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Identification and characterization of gibberellin-insensitive mutants selected from among dwarf mutants of rice

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Abstract. In rice, many dwarf mutants have been isolated and characterized. We have investigated the relationship between dwarfism and the gibberellin (GA)-mediated control of physiological processes. Twenty-three rice cultivars and mutants (9 normal, 3 semi-dwarf, 11 dwarf) were analyzed in terms of two GA-mediated processes, namely, elongation of shoots and production of α-amylase activity in the endosperm. As a result, we identified four different groups (groups N, T, D and E). Two-dimensional plotting of the extent of induction of α-amylase in the endosperm *versus* the extent of enhancement of shoot elongation upon treatment with exogenous gibberellic acid (GA_3) provided a useful method for the rapid allocation of large numbers of dwarf mutants of rice to the various groups.

Members of group N (normal type), which included all normal cultivars and semi-dwarf mutants, showed a slight increase in elongation of shoots and a remarkable increase in production of α-amylase with the application of GA_3 during germination. All of the dwarf mutants were classified as being members of the other three groups. Members of group T (Tan-ginbozu type), including three dwarf mutants, were highly responsive to exogenous GA_3 in terms of elongation of shoots and production of α-amylase, with associated lower levels of endogenous GA. In contrast, members of the other three groups, including group N, had normal levels of endogenous GAs. Members of group D (Daikoku type) were only slightly responsive to exogenous GA_3, an indication that they are GA-insensitive mutants. Members of group E (Ebisu type) had responses to GA_3 similar to those of group N, not only in terms of elongation of shoots but also in terms of α-amylase production, an indication that they are dwarf mutants that can be considered as neither GA-deficient nor GA-insensitive mutants.

We also examined a GA-insensitive mutant selected from among 19 near-isogenic dwarf lines of 'Shiokari', and we concluded that the *d-1* gene is associated with the phenotype of GA-insensitive dwarf mutants.

Key words: Rice – Dwarf mutant – α-Amylase – Gibberellin – *Oryza sativa*

Introduction

Gibberellins (GAs) play an important role in the regulation of many physiological processes in plant growth and development. The most extensive studies of these processes have involved examinations of shoot elongation (MacMillan and Phinney 1987) and the induction of hydrolytic enzymes in cereal aleurone layers (Jacobsen and Chandler 1987). The GA-elicited biochemical changes that are responsible for cellular elongation remain to be identified. In contrast, the molecular biology and biochemistry of the response of the cereal aleurone layer to GA is understood in some detail (Jacobsen and Chandler 1987). Among the best-characterized of the induced hydrolases are the α-amylases found in cereal seeds, such as in rice (Akazawa et al. 1990; O'Neill et al. 1990; Perata et al. 1992), barley and wheat (Ho 1985; Jacobsen and Chandler 1987). The genes for α-amylase are activated by GA at the transcriptional level (Jacobsen and Close 1991), and the mechanism of activation probably involves interaction between *cis*-acting promoter elements of the gene for

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α-amylase (Ou-Lee et al. 1988; Lanahan et al. 1992; Gubler and Jacobsen 1992; Rogers and Rogers 1992) and transacting protein transcription factors.

Dwarf mutants may be useful in clarifying the mode(s) of action of GA. In a number of plant species, such as rice (Suge and Murakami 1968; Murakami 1970), maize (Fujioka et al. 1988) and *Arabidopsis* (Koornneef and van der Veen 1980), mutants have been described in which the absence or altered composition of endogenous gibberellins results in dwarf plants, which are generally designated "GA-deficient dwarfs". In these mutants, the normal phenotype can be restored by the application of exogenous GAs. In some genotypes of wheat (Gale and Marshall 1975), maize (Fujioka et al. 1988) and *Arabidopsis* (Koornneef et al. 1985) dwarfism is associated with a decreased sensitivity to applied GA as compared to that of the tall wild type. Such mutants are designated "GA-insensitive dwarfs" and may prove useful in the analysis of GA-mediated processes.

For examination of responses to GA, dwarf mutants of cereal plants, which do not respond to GA, appear to provide a suitable experimental system. It is of interest to determine whether GA-insensitive mutants that do not respond to GA carry genetic loci that are capable of regulating all of the diverse effects of GAs. Numerous dwarf varieties of cereal plants exist, but most of these are of the GA-deficient or GA-independent type. A GA-insensitive mutant of wheat, D6899, having a mutation at a single locus, the *Rht3* gene, has been well characterized (Gale and Marshall 1975; Ho et al. 1981), as have mutants in maize (Fujioka 1988) and *Arabidopsis* (Koornneef et al. 1985). In rice, dwarf mutants have been studied in some detail (Kamijima 1974; Murai et al. 1982; Kitano et al. 1983), and the Daikoku dwarf mutant appears likely to be a GA-insensitive type of mutant (Murakami 1970). As a first step towards clarification of the mechanisms of responses to GA using GA-insensitive mutants of rice, we need to select as many such mutants as possible.

In this report, we describe a rapid method by which it is possible to classify dwarf mutants of rice as dwarf and GA-deficient, as dwarf and GA-insensitive and as dwarf without being either GA-deficient or GA-insensitive. We also describe the identification of a GA-insensitive mutant selected from among 19 near-isogenic dwarf lines of 'Shiokari'.

Materials and methods

Plant materials

Seeds of all rice (*Oryza sativa*) cultivars and mutants were provided by the University Farm in 1989, 1990 and 1991. All seeds of near-isogenic dwarf lines and the recurrent parent, 'Shiokari', were gifts from Drs. T. Kinoshita and H. Kitano.

Measurement of elongation of shoots

Elongation of shoots was quantified by a modified version of the "microdrop method" described by Murakami (1968). Twelve rice seeds were surface sterilized for 30 min with a 3% solution of NaClO that contained 0.1% Tween 20. They were then placed in wells of a 12-well plate (no. 3046; Falcon, N.J. USA), washed with sterile, distilled water and incubated for 2 days in 4 ml of distilled water at 30 °C. Ten of the germinated seeds were placed on a 1% agar plate (5 for application of gibberellic acid (GA_3) and 5 as controls) and grown at 30 °C under fluorescent light from until emergence of the second leaf sheath. After about 2 days, 1 µl of a solution of GA_3 (10 mg/ml) in ethanol was applied to the coleoptiles of rice seedlings at the first-leaf stage. After 3 days, the lengths of the second-leaf sheaths were measured. The results are shown as averages from 5 tested plants with the standard deviation.

Assay of α-amylase activity

Six embryo-less half-seeds were dehulled and surface sterilized for 15 min with a 3% solution of NaClO that contained 0.1% Tween 20 and then washed with sterile, distilled water. The seeds were placed in the wells of a 24-well plate (no. 3047; Falcon N.J.) and then incubated with 0.5 or 1 ml of culture medium (10 m*M* sodium acetate, pH 5.3, containing 2 m*M* $CaCl_2$) and allowed to germinate at 30 °C in darkness. The culture medium was supplemented with 1 µ*M* GA_3 (Table 1) or with GA_3 at the concentrations indicated in Fig. 3.

To measure the response of intact seeds to GA, as shown in Fig. 2, the seeds were dehulled and surface sterilized under the same conditions as described above. Six seeds were incubated in separate wells with 1 ml of the culture medium that had been supplemented with 1 µ*M* GA_3. After a 4-day incubation, the seeds were homogenized with the culture medium plus an additional 0.5 ml of homogenizing buffer (100 m*M* Tris-HCl, pH 7.6, containing 10 m*M* $CaCl_2$ and 0.1% Triton X-100), and the homogenate was then centrifuged at 18,500 *g* for 10 min. The supernatant was used for measurements of enzymatic activity.

α-Amylase activity was determined by the RBB-starch method of Hall et al. (1969) and Mitsunaga and Yamaguchi (1993). The sample (50 µl) and 450 µl of a suspension of substrate [2 *g* of RBB-starch (amylopectin azure; Calbiochem, Los Angeles, Calif., USA) in 50 m*M* sodium acetate buffer that contained 10 m*M* $CaCl_2$] were incubated for 10 min at 25 °C, and the reaction was terminated by the addition of 200 µl 18% acetic acid. After centrifugation at 18,500 *g* for 5 min, the absorbance of the supernatant was measured at 595 nm. Units of activity were defined by the following equation: units = As/ACu, where As and ACu represent the absorbance at 595 nm of the reaction mixture and of a 1 *M* solution of $CuSO_4$, respectively.

Results

Classification of dwarf mutants of rice

To characterize dwarf mutants of rice, we examined 23 different cultivars and mutants (Table 1), all of which were of the *Japonica* type and included 9 normal cultivars, 3 semi-dwarf mutants and 11 dwarf mutants.

We first examined the effects of GA_3 on the elongation of shoots and the induction of α-amylase activity in the endosperm, both of which are GA-promoted physiological processes. Elongation of shoots was quantified by a modified version of the "microdrop

Table 1. Effects of GA_3 on elongation of the shoots and production of α-amylase in various normal cultivars and dwarf mutants of rice

Number[a]	Cultivar and mutant	Type[b]	Elongation of shoot[c] (cm)		(ratio A) +GA/−GA	α-Amylase activity[d]	
			−GA	+GA		(Units/half-seed)(% B)[e] +GA	+GA
N1	Nipponbare	N	2.23 ± 0.30	3.98 ± 0.10	1.78	57.2 ± 0.1	(118.9 ± 0.2)
N2	Fujiminori	N	2.61 ± 0.16	4.32 ± 0.83	1.66	52.0 ± 0.3	(108.2 ± 0.6)
N3	Reimei dwarf	SD	2.70 ± 0.10	4.71 ± 0.29	1.74	48.1 ± 0.5	(100.0 ± 1.1)
N4	Jukoku dwarf	SD	2.46 ± 0.30	4.10 ± 0.31	1.67	48.1 ± 0.3	(100.0 ± 0.6)
N5	Tamanishiki	N	3.04 ± 0.05	5.52 ± 0.06	1.82	41.8 ± 2.3	(86.9 ± 4.8)
N6	Calrose 8989	N	3.60 ± 0.01	6.42 ± 0.01	1.78	55.0 ± 1.0	(114.5 ± 2.0)
N7	Calrose 76	SD	2.77 ± 0.12	5.62 ± 0.51	2.03	50.4 ± 1.4	(104.9 ± 2.9)
N8	Akage	N	3.29 ± 0.06	6.71 ± 0.11	2.04	55.4 ± 0.4	(115.3 ± 0.8)
N9	Norin 14	N	2.77 ± 0.21	4.62 ± 0.07	1.67	38.6 ± 1.2	(80.3 ± 2.5)
N10	Kinmaze	N	2.06 ± 0.12	3.74 ± 0.14	1.82	39.1 ± 1.0	(81.4 ± 2.2)
N11	Ginbozu	N	2.61 ± 0.17	4.28 ± 0.26	1.64	45.0 ± 1.3	(93.7 ± 2.8)
N12	M202	N	3.60 ± 0.28	5.35 ± 0.42	1.49	52.7 ± 0.6	(109.6 ± 1.2)
T1	Tan-ginbozu dwarf	D	1.90 ± 0.10	4.32 ± 0.36	2.27	53.3 ± 0.9	(110.9 ± 1.2)
T2	Waito C dwarf	D	1.68 ± 0.05	4.34 ± 0.44	2.58	39.6 ± 0.9	(82.4 ± 1.8)
T3	Kotake-tamanishiki dwarf	D	1.64 ± 0.50	3.64 ± 1.36	2.22	42.2 ± 0.7	(87.8 ± 1.5)
D1	Daikoku 1 dwarf	D	1.44 ± 0.05	2.26 ± 0.08	1.57	8.3 ± 0.4	(17.2 ± 0.8)
D2	Daikoku 4 dwarf	D	1.91 ± 0.18	3.04 ± 0.09	1.59	13.8 ± 0.9	(28.7 ± 1.8)
D3	Murasaki-daikoku dwarf	D	1.59 ± 0.13	2.85 ± 0.05	1.79	5.6 ± 2.3	(11.7 ± 4.8)
D4	Kanto dwarf	D	2.03 ± 0.09	2.88 ± 0.26	1.42	12.1 ± 2.4	(25.3 ± 5.1)
D5	Bonsai-daikoku dwarf	D	2.00 ± 0.09	2.37 ± 0.05	1.19	7.1 ± 0.4	(14.8 ± 0.8)
E1	Ebisu dwarf	D	2.16 ± 0.02	3.35 ± 0.28	1.55	52.8 ± 1.5	(109.8 ± 3.1)
E2	Norin 28 dwarf	D	2.15 ± 0.05	2.91 ± 0.07	1.35	46.8 ± 0.9	(97.2 ± 1.9)
E3	Fukei 71 dwarf	D	3.06 ± 0.06	4.70 ± 0.20	1.54	40.3 ± 2.4	(83.8 ± 5.0)

[a] Serial numbers were designated after the classification described in the text
[b] N, Normal cultivar; SD, semi-dwarf mutant; D, dwarf mutant
[c] For measurement of elongation of shoots, see text
[d] For assay of α-amylase activity in embryo-less half-seeds, see text
[e] Normalized α-amylase activity with the activity of Reimei dwarf (N3; 48.1 units/half-seed) taken as 100%

method" (Murakami 1968), and we calculated the ratio of the lengths of the second-leaf sheat that developed with and without prior application of 1 μg of GA_3. We also examined the effect of the application of 1 μ*M* GA_3 on the activity of the α-amylase from 4-day-incubated embryo-less half-seeds. The induction of α-amylase upon application of GA_3 in embryo-less half-seeds is a well-established system for assaying the responsiveness to GA of cells in the aleurone layers of the endosperm (Mitsunaga and Yamaguchi 1993). The two different indices, which represent the increase in length of the second-leaf sheath (ratio A in Table 1) and induction of α-amylase activity (% B in Table 1) caused by the application of GA_3 were plotted against each other, and as can be seen in Fig. 1, three distinct groups were formed, as indicated by the small circles. Most normal and semi-dwarf mutants (N1-6 and N9-12 in Table 1) fell within the circle of group N, the exceptions being 'Calrose 76' (N7) and 'Akage' (N8), while dwarf mutants (designated as ● in Fig. 1), with the exception of those in group E, fell outside the circle that identified group N (Fig. 1) but within two distinct circles that

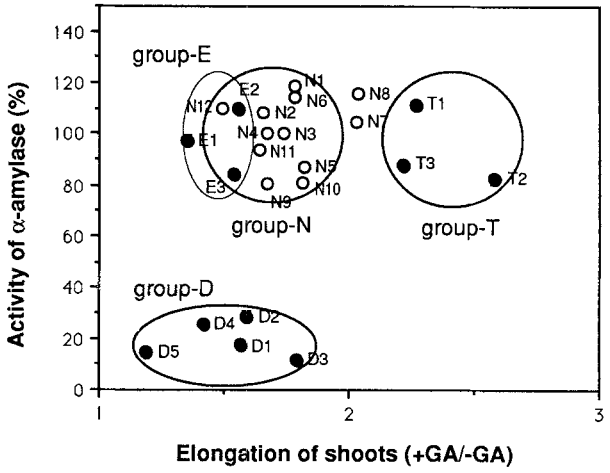

Fig. 1. Scatter diagram of all cultivars and mutants examined: the ratio of lengths of second leaf sheaths (ratio A in Table 1) is plotted against the development of α-amylase activity in embryoless half-seeds (% B in Table 1). Values (ratio A) obtained with and without the application of GA_3 are compared in each ratio. ○ Normal cultivars and semi-dwarf mutants, ● dwarf mutants

identified groups T and D. Thus, dwarf mutants could be classified into three different groups, designated group T, group D and group E, by reference to the names of the mutants, as follows: group T, Tan-ginbozu dwarf; group D, Daikoku dwarf; and group E, Ebisu dwarf (Table 1).

Characteristics of the classified dwarf mutants

Group N (normal type)

The elongation ratios, which represent the results of the application of GA_3 (ratio A), for all cultivars and mutants classified as members of group N were between 1.64 and 1.82, except in the case of M202 (N12), 'Calrose 76' (N7) and 'Akage' (N8) for which the ratios were 1.49, 2.03 and 2.04, respectively (Table 1). As shown in Fig. 1, no significant difference was observed in the development of α-amylase activity as a result of the application of GA_3 in the cultivars and mutants in group N. There are many reports on the presence of active GAs in the normal cultivars (Suge 1990), however, no active GAs have ever been found in the embryo-less half-seeds. Since the application of exogenous GA_3 to the normal cultivars and semi-dwarf mutants affected elongation of the shoots and production of α-amylase by cells in the aleurone layers, the cells of both the shoot and the aleurone layer appear to be able to respond to GA_3.

Group T (Tan-ginbozu type) dwarfs

The application of GA_3 to the mutants of group T caused significant enhancement of elongation of the shoots but little change in the production of α-amylase as compared with those in group N (Table 1). All 3 mutants in this group responded extremely vigorously to exogenously applied GA_3, a result that suggests that the mutants in group T are deficient in terms of endogenous GAs. Indeed, the Tan-ginbozu dwarf mutant (T1), as well as the Waito-C dwarf (T2) and the Kotake-tamanishiki dwarf (T3), have been demonstrated to be GA-deficient mutants in which one of the steps in the biosynthetic pathway to GAs is genetically blocked (Takahashi and Kobayashi 1990).

On the two-dimensional plot, 2 normal cultivars, namely, 'Calrose 76' (N7) and 'Akage' (N8), were located outside the circle that enclosed group N, close to that of group T (Fig. 1). To clarify the relationship between the normal cultivars and those in group T, we examined the activity of α-amylase from 4-day-germinated intact seeds to which either 1 μ*M* or no GA_3 had been applied. As shown in Fig. 2, no enhancement was observed in the development of α-amylase activity when GA_3 was applied to intact seeds of the cultivars in group N (N1, N7 and N8 in Fig. 2, data for other cultivars and mutants are not shown). However, the

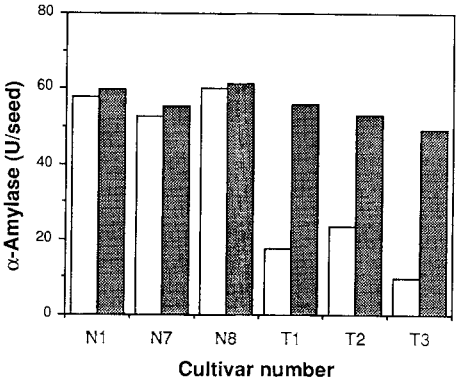

Fig. 2. Effects of GA_3 on various normal cultivars (group N) and dwarf mutants (group T) of rice in terms of development of α-amylase activity in intact seeds. Names of cultivars and mutants of rice: *N1* 'Nipponbare', *N7* 'Calrose 76', *N8* 'Akage', *T1* Tan-ginbozu dwarf, *T2* Waito-C dwarf, *T3* Kotake-tamanishiki dwarf. The assay for α-amylase activity is described in the text. The activities were measured after (▨) or without (□) the application of 1 μ*M* GA_3

application of GA_3 to intact seeds of mutants in group T was observed to cause significant enhancement of the development of α-amylase activity. The rate of production of α-amylase in intact seeds reflects the endogenous levels of active gibberellins (Mitsunaga and Yamaguchi 1993). These results suggest that the apparently atypical cultivars N7 and N8 are also members of group N.

Group D (Daikoku type) dwarfs

The mutants in group D had responses to exogenous GA_3 that were clearly distinct from those of cultivars and mutants in groups N, T and E (Table 1). Application of GA_3 did not cause complete recovery of the length of the second sheath, as compared with second sheaths of plants in groups N and T. These results suggest that members of group D are of the GA-insensitive type. To confirm this hypothesis, we examined the effects of GA_3 on these mutants in terms of the induction of α-amylase activity in embryo-less half-seeds (Fig. 3). Since embryo-less half-seeds fail to produce active GAs during imbibition, the production of α-amylase by cells in the aleurone layers is dependent on the application of exogenous GA_3 (Jacobsen and Chandler 1988). While the production of α-amylase by 'Nipponbare' (N1), 'Akage' (N8) and 'Ginbozu' (N11) in group N and by the Tan-ginbozu dwarf (T1) in group T and Ebisu dwarf (E1) in group E was dependent on the concentration of exogenous GA_3, with saturation of the effect at 10^{-8}*M* GA_3, no production of α-amylase by the Daikoku dwarf (D1), Kanto dwarf (D4) or Bonsai-daikoku dwarf (D5) was detectable at that concentration. The results indicate that the dwarf mutants in group D are GA-insensitive mutants.

Group E (Ebisu type) dwarfs

The dwarf mutants in group E exhibited minimal responses to GA in terms of growth. Therefore, the mutants in group E seem to have endogenous levels of GAs and sensitivity to GAs similar to those in group N, an indication that dwarfism in this case is associated with neither a deficiency in GAs nor insensitivity to GAs. Indeed, endogenous levels of GAs in the Ebisu dwarf (E1) are normal (Suge 1990), and this strain has a normal response to exogenous GA with respect to production of α-amylase (Fig. 3). Thus, it appears that group E is a dwarf mutant that is neither a GA-deficient nor a GA-insensitive mutant.

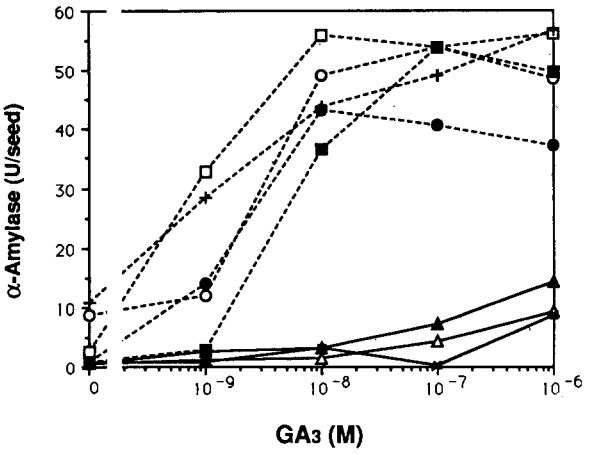

Fig. 3. Development of α-amylase activity in embryo-less half-seeds of the classified cultivars and mutants in response to the application of GA_3. Six embryo-less half-seeds were incubated in 1 ml of culture medium that contained 10 m*M* sodium acetate, pH 5.2, and 2 m*M* $CaCl_2$ with the indicated concentration of GA_3 at 30 °C. After 4 days, the seeds were extracted and α-amylase activity was measured as described in the text. ○ 'Nipponbare' (N1), ● 'Akage' (N8), □ 'Ginbozu' (N11), ■ Tanginbozu dwarf (T1), △ Daikoku 1 dwarf (D1), ▲ Kanto dwarf (D4), × Bonsai-daikoku dwarf (D5), + Ebisu dwarf (E1)

Identification of a GA-insensitive dwarf mutant from among near-isogenic dwarf lines of "Shiokari"

Dwarfing genes in rice were identified and characterized by Kinoshita and Shinbashi (1982). From their reports, some of the dwarf mutants used in this communication can be identified as being due to initiations in the following genes: Tan-genbozu dwarf (T1), *d-35*; Kotake-tamanishiki dwarf (T3), *d-18*^{k}; Daikoku dwarf (D1), *d-1*; Ebisu dwarf (E1), *d-2*; and Norin 28 dwarf (E2), *d-11*. Kinoshita and Shinbashi generated 21 near-isogenic lines from successive backcrossings with the 'Shiokari' cultivar as the recurrent parent (Table 2). We characterized a GA-insensitive dwarf mutant using their near-isogenic dwarf lines. The length of the culm of 19 distinct lines was within 30–60% of the parent with four exceptions namely, ID-7, -18h, -19 and -30 (Fig. 4A). As shown in Fig. 4B, the application of GA_3

Table 2. List of near-isogenic dwarf lines of 'Shiokari'

Number	Strain	Name of dwarf	Gene symbol	Back-crosses[a]
1	ID-1	Daikoku dwarf	*d-1*	11
2	ID-2	Ebisu dwarf	*d-2*	9
3	ID-3	Bunketsu-waito dwarf	*d-3, 4, 5*[b]	6
4	ID-6	Ebisumochi dwarf	*d-6*	7
5	ID-7	Heiei-daikoku dwarf	*d-7*	9
6	ID-10	Toyohikari bunwai dwarf	*d-10*	10
7	ID-11	Norin-28 dwarf	*d-11*	9
8	ID-12	Yukara dwarf	*d-12*	7
9	ID-13	Short-grained dwarf	*d-13*	7
10	ID-14	Kamikawa-bunwai dwarf	*d-14*	10
11	ID-17	Slender dwarf	*d-17*	7
12	ID-18^h	Hosetsu-waisei dwarf	*d-18*^{hc}	10
13	ID-18^k	Kotake-tamanishiki dwarf	*d-18*^{kc}	11
14	ID-19	Kamikawa dwarf	*d-19*	9
15	ID-27	Bunketsuto dwarf	*d-27*	9
16	ID-30	Waisei-shirasasa dwarf	*d-30*	9
17	ID-35	Tanginbozu dwarf	*d-35*	10
18	ID-42	Liguleless dwarf	*d-42*	9
19	ID-47	Dee-geo-woo-gen dwarf	*sd-1*	7
0	PAR	Shiokari		

All data in Table 2 are taken from Kinoshita and Shinbashi (1982) and Murai et al. (1982)

[a] Number of backcrosses

[b] Triplicate genes

[c] Multiple alleles in the order of $+ > d\text{-}18^k > d\text{-}18^h$

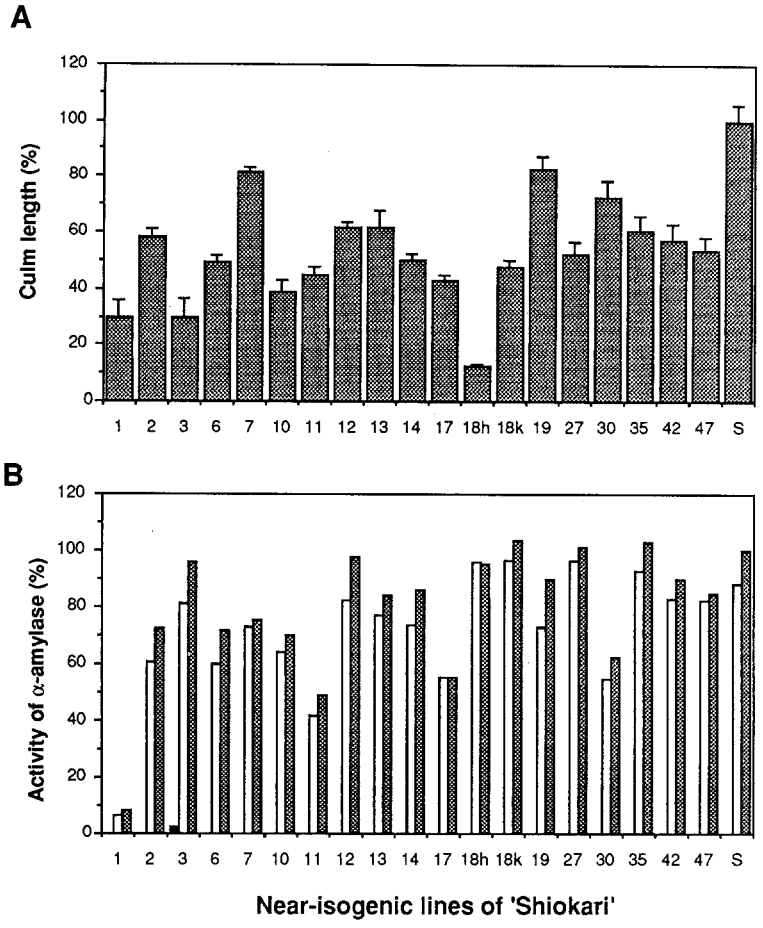

Fig. 4A, B. Length of culm (**A**) and development of α-amylase activity (**B**) in response to the application of GA_3 for near-isogenic dwarf lines of 'Shiokari'. **A** The seeds were grown in plots (1/5,000 are) at Nagoya University in 1991. Lengths of culm were compared after normalization, with that of 'Shiokari' being taken as 100%. *1* ID-1, *2* ID-2,...; *S* 'Shiokari', the recurrent parent. **B** Six embryo-less half-seeds of each of the near-isogenic dwarf lines and the parent were incubated in 1-ml aliquots of culture medium that contained 10 m*M* sodium acetate, pH 5.2, and 2 m*M* $CaCl_2$ with 0 (■), 0.01 μ*M* (□) and 1 μ*M* (▨) GA_3 at 30 °C. After 4 days, the seeds were extracted, and α-amylase activity was measured as described in the text

did not affect the production of α-amylase by embryo-less half-seeds in the ID-1 strain (column 1), indicating that the *d-1* dwarf gene is associated with decreased sensitivity to applied GA as compared to that of the parent and other dwarf mutants.

Discussion

Eleven dwarf mutants of rice were placed in three different groups on a two-dimensional plot (Fig. 1). Results were obtained by analyzing two distinct, GA-mediated physiological processes, namely, elongation of shoots and production of α-amylase by embryo-less half-seeds. We can conclude that these groups of dwarf mutants belong to three different categories: group T, GA-deficient; group D, GA-insensitive; and group E, dwarf with no relationship to either GA-deficiency or GA-insensitivity. Our reasoning for this conclusion is as follows: (1) Suge (1990) quantified endogenous levels of GAs in different dwarf mutants of rice using a bioassay, and the mutants in groups N, D and E had higher levels of GAs than those in group T; (2) the examination of the sensitivity to exogenous GA_3 of embryo-less half-seeds revealed that the seeds of group D are less responsive to exogenous GA_3 than the others (Fig. 3); (3) although mutants in groups D and E developed shoots of reduced length, as did those in group T after application of GA_3 (Fig. 1), only mutants in group E developed α-amylase activity to the same extent as those in group T (Fig. 1), suggesting that they are dwarf mutants in which the phenotype is not related to GA-deficiency (group T) or GA-insensitivity (group D).

Our approach has the advantage that differences among genetic backgrounds of the mutants did not cause divergence of the plotted points since most normal cultivars and mutants, with some exceptions, were located within circles (Fig. 1). Our method for classification of dwarf mutants of rice requires only small numbers of seeds (about 25 grains) and little time (about 1 week). Therefore, application of this approach to numerous dwarf mutants of rice may help us to characterize and classify them into different categories in terms of endogenous levels of GAs and sensitivity to GAs without any further complex analysis.

Dwarfing genes in rice were identified and characterized by Kinoshita and Shinbashi (1982). Kinoshita

and Shinbashi generated 21 near-isogenic lines from successive backcrossings with the 'Shiokari' cultivar as the recurrent parent. A trial classification was made on the basis of patterns of distribution of the elongated internodes, and the 21 near-isogenic dwarf lines were classified into nine groups. Takahashi and Takeda (1969) classified dwarf mutants of rice into five different types (dn, dm, d6, nl and sh) by analyzing the distribution of lengths of internode. The classification of the dwarf mutants in the present communication is different from theirs, because the present classification is based on an analysis of GA-mediated processes. From our attempt at the identification of the dwarf gene associated with the reduced sensitivity to GAs using 19 distinct dwarf lines of 'Shiokari', it appears that only the *d-1* gene is related to GA-insensitivity (Fig. 4). This result seems to make sense because the five distinct dwarf mutants (D1-5) in group D, classified as GA-insensitive mutants, are all derived from a mutant at the *d-1* locus (H. Kitano, personal communication).

GA-insensitive mutants of rice are of interest as materials for the analysis of the mode of action of gibberellins' as is the *Rht3* mutant in wheat. The genetic analysis of results of crosses between these dwarf mutants is now clearly necessary. On the basis of the present report, it appears that the *d-1* gene is associated with the Daikoku dwarf mutation that causes a reduction in the response to exogenous GA. The Daikoku dwarf mutant is characterized morphologically by a striking reduction in the length of the second internode and short round grains. It seems to be phenotypically different from the *Rht3* mutant in wheat. It has been reported that cold treatment of this GA-insensitive mutant of wheat during germination results in a significant increase in sensitivity to GA_3 (Singh and Paleg 1984). It will be of interest to examine the effects of cold treatment on the sensitivity to GA_3 of the Daikoku dwarf mutant as we continue our attempts to clarify the nature of the primary site of action of this plant hormone.

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