# SELECTION FOR FIELD SURVIVAL INCREASES FREEZING TOLERANCE IN FESTULOLIUM

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## ABSTRACT

Festulolium (*Festulolium braunii* K.A.) is marginally adapted to the north central and northeastern USA and southern Canada. The purpose of this study was to evaluate four festulolium populations selected for field survival under harsh winter conditions for their freezing tolerance in controlled environments. Progenies of all four populations showed some improvement in freezing tolerance compared to their parents. Improvements were dependent on the temperature at which measurements were made and varied among germplasms. Improvements were manifested in both decreased plant mortality and decreased injury to surviving plants. Genetic variation for freezing tolerance appears to be a viable mechanism for enhancing field survival of festulolium.

## KEYWORDS

Breeding, selection, winterhardiness, festulolium, ryegrass, fescue, freezing, tolerance

## INTRODUCTION

The lack of adaptation of festulolium and other ryegrasses (*Lolium* spp.) to the north central and northeastern USA and southern Canada severely limits their use in pasture systems. Severe cold, dessicating winds, and lack of snow cover cause severe winter injury and mortality, limiting stand life under these extreme conditions (Casler and Walgenbach, 1990).

Natural selection for survival was practiced in festulolium by digging and intercrossing survivors from swards that had been exposed to three winters (Novy *et al*., 1995). Selection increased progeny survival as measured by ground cover and botanical composition of mixed swards. Genetic variation for winterhardiness may be caused by genetic differences in fall dormancy, winter dormancy, soluble carbohydrate concentration, freezing tolerance, and possibly other factors. The objective of this study was to quantify freezing tolerance of four festulolium populations created by natural selection for field survival and their two parental populations.

## MATERIALS AND METHODS

In April, 1986, replicated 3-m row plots of 'Elmet', 'Prior', and 'Tandem' festulolium were planted near Arlington, Ashland, Marshfield, Prairie du Sac, and Spooner, WI in mixture with alfalfa, *Medicago sativa* L. (Casler and Walgenbach, 1990). Plots were harvested three or four times per year. 'Kemal' festulolium was seeded to a 2-ha pasture near Spring Green, WI. The pasture was set stocked for 5 yr, with 2 cows plus calves (*Bos taurus*) per $ha^{-1}$.

Following three winters, mean ground cover of the festulolium row plots ranged from 10 to 85% among the five locations (Casler and Walgenbach, 1990). The most vigorous survivors were selected from the row plots, in approximately equal frequencies among cultivars and locations. All selections were transplanted to a common location and intercrossed to create the WFL-a89C population. Approximately 4000 plants of this population were transplanted near Hubbard, OR in 1991. These plants were evaluated for stem rust (Puccinia graminis Pers.) resistance in June, 1992 and 100 of the most resistant plants were intercrossed to create the WFLr population.

Following five winters, festulolium plant density in the Kemal pasture was less than 1 plant $m^{-2}$. A random sample of 160 plants were dug from the pasture, 80 representing heavily grazed plants in open areas and 80 representing plants ungrazed and relatively hidden from cattle by tall perennial broadleaf weeds. These plants were transplanted near Hubbard, OR in August, 1991. In 1992, 11 clones were selected for resistance to stem rust and intercrossed in two separate groups. The progeny populations were named W4KG (five clones from the grazed group) and W4KU (six clones from the ungrazed group).

Seed from the populations W4KG, W4KU, WFL-a89C, and WFLr and the cultivars Kemal, Elmet, Prior, and Tandem were planted in a glasshouse in June, 1995. Each population was planted in twenty replicates of a completely randomized design. Each experimental unit was a rack of 67 30-mm x 130-mm cones, each with one seedling.

When seedlings reached a 56-d age, racks were placed in a controlled-environment chamber for 35 d of cold hardening at 2°C with an 8-hr photoperiod. After hardening, four replicates of each population were placed in one of five controlled-environment chambers, maintained at one of the following five constant temperatures: -5, -7, -11, -14, or -17°C. Plants were frozen for 3 d in the dark, after which they were removed and returned to the glasshouse, which was maintained at 18/13°C day/night.

After 30 d of recovery in the glasshouse, the number of living and dead tillers were counted on each plant. Tiller survival was computed on a plant basis and plant survival was computed on an experimental unit basis. Plants were harvested at a 0.5-cm cutting height, weighed, dried for 5 d at 60°C, and re-weighed to determine dry matter concentration. Data for each freezing temperature were analyzed by analysis of variance.

## RESULTS AND DISCUSSION

There were no plants which survived the -14 or -17°C freezing treatments. Mean dry matter for these two temperatures was 904 g $kg^{-1}$ and populations did not differ in dry matter at these two temperatures. The -11°C temperature allowed for considerable discrimination among populations, with mean plant survival of 32% and mean tiller survival of 39%. The -7 and -5°C temperatures were relatively mild, with mean plant survival of 98% and mean tiller survival of 88%. The latter two temperatures only allowed a small amount of discrimination among populations. It appears that future studies of freezing tolerance in festulolium should utilize a narrower range of temperatures with smaller increments between consecutive temperature treatments.

Selection for field survival in the Kemal pasture increased plant survival of the progeny when measured at -11, -7, and -5°C (Table 1). Progress from selection was large when measured at the -11°C temperature and W4KG had greater plant survival than W4KU ($P < 0.01$). The difference between W4KG and W4KU suggests that plants growing in relatively open areas of the pasture, where cattle had ready access to their herbage, were under greater selection pressure compared to those which were protected by perennial weeds. These results underscore the importance of interacting environmental factors in determining the ability of marginally hardy herbage plants to survive harsh environmental conditions (Charles et al., 1975).

Selection for survival in the replicated row plots of Elmet, Prior, and Tandem was also successful at increasing plant survival, but only when measured at -11°C (Table 1). The progress made in this germplasm appeared to be considerably less than that observed in Kemal. This appears to be due to the large differences in plant survival among the parental cultivars at -11°C (Elmet = 6%, Prior = 83%, and Tandem = 11%). The selected populations represented dramatic improvements in plant survival at -11°C over Elmet and Tandem, but not Prior. This was unexpected, because field survival of these three cultivars, following two winters at four locations ranged from 52 to 60% and were not significantly different (Casler and Walgenbach, 1990). Thus, the superior plant survival of Prior at this temperature did not confer superior field survival, compared to Elmet and Tandem. This is similar to results of Hides (1979) in which dramatic improvements in seedling cold tolerance led to highly variable and unpredictable changes in field survival. The field survival of Elmet and Tandem, observed by Casler and Walgenbach (1990) appears to be due to factors other than freezing tolerance.

Differences in tiller survival were similar to those for plant survival (Table 1). Selection progress was evident in tiller survival at all three of the warmest freezing temperatures for the Kemal selections and at -11 and -7°C for the Elmet, Prior, and Tandem selections. These changes indicate that improved freezing tolerance can be manifested in two ways - reduced plant mortality and reduced injury (tiller mortality) of surviving plants - and that these two responses are positively correlated with each other.

Dry matter concentration was determined as a measure of living tissue, on the assumption that the amount of living tissue was highly correlated with the concentration of moisture in the plants. Results for dry matter confirmed the combined results for plant and tiller survival, that selection for field survival increased freezing tolerance in both selection experiments, and that progress was greater for the grazed Kemal vs. the ungrazed Kemal (Table 1). Genetic variation for freezing tolerance is one mechanism by which adaptation of festulolium can be improved. Freezing tolerance can be increased in festulolium by field selection under either grazing or hay management. Although many interacting environmental factors combine to determine field survival of festulolium plants in harsh environments, selection of survivors can be a useful means of improving adaptation.

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**Table 1**
Mean plant survival, tiller survival, and dry matter concentration for two festulolium populations (Original K = Kemal and Original EPT = Elmet, Prior and Tandem) and four populations selected for field survival.

| Population | Freezing temperature (°C) -11 | -7 | -5 |
|---|---|---|---|
| | Plant survival (%) | | |
| Original K | 3 | 96 | 94 |
| W4KG | 58** | 98+ | 100+ |
| W4KU | 34** | 97 | 100+ |
| Original EPT | 33 | 98 | 97 |
| WFLr | 47** | 99 | 97 |
| WFL-a89C | 63** | 99 | 100 |
| | Tiller survival (%) | | |
| Original K | 25 | 70 | 82 |
| W4KG | 45** | 87** | 92** |
| W4KU | 35+ | 91** | 96** |
| Original EPT | 41 | 81 | 94 |
| WFLr | 43 | 87* | 96 |
| WFL-a89C | 55* | 81 | 97 |
| | Dry matter (g $kg^{-1}$) | | |
| Original K | 915 | 440 | 342 |
| W4KG | 724** | 375** | 327 |
| W4KU | 867 | 374** | 308+ |
| Original EPT | 841 | 412 | 328 |
| WFLr | 771* | 367* | 302 |
| WFL-a89C | 696** | 421 | 283 |

+, *, ** Mean significantly different from the respective original mean at the 0.10, 0.05, or 0.01 level.