Genetical and physiological studies on the dwarf mutants of the rice plants (Oryza sativa L.) : III. Effects of gibberellin A_3 on dwarf mutants of rice plants

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Genetical and physiological studies on the dwarf mutants of the rice plants (Oryza *sativa* L.)

III. Effects of gibberellin A_3 on dwarf mutants of rice plants

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There are many dwarf strains among the rice varieties kept in the laboratory of Kyushu University. In the previous paper (Nagamatsu *et al.* 1971), the authors grouped them genetically into 8 types and reported that these dwarf varieties were controlled by a single recessive gene and showed various phenotypes.

Van Overbeek (1934), Nakayama (1954), Galston (1959) and other workers have reported the relations of plant hormone in dwarf types in many species of plants. Recent investigations have shown that gibberellin derived from the fungus ***Gibberella fujikuroi*** Wr. increased the length of the leaves and internodes of normal plants in many species. Brian *et al.* (1954, 1955), Phinney (1956) and other investigators reported that sensitivity to the substance is higher in dwarf plants than in normal ones.

In the present paper, the responses of the dwarf mutants of rice plants to gibberellin A_3 (GA,) were dealt with.

Materials and Methods

The present experiment was performed with six dwarf varieties ; Daikoku, Chakei-daikoku, Waisei-shirasasa, Tankan-shirasasa, Kotake-tamanishiki and Bunketsu-to, which were controlled by a single recessive gene. Nôrin No. 18, a cultivar with relatively long culm, was used as the control plant.

Experiment 1. Effect of GA, on the elongation of the coleoptile

Seeds of each variety were soaked in petri dishes containing 100 ppm of GA, solution. Distilled water was used as the control. They were kept in an incubator maintained at ***25°C*** for ***42*** hours. After washing with distilled water, twenty seeds were placed on the petri dish (diameter 9 cm) containing 0.6 % agar medium which were autoclaved at 1.0 kg/cm' for 20 min., and they were kept in a dark incubator at a constant temperature of 33°C. When the coleoptile of the seedlings reached maximum length, fourteen plants were taken out to measure.

Experiment 2. Effect of GA, on the elongation of seedling height

The effect of GA, to the seedling growth was tested in the Kollowing two ways.

(i) To obtain uniform growth, seeds of medium size were selected. The seeds were soaked in the same way as in experiment 1. The concentrations of GA, employed were 1, 10 and 100 ppm. Sixteen seeds of each variety were sown on a small seedling- bed **(60 x 30.5 x** 8.5cm) and grown in a phytotron in a glass room of constant temperature (33°C).

(ii) Concentrations of GA, were **250, 500, 1000** and **2000** ppm. Nutrients, N, P and K, were given 15 days after sowing. Five days after sowing, ten plants, excluding border ones, were chosen to measure seedling height 6 times every 5 days.

Experiment 3. Continuous spray treatments with GA, solution

The seeds were sown on June 30 in a small seedling bed and also on August 1 three uniform seedlings of each variety were transplanted in one pot (22 x **24** cm) filled with sandy loam soil. They got wet thoroughly by spraying with 100 ppm solution of GA, containing spreading agent (Kino) 4 times every week. They were first sprayed on August 31. Control plants were sprayed with distilled water at the same time. The plant was measured every week after treatment and at harvest the morphological characteristics such as leaf length, culm length and internode length were measured. The three essential elements of plant nutrition, N, P and K were mixed beforehand with the soil.

Experiment 4. Effect of GA, on the double recessive plants

Thirty-five days after sowing, the seedlings were transplanted to water culture and grown under natural conditions. Kimura's solution was used in the present experiment and the solution was exchanged every four days. The application of GA, was the same as in experiment 3. At harvest, the morphological characteristics mentioned above were examined.

Results

Experiment 1. Effect of GA_3 on the elongation of the coleoptile

The response of the coleoptile in the dwarf rice plants to GA, is shown in Table 1. The elongation of the coleoptite was significantly increased in the plants treated as compared with the control ones. The degree of response, however, differed among different dwarf types. The dwarf variety "Kotake-tamanishiki" was most affected by GA_3, showing about a 90 % increase in the length of the coleoptile over the control plants. On the contrary, the dwarf variety "Tankan-shirasasa" hardly responded (3%). The other dwarf mutants and normal rice plant, Nôrin No. 18, were relativey weak in their response (13 to 30 %).

Table 1. Effects of GA_3 on the elongation of coleoptile in dwarf rice plants (mm).

	Daikoku	Chôkei-daikoku	Waisei-shirasasa	Tankan-shirasasa
Control	16.6± 1.4	18.8±1.7	22.5±1.7	23.3±1.3
GA_3 (100 ppm)	19.5± 1.5	21.2± 1.5	26.1±2.1	23.9±1.1
GA_3/Control X 100	(118)	(113)	(116)	(103)
	Kotake-tamanishiki	Bunketsu-to	Nôrin No. 18	
Control	16.1±1.3	22.9±1.7	20.2±2.1	
GA_3 (100 ppm)	30.4±1.5	29.7±1.7	22.9± 1.6	
GA_3/Control X 100	(188)	(130)	(113)	

Experiment 2. Effect of GA, on the elongation of seedling height

Seedling height of the plants treated with low concentrations of GA, were measured on the 5th and the 10th days after sowing respectively. The results are shown in Tables 2 and 3. From these tables, it was found that the response of dwarf rice plants to GA, was striking in the first 5 days and then it declined during the following 5 days. On the 5th day after sowing, Kotake-tamanishiki, Daikoku and Bunketsu-to treated with all concentrations of GA, showed a significant increase in seedling height over the control. The order of the sensitivity of the plants to 100 ppm of GA, on the 5th day after sowing was Kotake-tamanishiki> Daikoku> Bunketsu-to> Waisei-shirasasa>Norin No. 18>Chokeidaikoku>Tankan-shirasasa.

The response of the dwarf mutants to GA, at higher concentrations

Table 2. Effects of low concentration of GA_3 on seedling height of dwarf rice plants (cm). (5 days after sowing)

	Daikoku	Chôkei-daikoku	Waisei-shirasasa	Tankan-shirasasa
100 ppm	4.1 (158)***	3.7 (116)	4.5 (147)**	4.8 (123)**
50 ppm	3.6 (138)***	3.3 (103)	4.0 (128)**	4.6 (119)**
10 ppm	3.4 (131)**	3.6 (113)*	4.9 (134)”	4.9 (126)“”
1 ppm	3.2 (123)**	3.0 (94)	3.5 (109)	4.3 (110)
Control	2.6 (100)	3.2 (100)	3.2 (100)	3.9 (100)

	Kotake-tamanishiki	Bunketsu-to	Nôrin No. 18
100 ppm	5.8 (207)***	5.9 (148)***	6.0 (143)**
50 ppm	4.9 (161)***	5.2 (130)**	5.9 (145)”
10 ppm	4 4 (157)***	5.1 (128)**	5.1 (121)*
1 ppm	3.9 (139)**	4.7 (118)”	4.2 (100)
Control	2.8 (100)	4.0 (100)	4.2 (100)

*** Significant at 0.1 % level.
** Significant at 1 % level.
* Significant at 5 % level.

Table 3. Effects of low concentrations of GA_3 on seedling height of dwarf rice plants (cm). (10 days after sowing)

	Daikoku	Chôkei-daikoku	Waisei-shirasasa	Tankan-shirasasa
100 ppm	10.1 (135)*	8.4 (98)	11.1 (121)	14.9 (116)
50 ppm	9.2 (123)	7.6 (88)	10.2 (111)	13.0 (101)
10 ppm	9.0 (120)	8.8 (102)	10.0 (109)	13.7 (106)
1 ppm	8.4 (112)	7.7 (90)	8.7 (95)	12.2 (95)
Control	7.5 (100)	8.6 (100)	9.2 (100)	12.9 (100)

	Kotake-tamanishiki	Bunketsu-to	Nôrin No. 18
100 ppm	12.3 (184)***	11.5 (111)	11.2 (120)**
50 ppm	11.2 (167)***	12.3 (119)	11.9 (128)**
10 ppm	8.8 (131)**	11.7 (114)	11.8 (127)*
1 ppm	6.7 (100)	10.3 (100)	9.7 (104)
Control	6.7 (100)	10.3 (100)	9.3 (100)

*** Significant at 0.1 % level.
** Significant at 1 % level.
* Significant at 5 % level.

(2000 ppm) is shown in Fig. 1, from which it is found that the plant height showed acute elongation by GA, till 10 days after treatment and then tapered off. The plant height of Kotake-tamanishiki became higher than normal variety, Norin No. 18, under this treatment.

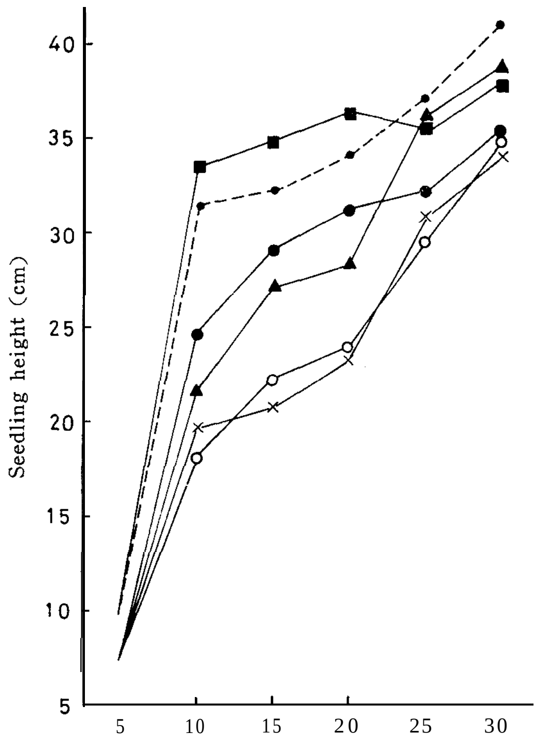

Fig. 1. Different response of the dwarf varieties in seedling height in GA_3 soaked seed (2000 ppm).

······ Nôrin No. 18 × Waisei-shirasasa
○ Daikok ▲ Tankan-shirasasa
● Chôkei-daikoku ■ Kotake-tamanishiki

The effects of GA, on seedling height on the 10th day after sowing increased with its concentration (Table 4). At 500 ppm Norin No. 18 showed the greatest elongation ratio (54 %), and Daikoku the least (5 %). The plants treated with 2000 and 1000 ppm solution showed highly significant over growth in comparison with the control ones for all the dwarf mutants and normal variety. Especially in the 2000 ppm plot, the seedling height of Kotake-tamanishiki surpassed that of

Table 4. Effects of high concentrations of GA, on seedling height of dwarf rice plants (cm). (10 days after sowing)

	Daikoku	Chôkei-daikoku	Waisei-shirasasa	Tankan-shirasasa
2000 ppm	18.1±1.3 (152)***	24.8±1.4 (216)***	19.7±2.1 (195)***	21.8±3.7 (176)***
1000 ppm	14.1±1.3 (119)**	21.1±3.5 (184)***	16.1±1.5 (159)***	16.9±1.3 (136)***
500 ppm	12.5±1.9 (105)	16.0±2.0 (139)***	14.3±1.7 (142)***	17.1±1.7 (138)***
250 ppm	12.0±1.9 (101)	14.8±1.8 (129)***	12.4±1.6 (123)***	15.6±1.6 (126)**
Control	11.9±1.2 (100)	11.5±0.8 (100)	10.1±1.4 (100)	12.4±2.9 (100)

	Kotake-tamanishiki	Bunketsuto	Nôrin No. 18
2000 ppm	33.7±2.6 (283)***	25.2±3.1 (169)***	31.8±4.0 (200)***
1000 ppm	21.6±2.4 (182)***	22.0±3.1 (148)***	25.3±2.0 (159)***
500 ppm	16.9±2.9 (142)***	18.9±1.2 (127)***	24.5±1.4 (154)***
250 ppm	16.0±2.3 (135)***	17.1±2.1 (115)***	20.2±1.1 (127)***
Control	11.9±0.7 (100)	14.9±1.8 (100)	15.9±0.6 (100)

*** Significant at 0.1 % level.
** Significant at 1 % level.
* Significant at 5 % level.

the normal variety, Nôrin No. 18, and its ratio to the control reached 283 %. Chôkei-daikoku showed 216 % in ratio to the control and the other dwarfs ranged from 150 to 159 %.

The response of the dwarf mutants to different concentrations of GA, is summarized as follows:

Kotake-tamanishiki: This variety shows the greatest response among the treated dwarf varieties and responds to both low and high concentration.

Chôkei-daikoku: Response is greater in the higher concentration but smaller in the lower one.

Waisei-shirasasa, Daikoku, Bunketsu-to and Nôrin No. 18: In both concentrations, these varieties respond relatively highly.

Tankan-shirasasa: This variety shows the least response among the dwarf mutants.

As mentioned above, Kotake-tamanishiki was most sensitive to GA_3. Table 5 shows the comparison of the response of "Kotake-tamanishiki" and its dwarf type of F_2 derivative DF, 38 and DF_2 26 which were selected from different F_2 populations and differ in their genetic background from each other and from the originated "Kotake-tamanishiki". These lines show a nearly similar response to that of Kotake-tamanishiki to GA,.

Table 5. Effects of GA, on the elongation of leaf sheath of "Kotake-tamanishiki" type of dwarf (9 days after sowing, 30°C).

		Kotake-tamanishiki	(DF_2 38)	(DF_2 26)
Length of 2nd leaf sheath (cm)	Control	3.2±0.3	3.4±0.3	3.2±0.4
	GA_3	5.3tO.5 (179)	5.5f0.9 (163)	4.8±0.5 (150)

( ) indicates GA/Control X 100.

Experiment 3. Effect of continuous spray treatments with GA, solution to plant growth

Fig. 2 shows the ratio of the plant height of each variety treated with GA, to the plant height of their control, measured on August 31 (61 days after sowing). It was found that the elongation of the culm length of the dwarf variety "Kotake-tamanishiki" was remarkably accelerated by the spray treatments and showed a rapid growth till September 28th. "Tankan-shirasasa" showed the lowest sensitivity and the other dwarf varieties showed an intermediate response.

The morphological characteristics measured after harvest are presented in Table 6. As shown in Table 6, the ratio of the culm length of the sprayed plant to its control was more 300 % in Kotake-tamanishiki and **180** % in Nôrin No. 18. The final culm length of the treated dwarf mutant "Kotake-tamanishiki" reached 119 cm, being 22 cm longer than the treated normal Nôrin No. 18. The ratio of culm length between plants with and without GA, for Daikoku and Chôkei-daikoku was about 180 % and for Waisei-shirasasa 150 %. In the case of Tankan-shirasasa, it was hardly affected by GA, and the culm length of the treated plant was only 20 % longer than its control. The ear length of the plants treated was longer than the untreated ones except for one variety "Chôkei-daikoku". Statistical significance was found in the case of Daikoku and Nôrin No. 18. The number of ears decreased in all varieties treated and in particular Kotake-tamanishiki decreased remarkably. The date of heading in plants treated was hastened than that of untreated ones, especially in Kotake-tamanishiki and Nôrin No. 18.

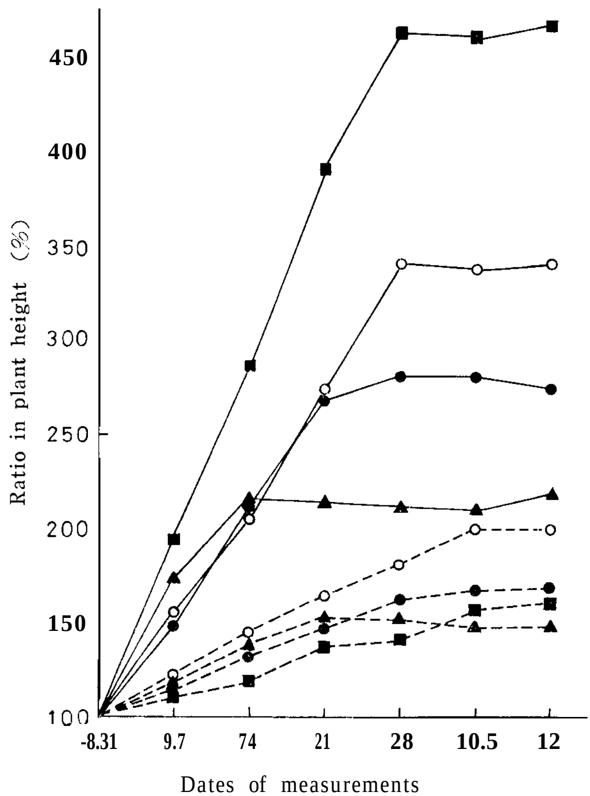

Fig. 2. Different response of the dwarf varieties by repeated spraying of GA_3 solution during growth.

■ Kotake-tamanishiki — Treated
○ Nôrin No. 18 ...... Control
● Daikoku
▲ Tankan-shirasasa

Experiment 4. The response of the double recessive plants to GA,

As above mentioned, there were differences of response to GA, among the dwarf rice plants. Table 7 shows the response of the plants possessing various dwarf genes double. From this Table, it is clearly found that the response was greater in the line possessing the dwarf gene of Kotake-tamanishiki than in those of other genes. The ratio of culm length between plants treated and untreated was 230 % in the former and 153 % in the latter.

Table 6. Effects of GA_3 to dwarf rice plants.

		Culm length (cm)	Ear length (cm)	No. of ear
Daikoku	Cont.	28.2± 4.2	8.9±1.7	3.3
	Treat.	49.7±10.2 (176)	11.6±1.7 (130)	2.0 (61)
Chôkei-daikoku	Cont.	43.7± 3.4	10.4±0.5	5.5
	Treat.	77.8t14.9 (178)	10.1±1.5 (97)	3.3 (60)
Waisei-shirasasa	Cont.	40.6± 3.4	10.1±1.4	5.5
	Treat.	60.8± 7.9 (150)	11.6±2.0 (115)	3.5 (64)
Tankan-shirasasa	Cont.	26.9± 0.6	9.71-o. 5	8.6
	Treat.	31.9± 1.5 (120)	11.0±1.2 (113)	5.0 (58)
Kotake-tamanishiki	Cont.	37.0± 4.4	14.3i0.8	5.5
	Treat.	119.1± 6.4 (322)	16.6±1.4 (116)	2.1 (38)
Nôrin No. 18	Cont.	53.6± 3.3	16.0±0.9	4.2
	Treat.	96.8± 6.1 (181)	19.4-i-1.7 (121)	3.1 (74)

	No. of days to heading	Dry weight of top (g)	Dry weight of root (g)
Daikoku	72.5t4.8	3.71	0.63
	68.3±2.4 (94)	4.38 (118)	0.83 (132)
Chokei-daikoku	76.5±1.1	6.95	1.09
	75.6±1.0 (99)	6.47 (93)	0.86 (79)
Waisei-shirasasa	72.711.2	6.68	0.87
	71.3±2.8 (98)	4.69 (72)	0.62 (71)
Tankan-shirasasa	71.7f1.2	9.14	1.05
	71.2f1.4 (99)	7.40 (81)	0.48 (46)
Kotake-tamanishiki	71.2t1.3	5.73	0.56
	67.3tl.0 (95)	5.42 (95)	0.45 (80)
Norin No. 18	74.5±1.0	5.40	0.67
	71.2t2.0 (96)	8.10 (150)	0.67 (100)

( ) indicates Treat./Cont. × 100.

Table 7. Effects of GA_3 to double recessive plants. (GA_3 100 ppm)

	Culm length (cm)	Ear length (cm)	No. of ear	No. of grain per panicle
DF_2 27 Control	27.9	10.1	9.2	46.8
Treatment	64.1 (230)	9.7 (96)	9.8 (107)	-*
DF_2 26 Control	26.2	9.6	14.5	38.5
Treatment	40.2 (153)	10.4 (108)	8.2 (57)	77.2 (201)

( ) indicates Treat./Cont. X 100.

* Not observed.

DF_2 27 ; Double recessive with Waisei-shirasasa and Kotake-tamanishiki genes.

DF_2 26 ; Double recessive with Tankan-shirasasa and Waisei-shirasasa genes.

Discussion

In the length of coleoptile, the differences between plants with and without GA, were statistically significant in all the varieties tested. Furthermore, there were the differences of response among the dwarf strains. The elongation of the coleoptile of the dwarf variety "Kotake-tamanishiki" was strikingly promoted by soaking the seed in GA_3 solution. It became twice as long as the control ones. On the contrary, the other dwarf variety "Tankan-shirasasa" hardly responded to GA,. In the other dwarf varieties situated between these two extremes, the ratio of elongation ranged from 10 to 30 %. The results obtained above were also confirmed by a comparison between the seedling height from seeds immersed in GA, solution and the culm length of adult plants continuously sprayed with its solution.

As to the effect of gibberellin on higher plants, a remarkable property of this substance is its ability to greatly stimulate the growth of some genetic dwarf plants. Brian *et al. (1954,* 1955) reported that the growth of the dwarf varieties of some species, such as French bean (*Phaseolus vulgaris* L.) was greatly accelerated by gibberellin. Phinney (1956) showed that dwarf maizes (*Zea mays* L.) regulated by a single recessive gene could be phenotypically restored to normal by treating with gibberellin. Cooper (1957) also showed that gibberellic acid brought the genetic dwarf types of *Lolium perenne* to the normal phenotype in leaf and inflorescence development.

In the present experiment, the rice variety "Kotake-tamanishiki" was phenotypically brought to the normal type by treatment with GA_3, but on the other hand "Tankan-shirasasa" was not affected. In the experiment using the dwarf plants in F_2 between "Kotake-tamanishiki" and normal type of rice varieties, it was shown that these dwarf plants responded to GA, like "Kotake-tamanishiki" in spite of the differences in their genetic background.

From the results obtained, it is suggested that the action of the dwarf gene in "Kotake-tamanishiki" is closely related to the metabolism of gibberellin-like substance.

Brian (1958) stated that plants might contain a hormone similar to gibberellic acid in its effects, and that some rearrangement in the metabolism of hormone was responsible for dwarfism.

Summary

The effect of gibberellin A, (GA,) on different dwarf rice mutants was investigated by the measurements of the length of the coleoptile

and the seedling height when seeds were soaked in the solution, and of the culm length when plants were sprayed with it. In Kotake-tamanishiki, the coleoptile, the seedling height and the culm length were remarkably elongated by GA, solution. On the contrary, Tankan-shirasasa is less responsive to this chemical solution and showed little increase of growth. The other dwarf types and normal type showed the intermediate response between Kotake-tamanishiki and Tankan-shirasasa.

It was also confirmed that the Kotake-tamanishiki type selected from the F_2 population showed similar response to GA,. These results may suggest that the action of the dwarf gene in Kotake-tamanishiki is closely related to the metabolism of gibberellin-like substance.

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