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Breeding for Freezing-Tolerance in Orchardgrass and Meadow Fescue

Hisaaki DAIDO, Hiroshi ARAKI* and Yasumichi TERADA**

Department of Forage Crop Breeding, Hokkaido National Agricultural Experiment Station (Hitsujigaoka, Sapporo, Hokkaido, 062 Japan)

Abstract

An artificial screening technique evaluating freezing tolerance in orchardgrass and meadow fescue was developed for the purpose of breeding winter-hardy cultivars. Four-leaf-stage seedlings were hardened at 3°C under 8-hr-day length for 14 days, followed by freezing treatment for 16 hr at −8 to −10°C for orchardgrass and at −12°C for meadow fescue. Visual scores for freezing damage were recorded 2 and 4 weeks after the treatment. Varietal differences in the freezing tolerance were significant. Scandinavian and Canadian cultivars of meadow fescue showed very high survival rates, which were in good agreement with the results of field trials, Effects of selection for freezing tolerance were clearly recognized among the progenies derived from the surviving seedlings. This technique was employed in the practical breeding program in Japan, under which Wasemidori for orchardgrass and Tomosakae for meadow fescue, both highly tolerant varieties to freezing, were successfully developed. The technique proposed in this paper could be applied to other temperate grasses for artificial selection.

Discipline: Grassland
Additional key words: breeding, Dactylis glomerata L., Festuca pratensis Huds.

Introduction

Orchardgrass (*Dactylis glomerata* L.) and meadow fescue (*Festuca pratensis* Huds.) are major temperate grasses in the cool region of Japan in general, particularly in Hokkaido, the northernmost island. Orchardgrass is a predominant grass species as is the case with timothy (*Phleum pratense* L.), and meadow fescue is cultivated in a mixture form with orchardgrass or timothy. Orchardgrass and meadow fescue are not as winter-hardy as timothy in eastern Hokkaido, where low temperature, thin snow cover and severe soil freezing usually take place in winter. Winter damage which is the most serious problem in this area, adversely affects grass yields in spring through the death of apical meristems, tillers and ultimately plants. Winter hardiness is therefore a major objective of the grass breeding program in Hokkaido.

The selection of superior winter-hardy plants under field conditions is not very effective due to the annual fluctuations in winter climate. To overcome these problems in breeding, an artificial selection technique for freezing tolerance first developed by Abe[1], was further improved and applied to the practical breeding program by the authors.

This paper attempts to review several conditions that affect the level of freezing tolerance of orchardgrass and meadow fescue under artificial conditions. Varietal differences in freezing tolerance of these grasses were evaluated and utilized for selection in the practical breeding program in Japan.

Present address:
* Sapporo branch, Takii Seed Company Ltd. (Sapporo, Hokkaido, 060 Japan)
** Department of Grassland, Kyushu National Agricultural Experiment Station (Nishigoshi, Kumamoto, 861-11 Japan)

Selection procedure and conditions affecting freezing tolerance

1) General procedure of selection for freezing tolerance

Plants were grown in 32 × 48 × 8 cm plastic boxes containing fertilized soil under the growth regime of 16-hr-day at 20°C and 8-hr-night at 10°C in a greenhouse. They were then transferred to a chamber for hardening and freezing (HR-256, Motoyama Manufacturing Co. Ltd.) for designated periods of time (Plate 1). Plants were hardened at 3°C under 8-hr-day length for 14 days. After the hardening treatment, the temperature was lowered to the test temperature at the rate of 2°C per hour. The seedlings were expored to freezing temperatures for 16 hr. The optimum freezing temperature for orchardgrass was −8 to −10°C, and −12°C for meadow fescue. After the freezing treatment, the chamber temperature was raised to 3°C at the rate of 2°C per hour, and thawed seedlings were transferred to the greenhouse. Plant damage was visually rated, and the survival rate was surveyed 2 to 4 weeks later.

2) Effect of seedling stage[2)]

Freezing tolerance determined by the above-stated artificial freezing method varied with the growth stages of the seedlings. In order to identify the optimum growth stage for comparing varietal differences, seedlings at 3-, 4- and 5-leaf-stages were frozen at −10°C for 16 hr after hardening. The materials used were the following three orchardgrass cultivars: Aonami, Kitamidori and Okamidori. The freezing tolerance increased with age (Fig. 1). It was recognized that the 4-leaf-stage of seedlings was most appropriate for identifying varietal differences. For individual selections at high intensity, however, the 3-leaf-stage would be more suitable in orchardgrass.

3) Effect of hardening duration

Another series of experiments was conducted to shorten the hardening period. Six orchardgrass cultivars were exposed to hardening for 7 and 14 days. Varietal differences were distinct in both treatments (Fig. 2). The survival rate of the plants hardened for 14 days was higher than that of the plants treated for 7 days, with a greater variation in the latter (Fig. 2). Fourteen days of hardening at 3°C under an 8-hr-day regime was required for hardening in orchardgrass.

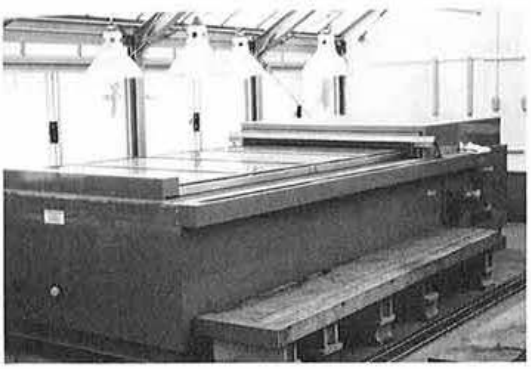

Plate 1. Chamber for hardening and freezing

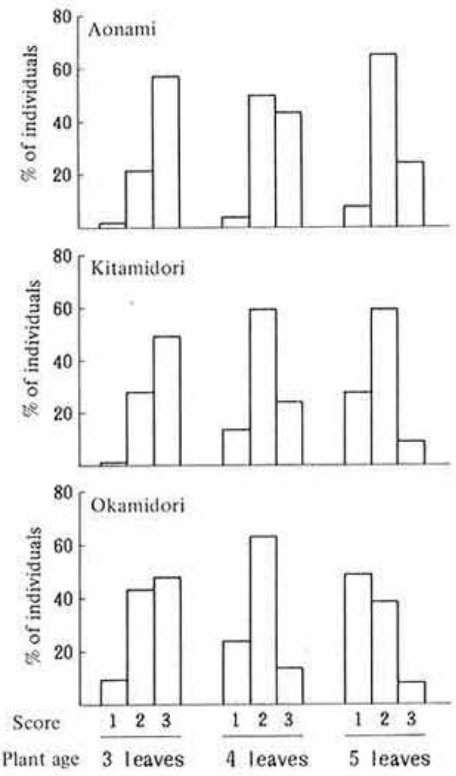

Fig. 1. Distribution of freezing tolerance in plants of different ages in orchardgrass
Freezing tolerance was scored on a scale of 1(good) to 3(dead).
Freezing temperature was −10°C.

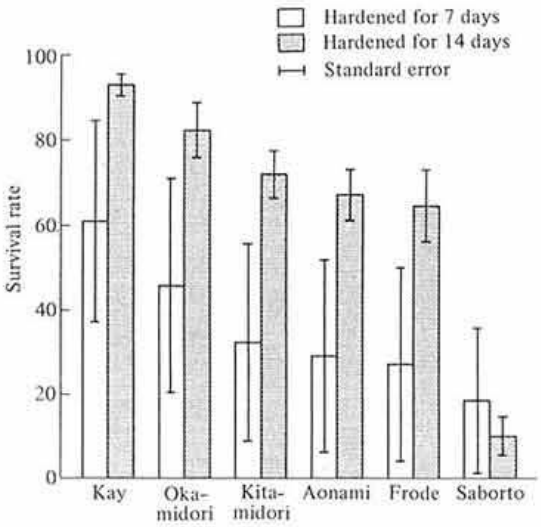

Fig. 2. Effect of hardening period on freezing in orchardgrass
Freezing temperature was −8°C.

4) Suitable conditions for artificial freezing

From the above results, it was concluded that the following conditions were suitable for comparing the freezing tolerance of breeding materials of orchardgrass. Clear differences among the strains of orchardgrass were recognized in 4-leaf-stage seedlings after the hardening treatment for 14 days at 3°C under an 8-hr-day regime followed by subsequent freezing for 16 hr at −8 to −10°C. In meadow fescue, the same conditions resulted in clear differences, except for freezing at −12°C.

As the survival rates varied significantly, the artificial freezing tests should be repeated under at least two levels of decreasing temperature (1 or 2°C) with 4–5 replications for identifying varietal differences.

Selection for freezing tolerance in meadow fescue

1) Freezing tolerance in meadow fescue cultivars[3]

Freezing tolerance was evaluated for 14 meadow fescue cultivars using the artificial freezing method described above. Plant survival and scores of freezing tolerance were compared under two levels of freezing temperature (−13 and −12°C) for 16 hr after hardening at 3°C for 14 days. As shown in Table 1, varietal differences in the freezing tolerance were significantly large, except for the survival rate after −13°C freezing. More than 70% of the tested plants of Scandinavian and Canadian cultivars, which also showed a good wintering habit under the field conditions in Sapporo[5], survived under −12°C

Table 1. Freezing tolerance in 14 meadow fescue cultivars

Cultivar	Origin	−13°C treatment		−12°C treatment	
		Score[a]	Survival[b] (%)	Score[a]	Survival[b] (%)
Salten	Norway	4.32 a[c]	17.7 n.s.	3.39 a	75.0 ab
Trader	Canada	4.34 a	15.0	3.60 ab	81.7 a
Tammisto	Finland	4.45 ab	8.3	3.63 ab	76.7 ab
Boris	Sweden	4.51 abc	13.9	3.87 bcd	53.3 c
B-14	Yugoslavia	4.67 bcd	8.3	3.67 abc	78.3 ab
Levocska	Czechoslovakia	4.68 bcd	6.8	4.49 e	26.7 de
Sequana	France	4.69 bcd	5.0	3.68 abc	68.3 b
Eridan	Germany	4.70 bcd	1.7	3.97 bcd	60.0 bc
Hokkai-1	Japan	4.72 bcd	8.3	4.02 bcd	53.3 c
First	Japan	4.78 cd	5.0	4.04 bcd	42.4 cd
Bundy	Netherlands	4.78 cd	0	4.20 de	41.0 d
Westa (4n)	Poland	4.79 cd	0	4.53 e	13.3 e
Prefest	Switzerland	4.90 de	3.3	4.10 cde	45.0 cd
Grombalia	Tunisia	5.00 e	0	4.95 f	3.3 f

a): Scored on a scale of 2 weeks after freezing; 1 (good)–5 (dead).
b): Observed 4 weeks after freezing.
c): Values within a column followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.

Table 2. Survival rates in Tammisto meadow fescue and a strain selected from Tammisto in freezing test

Cultivar and strain	Survival rate (%)		
	Exp. 1	Exp. 2	Exp. 3
Tammisto	6.8	61.0	38.3
Strain selected from Tammisto	24.6	88.0	69.8
LSD (P=0.05)	10.0	13.5	14.6

Freezing temperature was −12°C.

freezing. On the other hand, the plants from Tunisian, Polish and the Czechoslovakian cultivars survived under −12°C at rates of less than 30%.

2) Responses to selection using the proposed technique[4]

Selected plants (the selection rate was 28.9%) from Tammisto meadow fescue using the proposed screening technique were polycrossed to each other and the seeds were harvested to obtain a selected strain. To investigate the responses to selection, freezing tolerance of the strain derived and the control, Tammisto was compared using the same screening technique (Table 2). The survival rate of the strain from Tammisto was obviously higher than that of Tammisto, suggesting that freezing tolerance in seedlings is a heritable character. Larsen[7] estimated the broad sense heritability of freezing tolerance, which was 0.55 to 0.73 in orchardgrass, and 0.64 in meadow fescue. These results show that the freezing tolerance could be improved through selection. To achieve satisfactory selection effects in the breeding program, a large number of plants must be tested.

Breeding of winter-hardy cultivars

The artificial screening technique for freezing tolerance as proposed above, accompanied by field selection, was employed in the breeding program of orchardgrass and meadow fescue at the Hokkaido National Agricultural Experiment Station, Hokkaido, Japan. New winter-hardy cultivars were released: "Wasemidori" orchardgrass[8] in 1987 and "Tomosakae" meadow fescue[6] in 1988. These cultivars gave high yields in spring, as they were tolerant to winter damage or winter killing, which resulted in high spring vigor and yield. Their cold tolerance may be ascribed to the effective selection for artificial freezing in the breeding process such as individual selections and progeny tests. The artificial freezing technique in seedlings may therefore be highly effective in breeding cold-tolerant cultivars not only of orchardgrass and meadow fescue, but also of other temperate grasses.

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