 や GA_7 のように活性の高い GA だけでなく，イネ葉条やエンドウ芽ばえから抽出された GA 様物質でバイオアッセイの結果からジバン環の C-2 位に水酸基をもたないと推定される GA 様物質とも相乗的に作用した．これらのことから考えると，イネ芽ばえの初期伸長に内生 GA と内生エチレンは相互作用を通じて重要な調節作用をもつているものと推定される．