Development of Suitable Technique for Testing Resistance of Wheat Cultivars to Three Snow Mold Diseases

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Introduction

Snow mold diseases have been a significant problem to winter cereal production in the Hokuriku district in Japan, where snow falls heavily and persists for long periods. In the region, several kinds of snow mold fungi are pathogenic to winter cereals, and the most important snow mold fungi of them are *Pythium* species (*P. paddicum* Hirane, *P. iwayamai* S. Ito), *Typhula incarnata* Lash et Fr. and *Fusarium vivale* (Fr.) Ces.[12].

Snow mold resistant cultivars offer the most practical disease control. Therefore, the development of the screening technique is necessary for the selection of resistant cultivars. The resistance of wheat cultivars to snow mold diseases has been tested in the fields after snow melting, but the field tests have three problems as follows; 1) It is difficult to test the resistance of cultivars to a specific snow mold fungus because some snow mold fungi usually coexist in the fields. 2) There is a great variation from year to year in the amount of snow mold damage because the continuous snow cover duration is different every year. 3) It is impossible to carry out the field tests more than once a year and it takes a long time to finish the tests. Therefore, snow mold chamber methods, which have the ability to induce and regulate the occurrence of snow mold diseases at will, have been developed and modified by several workers[1,2,4,5,7,9,10]. When these methods are applied to testing the resistance of wheat cultivars to snow mold diseases, however, degrees of resistance of wheat cultivars are very changeable with experimental conditions such as the plant growth before the inoculation and incubation period at 0–1°C in the dark. Bruehl[3] and Jamallainen[8] reported that it is dfficult to detect small differences in resistance with the snow mold chamber methods, and that some results in the snow mold chambers are in conflict with those in the fields. Therefore, it is necessary to develop the suitable technique which makes it possible to detect small differences among wheat cultivars in resistance, and indicates close correlation with results of field tests.

In the present study, the resistance of wheat cultivars to snow mold fungi was tested in the field by controlling the duration of the snow cover, and with the two different snow mold chamber methods in order to develop a suitable technique for resistance of wheat cultivars to *P. paddicum*, *T. incarnata* and *F. nivale*.

Materials and methods

Three wheat cultivars, Nōrin No. 61, Tōsan No. 16 and Yukichabo, were used in all the experiments.

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1) Field tests for snow endurance

Each wheat cultivar was sown in rows 3 m in length and 0.25 m apart (30 seeds per row) in a field on October 4, 1984. Each cultivar was initially fertilized with 8 kg N, P and K/10 a. On December 19, the average numbers of tillers of Nōrin No. 61, Tōsan No. 16 and Yukichabo were 11.2, 18.3 and 19.9, respectively. The continuous snow cover began on December 22, and after 45, 60, 75, 90, 105 and 121 days, the plants under the snow cover were removed intact and transplanted to the pots (2 plants per pot). The pots were placed in a glasshouse at 15–25°C. The percentages of surviving tillers were recorded after a recovery period of 2 weeks.

2) Inoculum

Each of *P. paddicum* isolate W-82-15, *T. incarnata* isolate HT8301 and *F. nivale* isolate HF8301 was incubated in potato sucrose broth at 15°C for 10 days, transferred thereafter to sand-wheat bran-dextrose medium (sand 1,000 m*l*, wheat bran 1,000 m*l*, dextrose 14 g and distilled water 250 m*l*), incubated at 15°C for about 20 days and used as an inoculum. The origin of these fungi was described elsewhere[11].

3) Snow mold chamber method A

Five seeds of each cultivar were planted at the opposite sides of a plastic pot (13.3×28.4×9.0 cm) containing autoclaved sandy loam soil and fertilizer (N content: 0.76 g/pot). The plants were grown in a growth chamber at 9–15°C with a 16 hr photoperiod (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 26–28 days. Plants used for inoculation ranged from the 3 to 4 leaf stage with their growth state shown in Table 1. One hundred and ten g of inoculum was spread on the soil surface of each plastic pot. The leaves of the plants were bent on the inoculum and covered with moistened absorbent cotton. Each pot was wrapped in a polyethylen bag to keep humidity and incubated at 0.5°C in the dark for varying

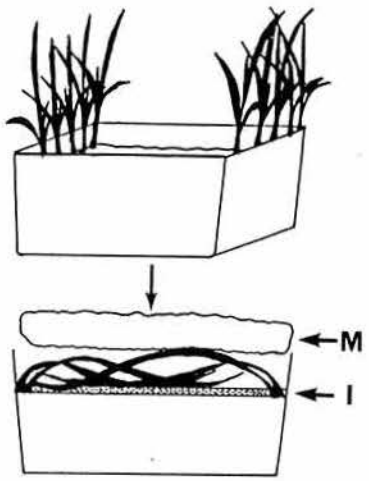

Fig. 1. Scheme showing artificial inoculation
M: Moistened absorbent cotton, I: Inoculum.

Table 1. The growth state of wheat cultivars used in snow mold chamber methods

Snow mold chamber method	Cultivar	Plant height (cm)	Number of tillers	Dry weight (g)	Dry matter (%)
A	Nōrin No. 61	36.6	1.5	0.08	10.0
	Tōsan No. 16	31.7	1.5	0.09	10.7
	Yukichabo	31.8	1.0	0.10	11.8
B (1986)	Nōrin No. 61	16.1	1.5	0.06	17.0
	Tōsan No. 16	13.3	2.5	0.07	16.5
	Yukichabo	13.2	1.7	0.06	18.2
B (1987)	Nōrin No. 61	16.4	1.6	0.07	15.2
	Tōsan No. 16	11.8	2.3	0.07	15.5
	Yukichabo	13.0	1.2	0.07	19.2

Values in the table are the means of 30 plants.

intervals (Fig. 1). At the end of the incubation period, the cotton was removed and the plants were transferred to the growth chamber. The percentages of surviving plants were recorded after a recovery period of 2 weeks.

4) Snow mold chamber method B

Wheat plants were grown outdoors from early November to early December of 1986 (for testing the resistance to *P. paddicum* and *F. nivale*) and 1987 (for *T. incarnata*) to allow plants to cold-harden under natural conditions. During these growth conditions, a minimum air temperature ranged from 0 to 8°C and a maximum air temperature ranged from 15 to 25°C. Plants used for inoculation ranged from the 3 to 4 leaf stage with their growth state shown in Table 1. The methods of artificial inoculation and assessment of resistance were the same as those of the method A.

Results

1) Field testings for snow endurance

Fig. 2 illustrates the percentages of surviving tillers of three wheat cultivars in the field after 45 to 121 days of the continuous snow cover. In this field *P. paddicum*, *P. iwayamai* and *T. incarnata* were isolated as snow mold fungi. The percentage of surviving tillers of each cultivar decreased with the continuous snow cover duration, however, there were significant differences among three cultivars in the rate of decrease of percent survival. Nōrin No. 61 exhibited 42% survival after 45 days and, almost all the plants died after 75 days. Atfer 45 days there was no difference in percent survival between Yukichabo and Tōsan No. 16, but when snow cover duration was extended to 60 days, the significant difference between two cultivars was detected. After 121 days 33% survival was

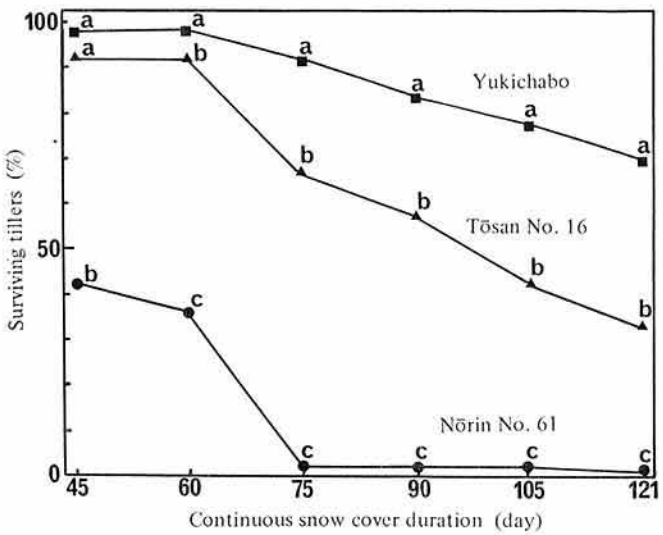

Fig. 2. Survival of wheat cultivars in the field after varying duration of continuous snow cover
Data are the means of 40 plants.
For statistical comparison, arc sin $\sqrt{\%}$ transformed values are used. Values at each snow cover duration followed by the same letter do not differ at P=0.05, using Duncan's multiple range test. The same statistical expression as above is adopted in Figs. 3 to 5.

observed in Tōsan No. 16, whereas Yukichabo exhibited 70% survival. On the basis of these results, degrees of snow endurance of Nōrin No. 61, Tōsan No. 16 and Yukichabo were assessed as low, middle and high, respectively.

2) Testings of resistance of wheat cultivars to three snow mold fungi with snow mold chamber methods

Fig. 3 illustrates the percentages of surviving plants of three wheat cultivars incubated with *P. paddicum* at 0.5°C in the dark for 1 to 5 weeks. In the method A, there were statistically significant differences in percent survival between Nōrin No. 61 and Yukichabo after 2, 3 and 4 weeks, and significant differences in percent survival between Tōsan No. 16 and Yukichabo were found after 3 and 4 weeks. However, significant differences among three cultivars were not found (Fig. 3A). In the method B the differences of percent survival among three cultivars were more distinct than in the method A (Fig. 3B). After 3 and 4 weeks, significant differences among three cultivars were observed.

The percentages of surviving plants of three cultivars incubated with *T. incarnata* for 3

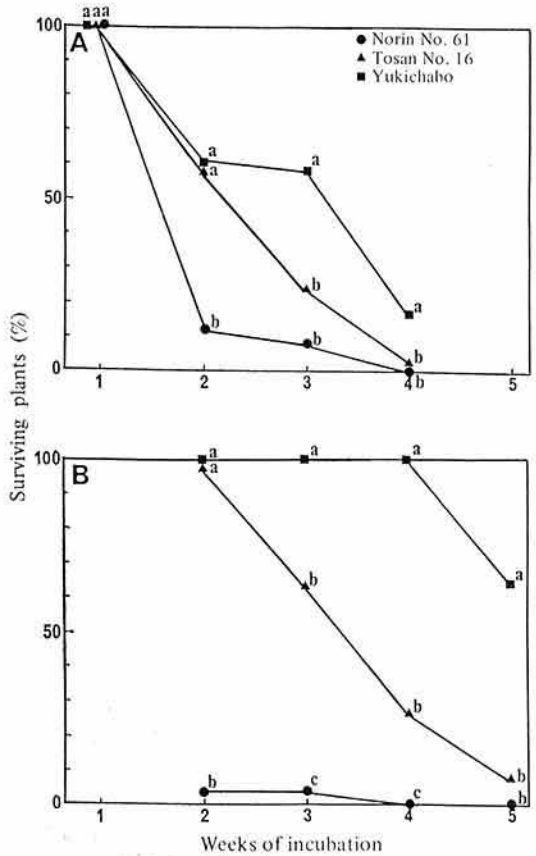

Fig. 3. Survival of wheat cultivars after incubation at 0.5℃ in the dark with *Pythium paddicum* isolate W-82-15 in snow mold chamber methods A and B
Data are the means of 5 pots (10 plants per pot).

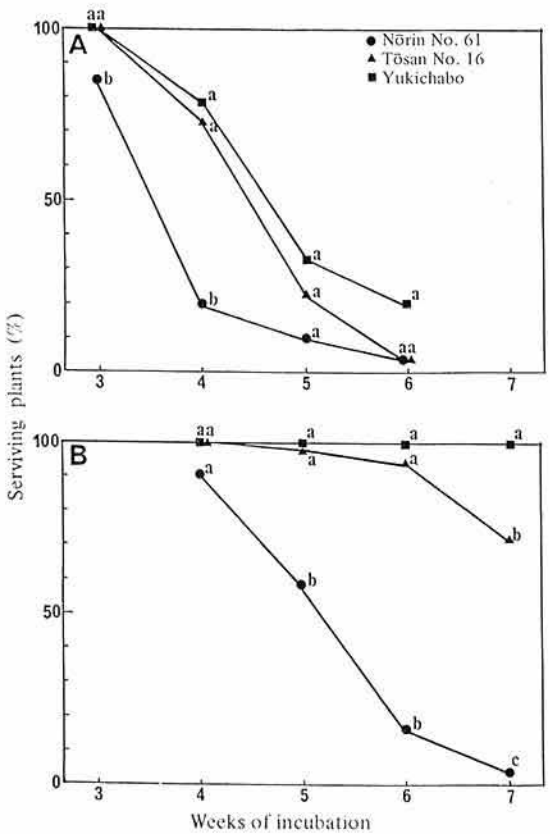

Fig. 4. Survival of wheat cultivars after incubation at 0.5℃ in the dark with *Typhula incarnata* isolate HT8301 in snow mold chamber methods A and B
Data are the means of 5 pots (10 plants per pot).

to 7 weeks are shown in Fig. 4. In the method A, there existed significant differences in percent survival between Nōrin No. 61 and Yukichabo after 3 and 4 weeks, but there were no differences between Tōsan No. 16 and Yukichabo after 3 to 6 weeks (Fig. 4A). In the method B, significant differences among three cultivars were found after 7 weeks (Fig. 4B).

Fig. 5 illustrates the percentages of surviving plants of three cultivars incubated with *F. nivale* for 1 to 4 weeks. The method A did not distinguish the difference among three cultivars in the resistance to this fungus after 1 to 3 weeks (Fig. 5A), whereas the statistically significant differences among three cultivars were found after 2 weeks in the method B (Fig. 5B).

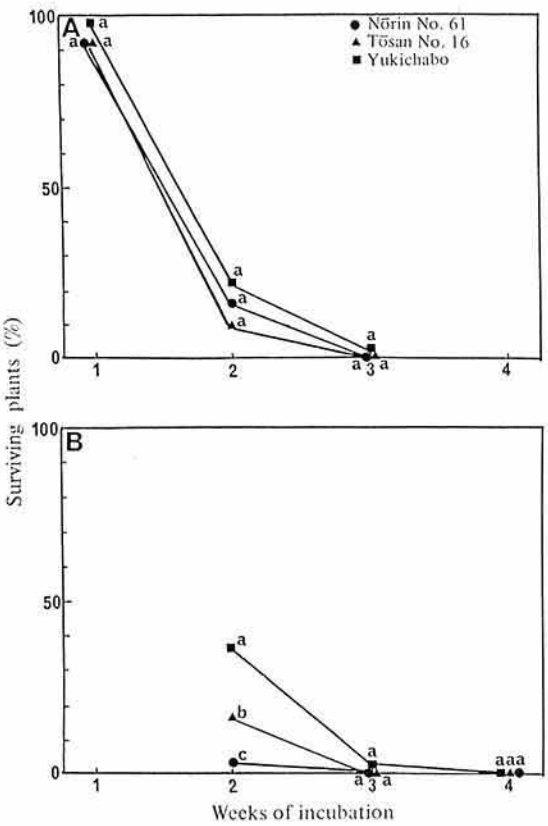

Fig. 5. Survival of wheat cultivars after incubation at 0.5℃ in the dark with *Fusarium nivale* isolate HF8301 in snow mold chamber methods A and B
Data are the means of 5 pots (10 plants per pot).

Discussion

The results of field test indicate that the rates of decrease of percent survival under the snow cover were distinctly different among low, middle and high snow endurance cultivars. The differences in snow endurance among three cultivars appear to be corresponding to those in resistance to *P. paddicum*, *P. iwayamai* and *T. incarnata* because these fungi were isolated as snow mold fungi. When the behavior of these fungi under the snow cover was observed, *Pythium* spp. had already infected the leaves of three cultivars at snow cover duration of 45 days, whereas *T. incarnata* was prevalent after 75 days, especially in the rotted leaves of Nōrin No. 61 (our unpublished observations). The results of snow mold chamber tests indicated that *P. paddicum* destroyed wheat plants at a more rapid rate than *T. incarnata*. These results suggest that *Pythium* spp. caused severe damage to wheats in the field.

The snow mold chamber method A was able to detect statistically significant differences in resistance between Nōrin No. 61 and Yukichabo, but not differences among three cultivars. In the method B the differences among three cultivars in resistance to three snow mold fungi, especially to *P. paddicum*, were distinct. The resistance of winter cereals to snow mold fungi increases under hardening conditions[1,6]. In the method B Yukichabo especially exhibited increased percent survival compared with those of Yukichabo in the method A, and therefore the degrees of resistance of three cultivars were distinguishable. This suggested that the natural hardening conditions would be the most effective on resistant wheat cultivars. However, the differences in resistance to *F. nivale* among three cultivars were less distinct than in both resistance to *P. paddicum* and *T. incarnata*. Resistance of these cultivars was assessed in the field where *Pythium* spp. and

T. incarnata were prevalent, but *F. nivale* was not. Therefore, it is reasonable to deduce that Yukichabo would not be a highly resistant cultivar to *F. nivale*.

The results of the snow mold chamber method B were correlated with the results of the field test, and this method was able to detect significant differences in resistance of wheat cultivars to *P. paddicum*, *T. incarnata* and *F. nivale* at the incubation period of 3–4, 7, and 2 weeks, respectively. This suggests that the snow mold chamber method B is a suitable technique for testing the resistance of wheat cultivars to these three snow mold fungi.

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