JARQ 40 (1), 1 – 12 (2006) http://www.jircas.affrc.go.jp

REVIEW

Effects of Dwarfing Genes on Chilling Tolerance at Seedling Stage in Rice

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Abstract

The effects of *sd1-d*, *sd1-r* and *sd1-c* at the *sd1* locus, *d18-k* (kotaketamanishiki dwarf) and *d12* (yukara dwarf) on the chilling tolerance at the seedling stage were examined. We used isogenic lines regarding the dwarfing genes/alleles, together with their parental varieties 'Fujiminori', 'Shiokari', 'Tamanishiki', 'Calrose', Taichung 65, Norin-PL8 and Norin-PL11. Chilling treatments at 2ºC for three, four and five days were conducted at the 1- and 2-leaf stages. The chilling tolerances of the lines were evaluated by survival percentages after the treatments. The *d12* lowered the chilling tolerance. The *sd1-d* and *sd1-c* lowered the chilling tolerance on the less tolerant genetic backgrounds of 'Fujiminori' and 'Shiokari', whereas this effect did not appear on the more tolerant genetic backgrounds of Taichung 65 and 'Calrose'. The *d18-k* enhanced the chilling tolerance on the less tolerant genetic backgrounds of 'Shiokari' and 'Tamanishiki', whereas the effect did not appear on the more tolerant genetic backgrounds of Norin-PL8 and Norin-PL11. Consequently, *d18-k* could be utilized to develop varieties adaptable to areas readily damaged by unseasonably low temperatures at all of the susceptible growth stages of rice, because *d18-k* is known to enhance the cool tolerances at both the booting and flowering stages also.

Discipline: Plant breeding
Additional key words: chilling injury, cool weather, direct seeding, *Oryza sativa*, transplanting

Introduction

In rice, the most cool-susceptible growing stage is the booting stage, particularly the young microspore stage, followed by the flowering stage[46]. Sterile-type cool damages sometimes bring about serious reduction of yield in northern Japan and other areas. Chilling injury at the seedling stage is also important, since seedling growth and transplanting are performed under low-temperature conditions in spring in many cases of cultivation. In California, USA, low temperatures diminish vigor of seedlings in a flooded direct-seeding system[19]. In northern Japan, direct seeding can not be conducted in flooded paddy fields when the daily mean temperature is below 10ºC[45]. In Nepal, double rice-cropping is restricted below an elevation of 700 m; because, above that elevation, seedlings in open-field nurseries often receive chilling injuries in February in the first crop[51]. Mechanical transplanting with young seedlings can be conducted when the daily mean temperature becomes higher than 11.5ºC in Hokkaido, the northernmost prefecture of Japan[34]. In the earliest rice cultivation of Kochi Prefecture located in southern Japan, chilling temperatures in spring inflict damages. For example, the minimum air temperature dropped to about 0ºC in early April in 1996, and just-transplanted seedlings withered or died. Thus, chilling injury readily occurs during the re-growth just after transplanting as well as during the seedling establishment in direct-seeding cultivation. Symptoms of chilling injury at the seedling stage of rice, viz. delay of growth, yellowing and white specks on leaf blades have been observed in several tropical and temperate countries[12].

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Received 6 May 2005; accepted 25 May 2005.

It is well-known that short-culm and lodging-resistant varieties carrying *sd1-d* originating from 'Dee-geo-woo-gen' are widely grown in Southeast Asia. A gamma-ray induced mutant 'Calrose 76' contains a dwarfing allele at the *sd1* locus, and it and its derivatives have been cultivated extensively in California[6,19,40]. 'Reimei' is a short culm mutant from 'Fujiminori'[7], which carries *sd1-r* at the *sd1* locus on chromosome 1[22,26]. 'Reimei' and its descendants have been widely cultivated in northern Japan until now[53]. A dwarf mutant Fukei 71 was exploited to develop two short culm varieties 'Fujihikari' and 'Katsurawase' which were cultivated in several districts[2,7]. Fukei 71 carries *d12* (yukara dwarf) or its allele[28] which is located on chromosome 2[47].

Murai et al.[23,24,26,28–31] demonstrated that *sd1-d* and *d12* lower the cool tolerances at both the booting and flowering stages; in contrast, *d18-k* (kotaketamanishiki dwarf) enhances the cool tolerances at these two stages due to its pleiotropic effects of increasing pollen number per anther and pollen fertility. The *d18-k* is allelic to *d18-h* (hosetsu dwarf)[50], being located on chromosome 1[8].

Murai et al.[32] concluded from the results of chilling treatments for isogenic lines of 'Shiokari' and other varieties that the dwarfing alleles at the *sd1* locus and *d12* lower the chilling tolerance at the seedling stage whereas *d18-k* enhances it. However, further experiments indicated that *d18-k* and *sd1-d* did not exert their effects on the chilling tolerance, neither enhancing nor lowering it, under certain genetic backgrounds. A part of the results was preliminarily reported by KC et al.[13]. In the present report, we have rearranged the results of the above two reports and unpublished data by the use of an *sd1-d* isogenic line of Taichung 65 and other isogenic dwarf lines. On the basis of the whole results, we examine the effects of the dwarfing genes/alleles on chilling tolerance at the seedling stage and the interaction between each gene/allele and genetic backgrounds.

Materials and methods

1. Dwarfing alleles at the *sd1* locus

The dwarfing alleles of 'Dee-geo-woo-gen' and 'Reimei' were registered as *sd1-d* and *sd1-r*, respectively[26]. Tanisaka et al.[55] tentatively designated the dwarfing allele of 'Calrose 76' as *sd1-c*. According to Murai and Yamamoto[22], the effects of the alleles on culm elongation are ranked in the order *sd1-d* ≤ *sd1-c* < *sd1-r* < *Sd1* (short < long ; *Sd1* is the dominant allele for long culm).

2. Isogenic dwarf lines

(1) Genetic background of 'Fujiminori'

'Fujiminori' is a tall variety grown in northern Japan during the 1960s. We used the following two isogenic lines of the recurrent parent 'Fujiminori'[22]. An isogenic line of *sd1-c* was developed after eight backcrosses, being denoted by "D^c". The isogenic line of *sd1-d* ("D^d") was developed after 11 backcrosses. 'Reimei' ("R") and Fukei 71 ("F-71") are gamma-ray-induced mutants from 'Fujiminori'[7]. F-71 carries *d12* or its allele[28]. We regarded R and F-71 as isogenic lines of 'Fujiminori'.

(2) Genetic background of 'Shiokari'

A variety of Hokkaido, 'Shiokari' and its isogenic lines regarding *d12*, *d18-k* and *sd1-d*, which were abbreviated as "S^{12}", "S^k" and "S^d", respectively, were used. S^d is one of the three *sd1-d* isogenic lines in the present study. Their morphological traits and dwarf donors were described in Murai et al.[27,30–32] and Murai[21]. The dwarf donor of S^{12} was N-62 ('Yukara dwarf') which is a mutant independent from F-71[28,54]. The numbers of backcrosses of S^{12}, S^k and S^d were 11, 15 and 12, respectively. The culm lengths of S^{12}, S^k and S^d at maturity (52.1, 38.7 and 49.5 cm, respectively) were shorter than that of 'Shiokari' (75.7 cm)[30]. Besides, *d18-k* decreases spikelet number per panicle but increases panicle number per plant, resulting in a notable panicle-number type in S^k.

(3) Genetic backgrounds of Taichung 65 and 'Calrose'

The *sd1-d* isogenic line of Taichung 65 (hereafter "T65") developed by ten backcrosses[31,33] was used, being abbreviated as "T^d". T65 is a Taiwanese *japonica* variety. 'Calrose 76' (hereafter "C^c") is a gamma-ray-induced mutant from a *japonica* variety 'Calrose' and the original donor of *sd1-c*[18].

(4) Other isogenic lines regarding *d18-k*

'Kotake-tamanishiki' (hereafter "KT") is a natural dwarf mutant found in a Japanese indigenous variety 'Tamanishiki', and is the gene source of *d18-k*. They were used in the present study.

Two isogenic lines of *d18-k* were developed, using Norin-PL8 and Norin-PL11 (hereafter "PL8" and "PL11") as recurrent parents[13,24,25]. The former and latter isogenic lines were denoted by "D8" and "D11", respectively. The number of backcrosses was seven for both D8 and D11. PL8 inherits high cool-tolerance at the booting stage from 'Silewah', an indigenous variety of Sumatra, Indonesia[1,41,42]. PL11 is extremely cool-tolerant at the booting stage: its cool tolerance was partially or fully originated from 'Padi Labou Alumbis' (a cool tolerant variety of the northern part of Borneo governed by Malaysia)[43,44]. PL8 and PL11 are not commercial variet-

ies, but intermediaries to transfer their cool tolerances into new *japonica* varieties.

3. Growing condition

Well-matured seeds were germinated in water at 28ºC for three days, after sterilization with Benlate® (E. I. du Pont de Nemours and Company). Transparent plastic cases with top and bottom inner dimensions of 6.5 × 6.5 cm and 5.9 × 5.9 cm, respectively, and a depth of 9.5 cm, were used for the experiments. The cases were filled with a commercial soil for rice seedlings, Biwako-ikubyo-baido® No. 2 (Biwako Sangyo Inc., Ibuki Town, Shiga, Japan), up to a depth of 3.5 cm, containing 0.025 g each of N, P_2O_5 and K_2O per case. For each case, 16 (4 × 4) well-sprouted seeds were sown. A semi-dry condition was kept by appropriate watering. The seeded cases were placed in an indoor-type growth chamber illuminated by both fluorescent lamps and electric bulbs at about 600 μ mol PAR m^{-2} s^{-1} from 5:30 a.m. to 5:30 p.m. which was set at 21/19ºC day/night temperatures with a relative humidity of 60 to 90%.

4. Chilling treatments

The incomplete leaf lacking its blade was excluded from the leaf age counting in the present study. Tajima et al.[52] reported that about half of the seedlings of 'Nipponbare' died after a 5ºC-10-day treatment at the 3-leaf stage. According to Kushibuchi et al.[16], the 1-leaf stage was more susceptible to a 1-day treatment at 2–4ºC than the following 2- to 4-leaf stages. Referring to these reports, we conducted the chilling treatments as described below.

The chilling treatments in the present study were conducted, using an incubator kept at 2ºC without illumination. The treatments were started at the following two growth stages. 1) 1-leaf stage—The 1st leaf blade just fully expanded in most of the seedlings, and the 2nd leaf blade started to appear from the 1st leaf sheath in some of the seedlings. Delayed seedlings with non-expanded leaf blades were marked and excluded from the survey described below. The material lines took seven or eight days from sowing to attain this stage. 2) 2-leaf stage—The 2nd leaf blade just fully expanded in most of the seedlings, and the 3rd leaf blade started to appear from the 2nd leaf sheath in some of the seedlings. Seedlings with delayed growth were excluded from the survey. The material lines took 12 to 14 days from sowing to attain this stage.

The treatments for three, four and five days at the 1-leaf stage, and those for four and five days at the 2-leaf stage were conducted. Twelve cases were used for each treatment in each line. After the treatments, the cases were returned back to the growth chamber. Cases for the control were placed in the growth chamber until the measurements described in the next section.

5. Survivability survey and measurements of morphological traits

Chilling tolerance was evaluated by survivability. Survival percentages were surveyed 22 days after the treatments. The main shoot was used to judge whether each seedling was surviving or dead. After the death of a main shoot, a tiller often appeared from the incomplete leaf in the treatments at the 1-leaf stage (Fig. 1); similarly, a tiller also appeared from the incomplete leaf or the 1st leaf sheath in the treatments at the 2-leaf stage. Seriously injured seedlings, which could not survive further, were judged to be dead. The morphological traits mentioned below were measured in the control and in the treatments at the 1-leaf stage. The leaf age, plant length and the 1st leaf blade length in the control were measured 22 days after the 1- leaf stage (29 or 30 days after sowing). The lengths of the 2nd to 5th leaf blades in the control were measured about 60 days after sowing. At each of these two measurement times, about 80 seedlings per line were sampled, and half of the seedlings, which had intermediate plant lengths, were measured. Similarly, about 45 seedlings for each treatment in the lines were sampled and measured on the day of the survivability survey.

Results and discussion

1. Survivability after chilling treatments

(1) Isogenic dwarf lines of 'Fujiminori', 'Shiokari', T65 and 'Calrose'

Table 1 shows survival percentages of 'Fujiminori', 'Shiokari', T65, 'Calrose' and their isogenic dwarf lines in the 3- to 5-day treatments at the 1- and 2-leaf stages. Numerals in brackets indicate the differences in survival percentage between the 1- and 2-leaf stages in the lines. Every line showed the tendency that the longer the treatment at the 1- or 2-leaf stage, the lower the survival percentage. In the 5-day treatment at the 1-leaf stage, the survival percentages of almost all lines were about 10% or less, excepting C^c (39.2%) and 'Calrose' (36.8%). In the 5-day treatment at the 2-leaf stage, the survival percentages of S^k, T^d and T65 were higher than 40%, while those of the other lines were lower than 30%. The survival percentage in the 4- or 5-day treatment at the 2-leaf stage was higher than or similar to that at the 1-leaf stage in each of the lines except C^c and 'Calrose'.

D^d, D^c and R, viz. *sd1*-allele carriers, had significantly lower survival percentages than 'Fujiminori' in the

4-day treatment at the 1-leaf stage, whereas they were not significantly different from 'Fujiminori' in the other treatments at the 1- and 2-leaf stages (Table 1-(1)). Similarly, S^d had a significantly lower percentage than 'Shiokari' only in the 3-day treatment at the 1-leaf stage (Table 1-(2)). However, no significant differences were noticed between T^d and T65 as well as between C^c and 'Calrose' throughout all treatments at the 1- and 2-leaf stages (Table 1-(3)).

F-71 had significantly lower survival percentages

Table 1. Survival percentages of 'Fujiminori', 'Shiokari', T65 and their isogenic dwarf lines, treated at the 1- and 2-leaf stages

(1) 'Fujiminori' and its isogenic dwarf lines

Treatment	D^d	D^c	R	F-71	**'Fujiminori'**
1-leaf stage					
3-day	87.5	90.2	97.1	63.7**	**88.6**
4-day	42.7**	35.2**	39.2**	8.1**	**74.6**
5-day	6.3	6.8	2.6	0.5	**1.6**
2-leaf stage					
4-day	91.4	72.7	84.7	84.9	**74.0**
5-day	7.4	8.0	14.3	9.2	**6.8**
Difference between 1- and 2- leaf stages					
4-day	[48.7**]	[37.5**]	[45.5**]	[76.8**]	[**0.6**]
5-day	[1.1]	[1.2]	[11.7*]	[8.7**]	[**5.2**]

(2) 'Shiokari' and its isogenic dwarf lines

Treatment	S^d	S^{12}	S^k	**'Shiokari'**
1-leaf stage				
3-day	52.0**	78.9	98.9	**92.3**
4-day	45.9	31.2	77.0**	**45.5**
5-day	0.0	6.0	10.2	**6.3**
2-leaf stage				
4-day	41.5	45.6	88.0**	**43.0**
5-day	26.9	15.7	52.9**	**24.1**
Difference between 1- and 2- leaf stages				
4-day	[–4.4]	[14.4]	[11.0]	**[–2.5]**
5-day	[26.9**]	[9.7*]	[42.7**]	**[17.8**]**

(3) T65, 'Calrose' and their isogenic dwarf lines

Treatment	T^d	**T65**	C^c	**'Calrose'**
1-leaf stage				
3-day	100.0	**100.0**	97.7	**100.0**
4-day	93.3	**93.3**	87.7	**95.7**
5-day	11.2	**6.6**	39.2	**36.8**
2-leaf stage				
4-day	–	–	–	–
5-day	40.2	**57.0**	16.5	**6.6**
Difference between 1- and 2- leaf stages				
5-day	[29.0**]	**[50.4**]**	[–22.7]	**[–30.2]**

*,**: Significantly different between each isogenic dwarf line and its parental variety at the 5 and 1% levels, respectively.
[ ]: 2-leaf stage – 1-leaf stage. Asterisks indicate significance of difference.
–: The 4-day treatment at the 2-leaf stage was not conducted.

Table 2. Survival percentages of KT, D8, D11 and their parental varieties, treated at the 1- and 2-leaf stages

Treatment	KT	**'Tamanishiki'**	D8	**PL8**	D11	**PL11**
1-leaf stage						
3-day	96.4	**95.1**	100.0	**98.9**	99.0	**96.5**
4-day	74.4*	**40.0**	88.5	**88.5**	90.5	**91.2**
5-day	3.1	**2.3**	11.5	**12.5**	43.6	**59.7**
2-leaf stage						
4-day	84.7*	**65.7**	–	–	–	–
5-day	35.6**	**2.3**	19.6	**19.8**	86.3	**66.8**
Difference between 1-and 2-leaf stages						
4-day	[10.3]	**[25.7]**	–	–	–	–
5-day	[32.5**]	**[0.0]**	[8.1]	**[7.3]**	[42.7**]	**[7.1]**

*,**: Significantly different between each isogenic dwarf line and its parental variety at the 5 and 1% levels, respectively.
[ ]: 2-leaf stage – 1-leaf stage. Asterisks indicate significance of difference.
–: The 4-day treatment at the 2-leaf stage was not conducted.

than 'Fujiminori' in the 3- and 4-day treatments at the 1-leaf stage, although significant differences between the lines were not noticed in the other three treatments (Table 1-(1)). The survival percentages of S^{12} were lower than those of 'Shiokari' in all treatments except the 4-day treatment at the 2-leaf stage, although not significantly (Table 1-(2)). Consequently, *d12* may lower the chilling tolerance on the two genetic backgrounds.

As shown in Table 1-(2), S^k had significantly higher survival percentages than 'Shiokari' in the 4-day treatment at the 1-leaf stage and the two treatments at the 2-leaf stage; the greatest difference (45.0%) was noticed in the 4-day treatment at the 2-leaf stage.

(2) Other isogenic lines regarding *d18-k*

Table 2 shows survival percentages of KT, D8, D11 and their parental varieties in the treatments. The lines showed the trend that the longer the treatment at the 1- or 2-leaf stage, the lower the survival percentage. In the 5-day treatment, the survival percentage was higher at the 2-leaf stage than at the 1-leaf stage in each of the lines except 'Tamanishiki'. 'Tamanishiki' and PL11 had the lowest and highest percentages, respectively, among the three parental varieties in every treatment except the 3-day treatment at the 1-leaf stage, reflecting their chilling tolerances.

KT had significantly higher survival percentages than 'Tamanishiki' in the 4-day treatment at the 1-leaf stage and the two treatments at the 2-leaf stage. However, no significant differences were noticed between D8 and PL8 as well as between D11 and PL11 throughout all treatments at the 1- and 2-leaf stages.

2. Interaction between *sd1-d*, *sd1-c* or *d18-k* and genetic backgrounds

The interaction of each dwarfing gene/allele with genetic backgrounds was examined for the survival percentages in the 4-day treatment at the 1-leaf stage; since all lines widely ranged from 8.1 to 95.7% in the treatment while they were almost maldistributed at high and low ranges in the 3- and 5-day treatments, respectively, at the 1-leaf stage (Tables 1 & 2).

Analysis of variance was conducted for the survivability data of 'Fujiminori', 'Shiokari', T65, and their *sd1-d* isogenic lines in the 4-day treatment at the 1-leaf stage (Table 3-(1)). The effects of *sd1-d*, genetic background and the interaction were all statistically significant. The parental varieties ranked in the order T65 (93.3%) > 'Fujiminori' (74.6%) > 'Shiokari' (45.5%) in the treatment (Table 1), reflecting their chilling tolerances. T^d had the same survival percentage as T65 in the treatment. On the other hand, D^d was significantly lower than 'Fujiminori'. S^d and 'Shiokari' had similar survival percentages in the treatment, but the former was significantly lower than the latter in the 3-day treatment at the 1-leaf stage. Consequently, *sd1-d* diminished the chilling tolerance on the two less tolerant genetic backgrounds, whereas this effect did not appear on the most tolerant genetic background.

Analysis of variance for the survivability data of 'Fujiminori', 'Calrose' and their *sd1-c* isogenic lines in the treatment indicated significant effects of *sd1-c*, genetic background and the interaction (Table 3-(2)). C^c (87.7%) was not significantly different from 'Calrose' (95.7%) whereas D^c (35.2%) was significantly lower than 'Fujiminori' (74.6%) in the treatment (Table 1-(1) & (3)). Thus, the effect of *sd1-c* on diminishing the chilling tol-

Table 3. Analysis of variance for survival percentages in the 4-day treatment at the 1-leaf stage, for examining the interaction between *sd1-d*, *sd1-c* or *d18-k* and genetic backgrounds

(1) 'Fujiminori', 'Shiokari', T65 and their *sd1-d* isogenic lines

Source of variation	Degrees of freedom	F- value
sd1-d	1	28.46**
Genetic background (G. B.)	2	4.08*
sd1-d × G. B.	2	4.45*
Error	66	

(2) 'Fujiminori', 'Calrose' and their *sd1-c* isogenic lines

Source of variation	Degrees of freedom	F- value
sd1-c	1	34.50**
Genetic background (G. B.)	1	16.94**
sd1-c × G. B.	1	6.88*
Error	44	

(3) 'Shiokari', 'Tamanishiki', PL8, PL11 and their *d18-k* isogenic lines

Source of variation	Degrees of freedom	F- value
d18-k	1	11.67**
Genetic background (G. B.)	3	16.30**
d18-k × G. B.	3	4.80**
Error	88	

*,**: Significant at the 5 and 1% levels, respectively.

erance appeared and disappeared on the less and more tolerant genetic backgrounds, respectively.

Analysis of variance for the survivability data of 'Tamanishiki', 'Shiokari', PL8, PL11 and their *d18-k* isogenic lines in the treatment indicated significant effects of *d18-k*, genetic background and the interaction (Table 3-(3)). The parental varieties ranked in the order PL11 (91.2%) > PL8 (85.5%) > 'Shiokari' (45.5%) > 'Tamanishiki' (40.0%) in the treatment (Tables 1 & 2), reflecting their chilling tolerances. D11 and D8 were similar to PL11 and PL8, respectively, in the treatment, whereas S^k and TK were higher than their respective parental varieties. Hence, *d18-k* enhanced the chilling tolerance on the two less tolerant genetic backgrounds, whereas the effect did not appear on the two more tolerant genetic backgrounds.

3. Morphological traits in the control

(1) Isogenic dwarf lines of 'Fujiminori'

Table 4-(1) shows morphological traits of D^d, D^c, R and F-71 in the control, compared with those of 'Fujiminori'. All of the dwarf lines except R had higher leaf ages than 'Fujiminori'. The plant lengths of D^d, D^c and R were about 90% of that of 'Fujiminori', while F-71 was similar to 'Fujiminori'. The 1st leaf blade lengths of the isogenic dwarf lines except R were somewhat longer than that of 'Fujiminori'. Regarding the 2nd to 5th leaf blade lengths, D^d, D^c and R had lower values than 'Fujiminori' (79 to 99%). The 2nd leaf blade length of F-71 was longer than that of 'Fujiminori', but the 3rd to 5th leaf blade lengths of F-71 were similar to or rather shorter than those of 'Fujiminori'. Thus, the *sd-1* alleles reduced lengths of plant and the 2nd to 5th leaf blades, whereas *d12* did not reduce plant length.

(2) KT and 'Tamanishiki'

Table 4-(2) shows morphological traits of KT and 'Tamanishiki' in the control. The leaf age of KT increased from that of 'Tamanishiki' by 0.4. The plant length of KT was 67% of that of 'Tamanishiki'. Regarding the 1st to 5th leaf blade lengths, KT was 62 to 78% relative to 'Tamanishiki'. Hence, *d18-k* drastically reduces the lengths of plant and the leaf blades.

4. Influences of chilling treatments on morphological traits

Table 5 shows influences of treatments at the 1-leaf stage on leaf age and plant length in 'Fujiminori', 'Tamanishiki' and their isogenic dwarf lines. The data on the 5-day treatment were excluded from the table, since the number of surviving seedlings in the lines was insufficient for statistical analysis.

The two treatments increased leaf age by 30 to 68%, relative to the control values, in the seven lines. As shown in Table 5-(1), the percentage increase was lower in D^d than in 'Fujiminori' for the 3-day treatment, whereas the reverse result was obtained for the 4-day treatment. In the other three isogenic dwarf lines of 'Fujiminori', also, inconsistent and/or nonsignificant differences from 'Fujiminori' were noticed. KT was not significantly different from 'Tamanishiki' in the 3- and 4-day treatments (Table 5-(2)).

Each line had a more reduced plant length in the 4-day treatment than in the 3-day treatment (Table 5). Regarding this trait, the percentages of D^c and R were higher than that of 'Fujiminori' in the 3-day treatment (Table 5-(1)). In the 4-day treatment, however, the percentages of D^d, D^c and R were similar to that of 'Fujiminori'. F-71 had lower percentages than 'Fujiminori' in both treatments. As shown in Table 5-(2), the percent-

Table 4. Morphological traits of 'Fujiminori', 'Tamanishiki' and their isogenic dwarf lines in the control

(1) 'Fujiminori' and its isogenic dwarf lines

Trait	D^d	D^e	R	F-71	**'Fujiminori'**
Leaf age[a)]	3.5 (107)**	3.4 (104)**	3.3 (100)	3.6 (109)**	**3.3**
Plant length[a)] (mm)	361.1 (90)**	369.2 (92)**	356.7 (89)**	410.4 (102)**	**402.4**
Leaf blade length (mm)					
1st	23.1 (116)**	20.9 (105)*	20.1 (101)	22.4 (112)**	**20.0**
2nd	106.9 (99)	102.4 (95)**	98.3 (91)**	120.6 (111)**	**108.1**
3rd	225.0 (88)**	228.4 (90)**	221.9 (87)**	254.7 (100)	**254.4**
4th	227.7 (89)**	234.8 (92)**	247.5 (96)**	253.5 (99)	**256.6**
5th	187.1 (79)**	198.8 (84)**	218.3 (92)**	226.5 (96)**	**237.1**

(2) KT and 'Tamanishiki'

Traits	KT	**'Tamanishiki'**
Leaf age[a)]	4.1 (111)**	**3.7**
Plant length[a)] (mm)	266.6 (67)**	**396.1**
Leaf blade length (mm)		
1st	15.0 (78)**	**19.3**
2nd	67.9 (66)**	**120.7**
3rd	137.3 (62)**	**220.9**
4th	195.5 (73)**	**269.2**
5th	152.3 (68)**	**223.7**

( ): Percentage of each isogenic dwarf line to its parental variety for each trait.
a): 22 days after the 1-leaf stage.
*,**: Significantly different between each dwarf line and its parental variety at the 5 and 1% levels, respectively.

ages of KT and 'Tamanishiki' were similar to each other in both treatments.

Table 6 shows influences of treatments at the 1-leaf stage on the 2nd to 5th leaf blade lengths in 'Fujiminori', 'Tamanishiki' and their isogenic dwarf lines. The 1st leaf blade was not included in the table, since it already attained its full length at the treated time. In the 3-day treatment, all of the seven lines showed apparent reductions in all the four traits, excepting the 5th leaf blade length of D^e. In each line, the percentage of the 5th leaf blade length was higher than those of the 2nd to 4th leaf blade lengths. In each line, the 2nd to 5th leaf blade lengths were shorter or at least similar in the 4-day treatment than in the 3-day treatment. In every combination of treatment and line, the percentage of the 3rd leaf blade length was the lowest among those of the 2nd to 5th leaf blade lengths. Furthermore, degeneration and malformation of the 3rd leaf blades, as shown in Fig. 2 and Fig. 3, were frequently observed in the 4- and 5-day treatments for all lines in the present study. The following inference can be drawn: the 3rd leaf blade was at the early stage of elongation at the 1-leaf stage, so that this organ was underdeveloped and/or malformed; and so, the developments of the 4th to 6th leaves were accelerated to compensate the damage resulting in the increase of leaf age (Table 5). In the 3-day treatment, the percentages of D^d, D^e and R were higher than that of 'Fujiminori' in each of the leaf blade lengths, exclusive of the 2nd leaf blade length of D^d (Table 6-(1)). In the 4-day treatment, however, the percentages of D^d, D^e and R were lower than that of 'Fujiminori' in the 3rd to 5th leaf blade lengths. F-71 had lower percentages than 'Fujiminori' for all of the leaf blade lengths in both treatments, exclusive of the 3rd leaf blade length in the 3-day treatment. KT had a significantly lower percentage of the 4th leaf blade length than 'Tamanishiki' in both treatments, but the former had a higher percentage of the 2nd leaf blade length than the latter in the 3-day treatment (Table 6-(2)). Regarding the 3rd and 5th leaf blade lengths, no significant differences between the two lines were noticed in both treatments.

Consequently, the chilling treatments notably influenced the morphology of seedlings. F-71 had more reduced lengths of plants and the leaf blades in the treatments than 'Fujiminori'. However, differences in response between the *sd1*-allele carriers and 'Fujiminori' as well as between KT and 'Tamanishiki' were either nonsignificant or significant but inconsistent between the two treatments.

Table 5. Influences of the 3- and 4-day treatments at the 1-leaf stage on leaf age and plant length in 'Fujiminori', 'Tamanishiki' and their isogenic dwarf lines: Actual values in the treatments are converted into the percentages relative to control values

(1) 'Fujiminori' and its isogenic dwarf lines

Trait / Treatment	D[d]	D[c]	R	F-71	**'Fujiminori'**
Leaf age[a)]					
3-day	147**	135**	154*	156	**162**
4-day	168**	160	153	152	**153**
Plant length[a)]					
3-day	74	86**	83*	62	**72**
4-day	65	64	63	33**	**62**

(2) KT and 'Tamanishiki'

Trait / Treatment	KT	**'Tamanishiki'**
Leaf age[a)]		
3-day	131	**132**
4-day	133	**130**
Plant length[a)]		
3-day	77	**77**
4-day	55	**59**

a): 22 days after the treatments at the 1-leaf stage.
*,**: Significantly different between each dwarf line and its parental variety at the 5 and 1% levels, respectively.

Table 6. Influences of the 3- and 4-day treatments at the 1-leaf stage on the 2nd to 5th leaf blade lengths in 'Fujiminori', 'Tamanishiki' and their isogenic dwarf lines: Actual values in the treatments are converted into the percentages relative to control values

(1) 'Fujiminori' and its isogenic dwarf lines

Leaf blade length	D[d]	D[c]	R	F-71	**'Fujiminori'**
3-day treatment					
2nd	38**	48	54**	39**	**44**
3rd	11	25**	18*	9	**7**
4th	30	64**	50**	19	**28**
5th	76	94**	71	52	**59**
4-day treatment					
2nd	40	44	43	29**	**41**
3rd	8	5*	7	2**	**10**
4th	18**	13**	25	7**	**33**
5th	53	54	58	28**	**71**

(2) KT and 'Tamanishiki'

Leaf blade length	KT	**'Tamanishiki'**
3-day treatment		
2nd	54	**51**
3rd	24	**33**
4th	46*	**61**
5th	90	**88**
4-day treatment		
2nd	26*	**21**
3rd	8	**13**
4th	16**	**32**
5th	51	**63**

*,**: Significantly different between each isogenic dwarf line and its parental variety at the 5 and 1% levels, respectively.

5. Partial chlorosis

Partial chlorosis across the 2nd leaf blade, as shown in Fig. 4, was noticed in all seedlings of the lines treated at the 1-leaf stage. Similar chlorosis across the 3rd leaf blade was observed in every seedling treated at the 2-leaf stage. However, this partial chlorosis did not appear in other leaf blades than the 2nd/3rd leaf blade. The chlorosis part was inside the 1st or 2nd leaf sheath at the treated time and appeared immediately after the treatments. It is known in rice that chlorophyll content in leaf blades is decreased by chilling temperatures[10,11,48,49]. Otani and Doi[39] reported that similar partial chlorosis was induced by 2 or 5ºC treatments at the seedling stage. It seems that chilling temperatures at about 2ºC hinder the chlorophyll formation at the part of a leaf blade just before appearing from the previous leaf sheath.

General discussion

In general, *japonica* varieties are more chilling-tolerant at the seedling stage than *indica* varieties[4,5,11,20,37,38]. Komatsu et al.[15] reported that a protein fragment of RuBisCO (the photosynthetic CO_2 fixation enzyme) induced by a chilling stress was more phosphorylated in chilling-tolerant *japonica* varieties than in susceptible *indica* ones. Kwak et al.[17] and Nagamine[36] drew similar conclusions, viz. single-genic control and the dominance for the chilling tolerance at the seedling stage, from results of F_2 populations between tolerant *japonica* and susceptible *indica* varieties. This gene(s) may be a causative factor in the geographical distribution of the two subspecies. The present study deals with pleiotropic effects of the dwarfing genes/alleles on the chilling tolerance under the *japonica* genetic backgrounds.

Under the genetic backgrounds of 'Fujiminori' and 'Shiokari', the *sd1* alleles lowered chilling tolerance at the 1-leaf stage (Table 1). The study of Kushibuchi et al.[16] indicates that the 1-leaf stage is the most chilling-susceptible during the growth of seedling, being consistent with a lower survivability at the 1-leaf stage than at the 2-leaf stage in most of the lines (Tables 1 & 2). Hence, the effect of the *sd1* alleles may accelerate the chilling damage at the 1-leaf stage, when direct seeding or open-field nursery cultivation is performed in cool-weather regions. However, this effect did not appear in the treatments at the 2-leaf stage (Table 1). Seedlings older than the 2-leaf stage are mechanically transplanted, ordinarily, in northern Japan, so that the cultivation of *sd1-r* carriers like R may not cause the chilling damage, practically. The *sd1-d* and *sd1-c* did not lower the chilling tolerance under the genetic backgrounds of T65 and 'Calrose', respectively (Table 1-(3)). According to Murai et al.[29,30], the effect of *sd1-d* on reducing the cool tolerance at the booting stage was great and small on the genetic backgrounds of less-cool-tolerant 'Shiokari' and more-cool-tolerant T65, respectively. Consequently, the effect of *sd1-d* on the chilling tolerance at the seedling stage and its interaction with the two genetic backgrounds are almost identical with those at the booting stage.

S^k and KT had higher survival percentages than their respective parental varieties in all treatments at the 1- and 2-leaf stages (Table 1-(2) & Table 2). However, the tolerance-enhancing effect of *d18-k* did not appear under the more-chilling-tolerant genetic backgrounds of PL8 and PL11. An experimental result of Kabaki et al.[10] showed that KT recovered its growth after transplanting under a low temperature condition, more immediately than tall varieties. This quick recovery of growth may be due to not only the tolerance-enhancing effect of *d18-k* but also the short stature and small leaf area caused by this gene (Table 4-(2)). Anyway, *d18-k* carriers have an advantage in avoiding the chilling damage, due to the tolerance-enhancing effect and/or the short-sturdy seedling-stature. According to Murai et al.[24,25,29,30], *d18-k* enhanced the cool tolerance at the booting stage on all of the four genetic backgrounds common with the present study, inclusive of extremely-cool-tolerant LP11, so that a super-high cool-tolerance at the booting stage was realized by the *d18-k* isogenic line of LP11, viz. D11. Besides, developing panicles on short culms in *d18-k* isogenic lines can be more easily protected from cool air by relatively warm irrigation-water than in their tall parental varieties[14,25,30]. Moreover, *d18-k* enhances the cool tolerance at the flowering stage[23]. Hence, *d18-k* could be utilized to develop varieties adaptable to areas readily damaged by unseasonably low temperatures at all of the susceptible growth stages of rice.

According to Ashikari et al.[3], *SD1* encodes a gibberellin biosynthetic enzyme, GA20 oxidase; the gibberellin activity in 'Dee-geo-woo-gen' was low; and *sd1-r* and *sd1-d* have different structures at the molecular level. Itoh et al.[9] reported that *D18*, the dominant allele for tallness, encodes gibberellin 3β-hydroxylase which catalyzes the biosynthetic pathway from GA_{20} (immediate precursor) to GA_1 (active gibberellin). The *d18-k* blocks the final step of the active gibberellin biosynthesis[35]. Although both *sd1-d* and *d18-k* lower gibberellin activity in plant body, why does the former lower the chilling tolerance at the seedling stage but the latter enhance it? Further physiological and biochemical researches are necessary to answer this question.

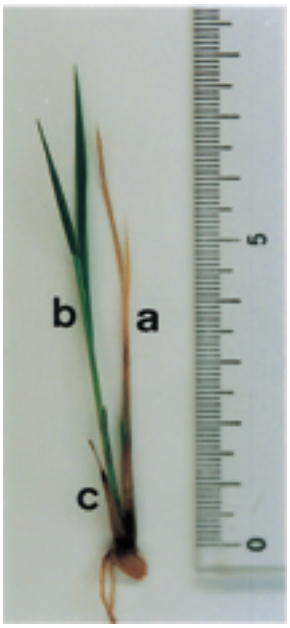

Fig. 1. Development of the tiller from the inside of the incomplete leaf after the death of a main shoot (4-day treatment at the 1-leaf stage for PL11)
a: Main shoot.
b: Tiller.
c: Incomplete leaf.

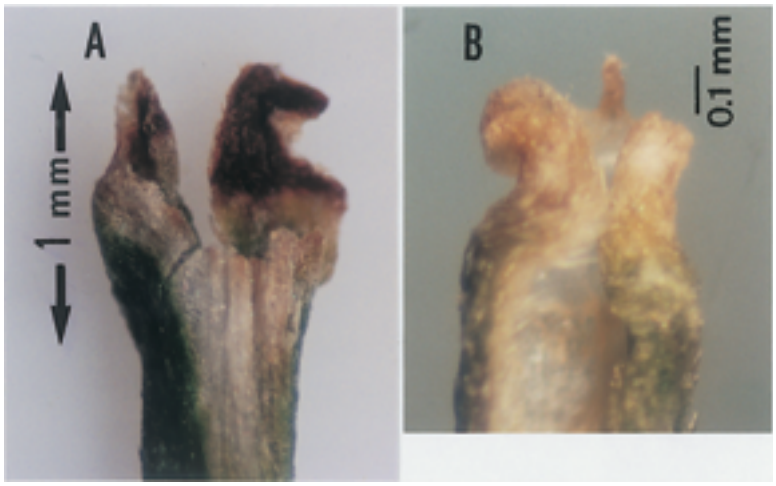

Fig. 2. Degeneration of the 3rd leaf blade induced by the 4-day treatment at the 1-leaf stage
A: Extreme reduction of the leaf blade less than 1 mm, lacking its midrib, in D^c.
B: Almost complete degeneration of the leaf blade, except for a little midrib, in D^d.

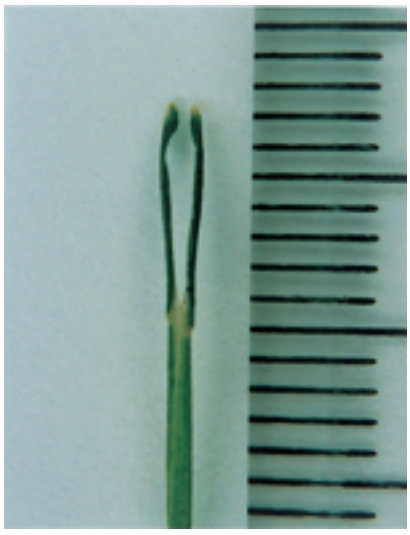

Fig. 3. Malformation of the 3rd leaf blade, forked into two parts at its midrib (4-day treatment at the 1-leaf stage for D^c)

Fig. 4. Partial chlorosis across the 2nd leaf blade (3-day treatment at the 1-leaf stage for PL11)

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