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New Rapid Seedling Tests for Freezing Tolerance and Resistance to *Typhula ishikariensis* Imai in Meadow Fescue (*Festuca pratensis* Huds.)

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人工気象室を利用したメドウフェスクの耐凍性および雪腐黒色小粒菌核病抵抗性検定法

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Summary

New rapid seedling tests for freezing tolerance and resistance to *Typhula ishikariensis* Imai have been developed using a controlled environment facility for all procedures. Seeds are germinated at 20/30℃ with a photoperiod of 12h for 7 days. Seedlings are grown at 15/20℃ with a photoperiod of 15h for 14 days. Hardening is then carried out at 2℃ with a photoperiod of 10h for 14 days. Freezing treatment is conducted using a programmed freezer. Plants are placed in the greenhouse and surviving plants are evaluated after four weeks. For the test of resistance to *Typhula ishikariensis*, seedlings are inoculated with the pathogen, and incubated at 3℃ for 8, 10, and 12weeks. The plants are then transferred to a chamber at 12℃ with an continuous light. Plant survival is evaluated after two weeks. The results of the new seedling test correlated significantly ($r=0.92$, $P<0.01$) with those of the conventional freezing test using crown tissues under natural hardening conditions. The results of the new seedling test also correlated significantly ($r=0.83$, $p<0.05$) with those of the conventional test of resistance to *T. ishikariensis* involving the inoculation of pathogens and incubation under natural snow cover. The new seedling tests for freezing tolerance and resistance to *T. ishikariensis* enable the evaluation of 16 cultivars/strains at once, in 63 days and 133 days, respectively. These techniques are promising as a routine work for evaluating the winter hardiness of meadow fescue.

Key words：Freezing tolerance, Meadow fescue, Phytotron, Resistance to snow mold, *Typhula ishikariensis* .

キーワード：人工気象室、耐凍性、メドウフェスク、雪腐黒色小粒菌核病菌、雪腐病抵抗性.

Introduction

The National Agricultural Research Center for the Hokkaido Region (NARCH) is responsible for breeding cool-season grasses such as orchardgrass (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis Huds* .). Our main breeding objective is the improvement of winter hardiness to the level of timothy. We usually evaluate tolerance and resistance using germplasm and plant strains grown in the field under natural hardening[5] only once a year.

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ARAKI 1) and Daido *et al*.[2] have developed simple methods for enhancing freezing tolerance and resistance to snow mold in the greenhouse. However, this method could not be practiced year-round because of the lack of the constant environment as temperature and photoperiod provided by a greenhouse. The present paper describes new rapid seedling tests for freezing tolerance and resistance to *Typhula ishikariensis* of meadow fescue using a controlled environmental facility during all procedures.

Materials and Methods

Plant materials

Plastic boxes (30×30×3.5 cm) having 49 wells were used for the establishment and treatment of plant materials. We allotted three wells to a strain. Dozens of seeds were sown in wells filled with commercial nursery soil.

Seeds were germinated at 20/30 ℃ with a photoperiod of 12h and light intensity of 3,000lx. After 7 days, the seedlings were grown at 15/20 ℃ with a photoperiod of 15h and light intensity of 20,000lx. for 14 days. Hardening treatment was then carried out at 2℃with a photoperiod of 10h and light intensity of 10,000lx. for 14 days.

Freezing tolerance

Eight cultivars of meadow fescue were used in the present study (Table 1). In the new seedling test, the bases of hardened seedlings were cut at 2.5 cm above ground level and placed in a programmed freezer (Tabai Co. LU-112). After ice nucleation at −3℃ for 8hours, the temperature was reduced by 1℃ per hour and maintained for three hours at the designed temperature (−10, −11, −12, −13 and −14℃). Samples were kept in a freezer at 2℃ overnight. After regrowth of the samples for four weeks in the greenhouse, the number of surviving plants with new shoots was counted.

The conventional freezing test was carried out according to almost the same procedures as described by MORIYAMA *et al*.[3]. Seeds were sown in plastic boxes (50×35×8 cm) filled with commercial nursery soil on 2 September 1999 in a greenhouse, and placed outdoor for hardening on 20 September. One hundred crown tissues of 3 cm length of each cultivar were randomly allocated to 5 freezing treatments [−18, −20, −22 −24 and 26℃ (except former USSR cultivars); −20, −22 −24, 26 and −28℃] on 6-8 December. Samples were placed in a programmed freezer. After ice nucleation at −3℃ for 8 hours, the temperature was reduced by 1℃ per hour. Samples were removed at the designed temperatures and kept in a freezer at 2℃ overnight. After regrowth of the samples for three weeks in the greenhouse, the number of surviving plants with new shoots was counted.

Each experiment was carried out with two replications. Freezing tolerance was expressed as LT_{50} (the median lethal temperature that kills 50% of plants).

Table 1. Plant materials used for freezing tolerance and resistance to *Typhula ishikariensis*

No.	Accessions	Origin	Freezing tolerance	Resistance to *Typhula ishikariensis*
1	Barpresto	Netherlands	○	○
2	First	Japan	○	○
3	Harusakae	Japan	○	○
4	Hokkai 9	Japan		○
5	Hokkai 13	Japan		○
6	Karelskaya	former USSR	○	
7	Merifest	Belgium	○	
8	Raduga	former USSR	○	
9	Severodvinskaya	former USSR	○	
10	Tomosakae	Japan	○	○

○: tested. blank: not tested.

Resistance to *Typhula ishikariensis*

Six cultivars/strains of meadow fescue were used in the present study (Table 1). In the new seedling test for resistance to *T. ishikariensis*, the bases of hardened seedling plants were cut into 2.5 cm lengths and inoculated with the 100mℓ pathogens in each boxes. The boxes were covered with cut leaves and wet cotton, wrapped in black vinyl bags, and incubated at 3℃. Inoculum of *T. ishikariensis*, biotype B was cultured at 8℃ for about one month in a medium of oat seeds − distilled water (3:4, v/v) and stocked in −20℃. At 8, 10, and 12 weeks after inoculation, the plants were transferred to a chamber at 12 ℃with continuous light. The plants' survival rate was evaluated after two weeks. Snow mold resistance was evaluated by means of the plants' survival rate.

The conventional test for resistance to *Typhula ishikariensis* was carried out according to almost the same procedures as those described by NAKAYAMA and ABE [5]. Seeds were sown (1 seed per 9cm²) in plastic boxes filled with commercial nursery soil on 31 August 1999 in the greenhouse, and placed outdoors for hardening on 21 September. The naturally hardened plants were inoculated on 24 December. *T. ishikariensis* (biotype B) was cultured at 8 ℃ for about one month in a medium of wheat bran − vermiculite − distilled water (10:10:9, v/v/v). The inoculum was spread onto the soil surface at the ratio of 0.06 g/cm². The plants were then covered with a snow layer of approximately 50 cm snow. At 67, 81, and 102 days after inoculation, the plants were transferred to a greenhouse. Snow mold resistance was evaluated by means of regrowth vigor scored on a scale from 1-5 with 1 being poorest and 5 being best at four weeks after transfer.

Each experiment was carried out with two replications.

Results

Figure 1 shows the freezing tolerance of 8 cultivars/strains in meadow fescue as expressed by LT_{50} by the two freezing tests. The LT_{50} in the new seedling test ranged from −9.6 (Tomosake) to −13.5 ℃ (Karelskaya), and the difference between them was 3.9 ℃. The LT_{50} in the conventional freezing test ranged from −19.9 (First) to −29.2 ℃ (Karelskaya), and the difference between them was 9.3 ℃. The LT_{50} Value in the new seedling test was higher than in the conventional test. The range of LT_{50} in the new seedling test was narrower than that of the conventional test. A high correlation was observed ($r=0.92$, $p<0.01$) between the two tests in regard to LT_{50}.

Figure 2 shows the resistance to *T. ishikariensis* of 6 cultivars/strains in meadow fescue by the two snow mold resistance tests. The plant survival rate in the new seedling test ranged from 17 (First) to 89 (Hokkai 13), with a difference between them of 72 points. Regrowth in the conventional test ranged from 3.0 (First) to 4.7 (Hokkai 13), with a difference of 1.7. A high correlation was observed ($r=0.83$, $p<0.05$) between the two tests in regard to snow mold resistance.

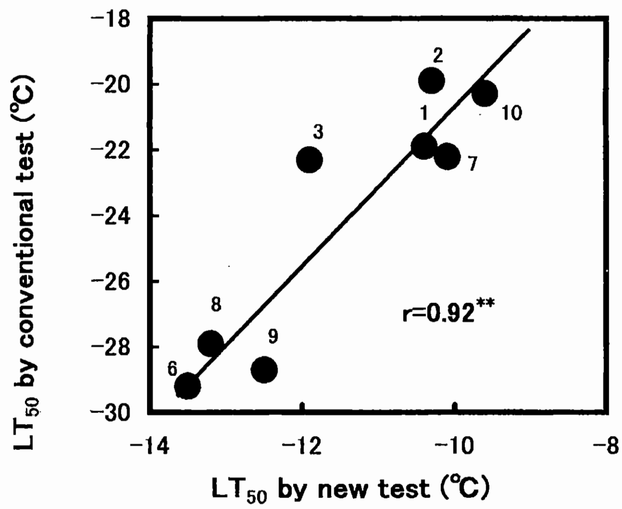

Fig. 1. Comparsion of freezing tolerance measured by new seedling test and conventional test. The numeral by each circle indicates cultivar in Table 1. LT_{50}: the lethal dose temperature that kills 50% of plants.

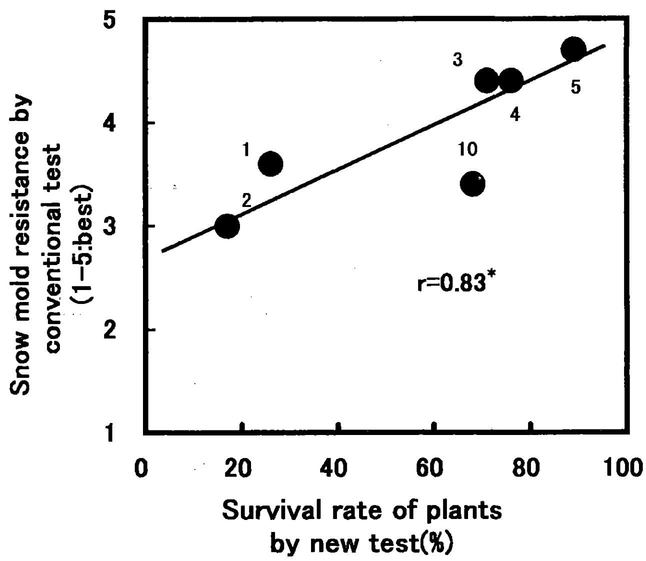

Fig. 2. Comparsion of resistance to *Typhula ishikariensis* measured by new seedling test and conventional test. The numeral by each circle indicates cultivar in Table 1.

Discussion

The results of the present new seedling test coincided with those of conventional tests of freezing tolerance and resistance to *T. ishikariensis*. The new test has the advantage year-round evaluation in the short term and does not require the use of plant crowns. However, the disadvantages of the new test include a narrower LT_{50} range, and a higher LT_{50} temperature than does the conventional test. Higher LT_{50} seems to indicated a shorter growth term and higher temperature in the hardening period. MORIYAMA *et al.* [3] reported that freezing tolerance was increased by lower temperature with a short photoperiod under natural conditions, and most species reached maximum freezing tolerance in December (before snow cover). They also reported that lower temperatures of less than 0℃ increased freezing tolerance in winter-hardy species, while it did not in non-hardy species. In general, the shortening of the plant growth period before tests for winter hardiness was to decrease freezing tolerance as well as snow mold resistance [1,4].

Though the new seedling test did not estimate actual freezing tolerance in December under natural conditions, the LT_{50} in this test correlated closely with the one in the conventional test. The new seedling test is promising as a routine technique for evaluating the freezing tolerance of germplasms of meadow fescue because of the short experiment term (63 days) and it ability to deal with many samples (16 cultivars/strains at a once) using a simple procedure (crowns do not need to be taken from plants).

In the conventional test for resistance to *T. ishikariensis* (seeds were sown on late August or early September and plants were exposed to natural hardening), orchardgrass showing higher resistance to snow mold was evaluated in terms of regrowth vigor after incubation because most of the plants survived this method[5]. The new seedling test adopted the plants' survival rate, tending to a more severe condition. Two factors appeared significant in such situation. One was the short period of plant growth before inoculation (as described above), and the other was high incubation temperature after inoculation. It has been reported that the incubation of plants inoculated with *Microdochium nivalis* at an optimum growth temperature of 15℃ could reduce the incubation period [4].

The average survival rate of plants in the new test ranged from 41% at 12 weeks to 77% at 8 weeks. The duration of incubation necessary for a 50% lethal effect was about 10 weeks in the present experiment conditions. NAKAJIMA [4] used LI_{50} as the quantitative determination of the degree of resistance (the number of incubation days when 50% of the plants are killed). Further examination is necessary to determine the incubation period (ejection frequency and interval) and temperature in order to evaluate resistance to *T. ishikariensis* expressed as LI_{50}.

In conclusion, the results of present seedling tests coincided closely with those of the conventional tests. The new technique is promising as a routine work for evaluating freezing tolerance and resistance to *T.*

ishikariensis mold in meadow fescue germplasms

References

1) ARAKI,H. (1985) Screening techniques for freezing tolerance and snow mould disease resistance in orchardgrass. *Res. Bull. Hokkaido Natl. Agric. Exp. Stn* . 143, 105-114.

2) DAIDO,H. H.ARAKI and Y.TERADA (1992) Breeding for freezing tolerance in orchardgrass and meadow fescue. *JARQ* 26,192-195.

3) MORIYAMA, M., J.ABE, M. YOSHIDA, .TSURUMI and S.NAKAYAMA (1995) Seasonal changes in freezing tolerance, moisture content and dry weight of three temperate grasses. *Grassland Science* . 41, 21-25.

4) NAKAJIMA,T. (1998) Studies on the resistance to pink snow mold in winter wheat. *Bull. Tohoku Natl. Agric. Exp. Stn* . 94, 53-98.

5) NAKAYAMA, S. and J. ABE (1996) Winter hardiness in orchardgrass (*Dactylis glomerata* L.) populations introduced from the former USSR. *Grassland Science* . 42, 235-241.
:In Japanese with English summary.

摘　　要

人工環境下で生育させた若齢な幼苗を用いることにより，１年中検定が可能なメドウフェスク用の簡便な耐凍性および雪腐病抵抗性検定法を開発した。発芽試験器（20／30℃，12時間日長）で１週間，常温室（15／20℃，15時間日長）で２週間，ハードニング室（２℃，10時間日長）で２週間生育させた幼苗を用いて耐凍性および雪腐病抵抗性検定を行った。メドウフェスク遺伝資源を供試した試験から，新しい耐凍性検定法は，従来の自然環境下で生育，ハードニングさせ12月に耐凍性検定する方法に比べて品種間差異は小さく，耐凍度は低かったが，両検定法間の相関は高かった（ｒ＝0.92，Ｐ＜0.01）。また，新しい雪腐病抵抗性検定法は，自然環境を用いる従来の方法に比べて短期間（70日前後の処理期間）で評価でき，両検定法間の相関は高かった（r＝0.83，Ｐ＜0.05）。新しい幼苗検定法は，今後メドウフェスク遺伝資源の耐冬性評価に常法として利用が可能である。