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IV. The amounts of gibberellin-like substances in the dwarf mutants of rice plants

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It is known that gibberellin promotes the elongation of internodes and growth of leaves in higher plants. Brian ***et al.* (1955, 1956)** and Phinney (1956) have reported that the action of this substance is stronger in dwarf than in normal plants, such as *Pisum* ***sativum, Vicia*** *faba*, ***Zea*** *mays*. Since Phinney (1957) first reported that gibberellin-like substances were present in higher plants, many investigators indicated that these substances were widely present in various plant species. In our previous paper (1971), we reported that there were differences among the dwarf rice varieties in their response to gibberellin A_3 (GA,). These facts may provide an interesting problem in connection with the growth of genetic dwarf mutants and the action of gibberellin.

The present paper was designed to analyze the growth of the dwarf mutants of rice plants in comparison with the amounts of gibberellin-like substances in normal and dwarf rice plants.

Materials and Methods

Four varieties of dwarf rice mutants and two normal varieties were used in the present experiment ; Kotake-tamanishiki, Tankan-shirasasa, Miyazaki No. 3 and Daikoku as dwarf types and Nôrin No. 18 (relatively long culm variety) and Kinmazc (relatively short culm variety) as normal type.

1. Seedling stage

The plants were grown on the sand for 14 days under fluorescent light (about 50 lux) at 30°C. Detached shoots were washed with tap water. Ten grams fresh weight of the leaves were ground in a mortal, extracted with 25 ml of **80** % ethanol at room temperature, and filtrated. The extraction (the manner described by Ogawa, 1963) was repeated every hour for six times with fresh ethanol. The combined filtrates were evaporated to dry under reduced pressure. The resulting residues were dissolved in 2 ml of 60 % ethanol, spotted on a sheet of Tôyo No. 51 filter paper and developed with a mixture of iso-propanol, 7 N-ammonia and water (8 : 1 : 1) by the ascending method at about 30°C. The dried chromatogram was cut into 10 pieces and each segment was then placed in a glass tube (12 × 2 cm) with 1 ml of distilled water. "Kotake-tamanishiki", dwarf rice plant, was used as a test plant. When the seeds of this variety began to germinate in a petri dish with distilled water, they were placed in the glass tube containing the test solution, and grown under fluorescent light (about 50 lux) at 30°C. The seedling was supported with glass fiber and the top of the tube was covered with a sheet of paraffin paper in order to prevent drying. After 7 days, length of the second leaf sheath was measured. One plot consisted of seven seedlings.

2. Internode elongating stage

Three terminal leaves of plants under the rice field of natural conditions were collected as material for extraction, during the stage of internode elongation. Five grams fresh weight of the leaves were ground in a mortal using dry ice acetone. After evaporating off the acetone, the extraction was prepared with the methods mentioned above.

3. Effects of GA, on the elongation of the internodes of dwarf rice plants in darkness

In order to examine the elongation of the internodes in dwarf and normal rice plants under the dark condition, those varieties above mentioned were grown in test tubes (35 x **2.4** cm) under aseptic condition. The basic culture medium is a modified White's medium containing $MgSO_4 \cdot 7H_2O$ **260** mg, $Ca(NO_3)_2 \cdot 4H_2O$ **200** mg, $Na_2SO_4 \cdot 10H_2O$ **200** mg, KNO_3 80 mg, KC1 65 mg, $NaH_2PO_4 \cdot 12HO$ 16.5 mg, $MnSO_4$ 4.5 mg, $ZnSO_4$ 1.5 mg, H_3BO_4 1.5 mg, KI 0.75 mg, Fe-citrate 4 mg, ebios 25 mg, sucrose 15 mg and agar 6 g in 1000 ml distilled water. The test tube, each containing about 40 ml of the culture medium, were autoclaved at 1.0 kg/cm^2 overpressure for 20 min. Seeds were sterrilized by immersing them in 75 % alcohol for 30 sec., then in 10 % calcium hypochlorite solution for 25 min. and finally in 3 % hydrogen peroxide for 20 min.

One seed was sown in each tube. The concentration of GA, was maintained at 50 ppm in the tube. The test tube was kept at 25°C for 60 days in the dark and taken out to measure the elongation of the internodes.

4. The amounts of gibberellin-like substances in the dwarf rice plants grown in the dark

Five grams fresh weight of etiolated seedlings grown in a incubator at 30°C from 12 to 14 days were extracted and extractions bioassayed in the same methods used in the seedling stage.

Results

1. Elongation of the second leaf sheath by gibberellin known concentration and Rf value

Fig. 1 shows response curve of the rice seedling test (dwarf mutants of rice plants, "Kotake-tamanishiki") for gibberellin, plotting the log. as excess in length over the control versus the log. dosage. As shown in Fig. 1, the response curve is almost linear 1 to 0.001 ppm of concentrations in GA,. The solution containing GA, was chromatographed by the above mentioned method and bioassay was carried out. The results obtained are shown in Fig. 2. From this figure, the active zone is found at Rf 0.5-0.7 and it seems to coincide with GA,.

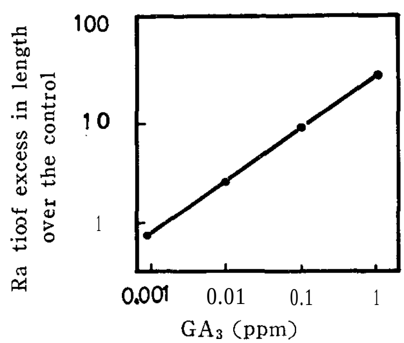


Fig. 1. Response of the rice to GA_3.

2. Seedling stage

1) Normal rice plants ; Two active zones were found in the ethanol extracts of normal rice plants. Namely, the first zone was found at Rf value 0.1-0.3 and the other zone at Rf **0.4-0.6.** The latter seems to

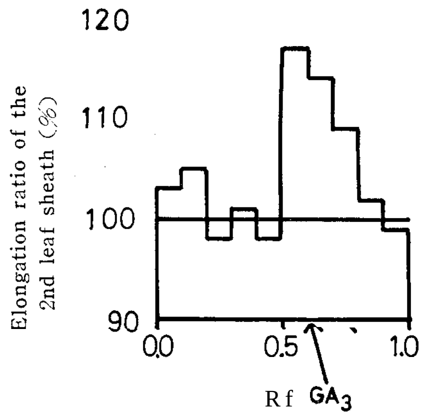


Fig. 2. Growth response of leaf sheath to chromatographic separation of gibberellin development with ammonical iso-propanol.

correspond with GA,. The former is supposed to differ from GA_3 and to be another gibberellin-like substance. In the short culm variety "Kinmaze", a growth inhibiting substance which inhibits elongation of the second leaf sheath was found at Rf 0.8–1.0, but this substance was not found in the relatively long culm variety "Norin No. 18" (Fig. 3).

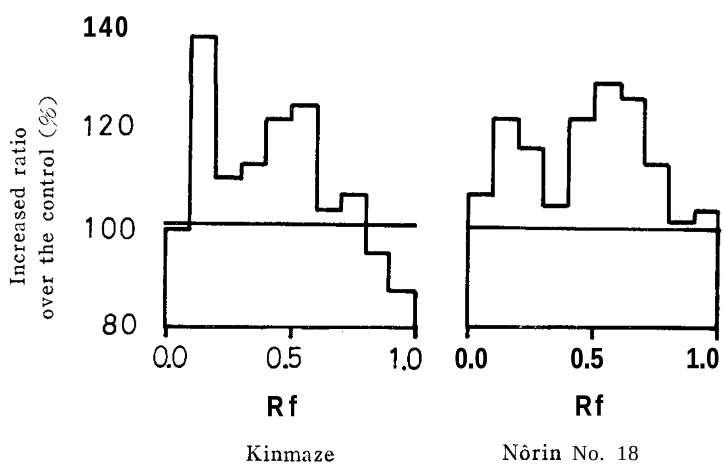


Fig. 3. Histograms showing the activities of gibberellin-like substances in the ethanol extracts obtained from seedlings of normal rice plants.

2) Dwarf mutants of rice plants ; As shown in Fig. 4, an active zone was found in the 0.4-0.7 region in all dwarf plants used in the present experiment and also the active substance seems to correspond to GA,. The level of the gibbercllinlilce substance is higher in "Daikoku" than in other dwarf rice plants. The inhibitor which was contained in a normal rice, "Kinmaze", is also found at Rf 0.8-1.0 in the dwarf rice plants. The occurrence of the growth inhibiting substance is remarkably high in "Daikoku", and furthermore, is found at 0.1-0.3. In the dwarf rice plants, the other gibberellin-like substance (Rf 0.1-0.3) which was present in normal ones is not evident.

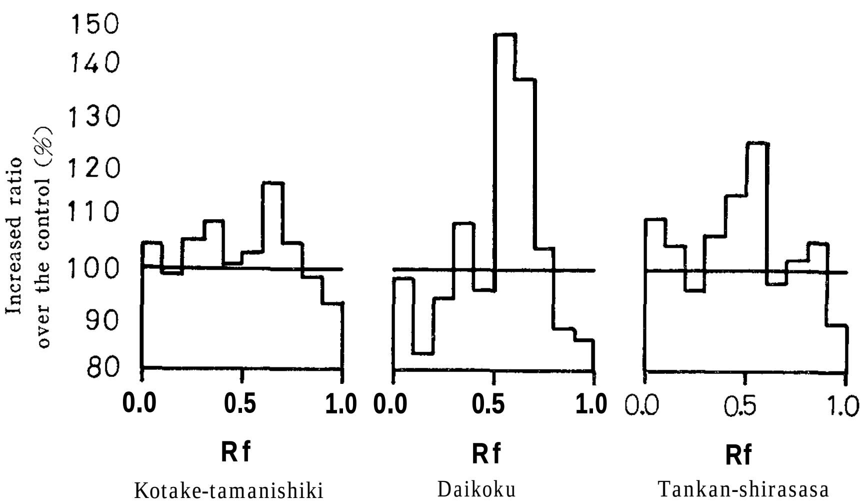


Fig. 4. Histograms showing the activities of gibberellin-like substances in the ethanol extracts obtained from seedlings of dwarf rice plants.

3. **Internode elongating stage**

1) Normal rice plants ; The same two active zones as determined in the extracts of seedlings were also found in the internode elongating stage of normal rice plants (Fig. 5). That is, they arc found at Rf 0.0-0.3 and 0.4-0.7 respectively. As mentioned previously, the latter seems to bc GA, and the former some other gibberellin-like substance. The former is greater than the latter in action. In Kinmaze, the growth inhibiting substance which was found in the ethanol extracts of the seedling stage was not present in the internode elongating stage (Fig. 5).

2) Dwarf rice plants ; Two active zones were also found at 0.0-0.3

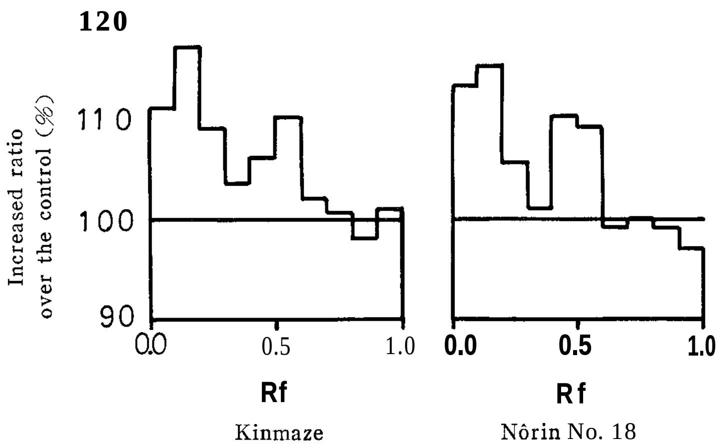


Fig. 5. Histograms showing the activities of gibberellin-like substances in the ethanol extracts obtained from internode elongating stage of normal rice plants.

and 0.4-0.7 in the extracts of the internode elongating stage of dwarf rice plants. The latter is another gibberellin-like substance which is not present in the extracts of the seedling stage (Fig. 6).

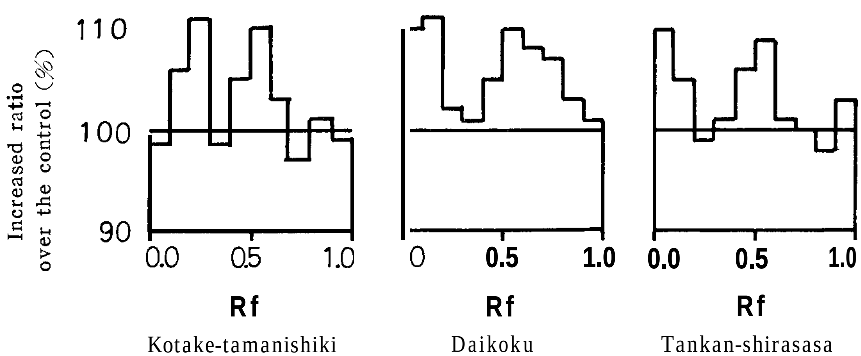


Fig. 6. Histograms showing the activities of gibberellin-like substances in the ethanol extracts obtained from internode elogating stage of dwarf rice plants.

4. Effects of GA, on the elongation of the internodes of the dwarf rice plants in darkness

In considering the effect of GA,, the seedling height of Nôrin No. 18 and Daikoku were increased by GA,. The treated plants surpassed the control plants by 44 %. Kinmaze and Miyazaki No. 3 were 35 %

higher. On the contrary, Tankan-shirasasa was hardly affected by GA, and seedling height in this variety was only 4% higher than the controls.

Regarding the elongation of internode two dwarf varieties Miyazaki No. 3 and Tankan-shirasasa grown in the dark did not differ so much from the normal varieties in the length of the first (lower position) and second internodes, but those of Daikoku were remarkably small. The elongation of the second internode of Nôrin No. 18 and Kinmaze by GA, was strikingly promoted, but Tankan-shirsasa and Miyazaki No. 3 were not affected (Fig. 7). The seedling height of the normal rice varieties Nôrin No. 18, "Kinmaze" and dwarf variety, Miyazaki No. 3, were greater than that of the other dwarf varieties. The dwarf variety, Daikoku is only 19 cm in height and is the shortest of the dwarf rice varieties. The dwarf variety "Tankan-shirasasa" were 23 cm (Table 1).

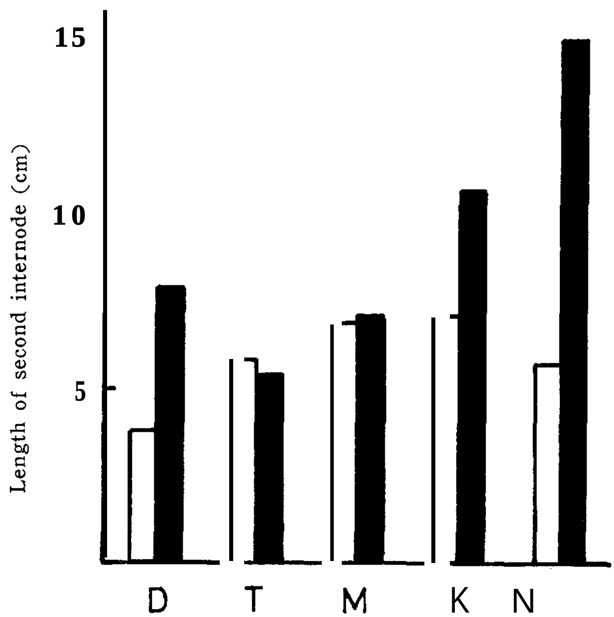


Fig. 7. Effects of GA_3 and darkness on elongation of 2nd internode in dwarf and normal rice plants.

■ Treated D Daikoku K Kinmaze
□ Control **T** Tankan-shirasasa N Nôrin No. 18
M Miyazaki No. 3

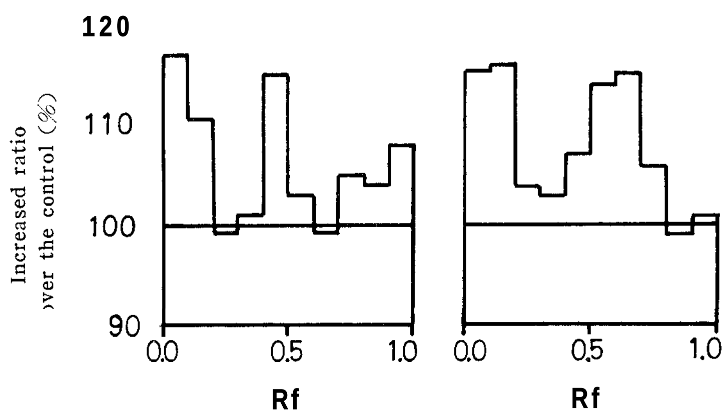
Table 1. Response of dwarf rice plants to the dark and gibberellin A_3.

Variety	Treatment	Seedling height(cm)	Length of internode (cm) First inter.	Second inter.	Third inter.
Daikoku	Cont.	18.7	0.4	3.7	4.4
	GA_3	26.9(144)	1.0(250)	7.6(206)	4.7(107)
Tankan-shirasasa	Cont.	23.2	1.2	5.4	3.1
	GA_3	25.1(108)	2.0(167)	4.8(89)	3.7(119)
Miyasaki No. 3	Cont.	30.7	1.2	6.4	4.1
	GA_3	41.6(135)	3.9(325)	6.8(106)	
Nôrin No. 18	Cont.	33.2	1.8	5.5	3.2
	GA_3	48.2(145)	5.8(322)	15.0(273)	11.7(366)
Kinmaze	Cont.	27.9	1.5	6.7	5.7
	GA_3	38.2(137)	5.4(360)	10.5(157)	7.6(133)

( ) indicates Treat./Cont. X 100.

5. The amounts of gihberellin-like substances in dwarf rice seedlings grown in the dark

As mentioned above, the lengthening of seedling height and internodes in the dwarf rice variety "Tankan-shirasasa" is induced in the dark (Table 1). A histogram showing the activity of the ethanol extracts in seedlings grown in the dark is presented in Fig. 8. As shown in Fig. 8, two activity zones are present in dwarf and normal



Tankan-shirasasa Kinmaze

Fig. 8. Histograms showing the activities of gibberellin-like substances in the ethanol extracts obtained from etiolated seedling of dwarf and normal rice plants grown in the dark.

rice plants. The results suggest that the activities of these growth promoting substances were hardly different from the ethanol extracts in dwarf and normal rice plants.

Discussion

The growth promoting substances which induce the growth of the second leaf sheath were found in the ethanol extracts of the seedlings of the dwarf and normal rice plants. Murakami (1959, 1961) and Ogawa (1963) reported that the action of the gibberellin-like substance was observed at Rf 0.4-0.7 on a paper chromatogram developed in a mixture of iso-propanol, 7-N amonia water and distilled water (8 :1:1). In the present experiment, the growth promoting substance existing in the range of Rf value 0.4-0.7 seems to be GA,. Suge et al. (1963) have reported the difference in contents of gibberellin-like substance among the dwarf mutants of rice plants. Comparing the amounts of this active substance in the seedlings of the dwarf and the normal rice plants, the activity of the dwarf variety, Daikoku, is greater than the normal rice variety, Kinmaze. However, the other dwarf rice varieties showed slightly smaller amounts as compared with normal ones.

Two active zones which cause the elongation of the second leaf sheath were observed on a paper chromatogram in the ethanol extracts of seedlings of normal rice varieties. Namely, they were found at Rf 0.0-0.3 and 0.4-0.7 respectively. The latter range, as mentioned previously, seems to correspond to GA,. Murakami (1966) reported that the extracts from rice seedling contained at least two gibberellin-like substances. The growth promoting substance in the range of Rf 0.0-0.3 was not found in the extracts of the seedlings of the dwarf rice varieties used in the present experiment.

On the other hand, the growth inhibiting substance which inhibits the elongation of the second leaf sheath is found at range of Rf 0.8-1.0 in both dwarf rice varieties and normal one, "Kinmaze". Further, in Daikoku, the inhibitor was also found at Rf 0.0-0.3. Ogawa (1963) reported that the second leaf sheath of rice plants responded specifically to gibberellin. From these facts, it is assumed that the growth inhibiting substance which inhibits the elongation of the tissue may have an antagonistic relation with the action of gibbcrellin. The small growth rate of the dwarf rice variety "Daikoku" may be due to a higher content of the growth inhibiting substance than in normal and other dwarf rice varieties.

In the histogram of the extracts in the internode elongating stage

(Figs. 5 and 6) two active zones were found at Rf 0.1-0.3 and 0.4-0.7 respectively. The difference in the amounts of the GA, like substance between normal and dwarf rice plants is not clear, but it is supposed that there are higher levels of other gibberellin-like substances (Rf value 0.1-0.3) in normal rice plants than in dwarf ones. There are no difference among the dwarf rice varieties in the amounts of both GA_3 and other gibberellin-like substances.

In our previous paper (1971), it was reported that there was a difference between the dwarf rice varieties in their response to GA,, and that the dwarf variety "Kotake-tamanisiki" could revert to a normal phenotype by continuous spray with its solution. Furthermore, from other experiments (1971) it has been confirmed that the dwarf variety "Koatake-tamanishiki" responded specifically to GA,. These facts give promise that the differences of growth rate between normal rice varieties and the dwarf variety "Kotake-tamanishiki" may be due to the presence in the former of a growth promoting substance similar to GA,. As mentioned above, however, the difference in the amounts of gibberellin-like substance among the dwarf variety "Kotake-tamanishiki", the normal and other dwarf rice varieties is not clear. Radley (1958) reported the amount of gibberellin-like substance was not different between normal and dwarf peas. As to the difference of growth rate between normal and dwarf peas, she stated that in the dwarf plants the substance was present in a form unable to exert any influence on cell growth or that there might be present inhibitors which prevent its action. The same idea may be applied to the dwarf rice variety "Kotake-tamanishiki" employed in the present experiment.

The relation between light and plant growth, with special reference to the growth of dwarf plants have been investigated in detail by Lockhart (1958, 1959) and Kende et ***al.*** (1963). Lockhart (1959) reported that growth of some dwarf plants in ***Phaseolus*** *vulgaris* were accele-erated by the illumination of light. Furthermore, Kende *et* ***al.*** (1963) showed that in dwarf peas no difference in the levels of gibberellin-like substance could be detected between the plants grown under light and those under darkness, and that illumination lowered the sensitivity of the tissue to gibberellin A_5 (fraction I) while producing no effect on the sensitivity to gibberellin A, (fraction II). The response to light of dwarf peas was 5 times greater than that of normal Alaska peas.

In our previous paper (1971), it was suggested that the dwarf variety "Tankan-shirasasa" grown under natural conditions had less elongation of lower internodes and that the dwarf gene of this variety might be closely related to the elongation of these tissues. When the dwarf rice variety was grown in the dark, the promotion of seedling height and lower internodes was remarkable and no difference could be detected between the dwarf rice variety "Tankan-shirasasa" and normal rice variety. There were no differences between the amounts of gibberellin-like substance (Rf 0.1-0.3) extracted from normal (Kinmaze) and dwarf rice (Tankan-shirasasa) grown in the dark, but the levels of the promoting substance extracted from those grown in light condition was higher in the normal type than in the dwarf one.

These results suggest that the dwarf gene of "Tankan-shirasasa" was closely related to the elongation of the lower internodes, that the elongation of these tissues might be promoted by a gibberellin-like substance (Rf value 0.0-0.3), and that this substance might be disturbed by light illumination.

Summary

The existence of gibberellin-like substances in ethanol extracts of the seedlings, the upper leaves at the internode elongating stage and the etiolated seedlings grown in darkness of dwarf and normal varieties of rice plants were examined by paper chromatography with ammonical iso-propanol. The effects of the darkness and GA, on the internode elongation of these plants were also investigated.

In the seedling stage, the gibberellin-like substance (Rf 0.4-0.7) was found in both dwarf and normal rice plants and this substance corresponded to GA,. However, its amounts differed from each other. Specifically, Daikoku contained more this growth promoting substance than Kotake-tamanishiki. In all the dwarf varieties used in the present experiment and in the normal variety "Kinmaze", an inhibiting substance was found in the region of Rf value 0.8-1.0. This substance was especially pronounced in "Daikoku". On the other hand, another growth promoting substance appeared in the region of Rf value 0.0-0.3.

Extracts of the upper leaves in the internode elongating stage contained two growth promoting substances in both dwarf and normal rice varieties (Rf 0.0-0.3 and 0.4-0.7). It was found that there was no difference between dwarf and normal rice plants in the amounts of GA_3, but the other gibberellin-like substance (Rf 0.0-0.3) was more abundant in normal than in dwarf rice plants.

In the dark condition, the dwarf type, " Tankan-shirasasa ", shows a great elongation of the internode that is nearly equal to the normal type, but in this dwarf type the elongation of the internode is not

promoted by the GA, treatment.

The amount of gibberellin-like substance (Rf 0.4-0.7) in the dwarf types grown in the dark was not different from that of the normal ones.

These results may suggest that the dwarf gene of the dwarf rice variety "Tankan-shirasasa" is closely connected with the elongation of the lower internode, and that the elongation of it was promoted by a gibberellin-like substance (Rf 0.0-0.3), although in this variety this substance was disturbed by light illumination.

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