Genotypic Variation for Cold Tolerance in Winter and Facultative Barley

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ABSTRACT

Improved winter survival of barley (*Hordeum vulgare* L.) may be achieved by developing genotypes that maintain an acceptable level of cold tolerance despite fluctuations in air and soil temperature. The cold tolerance of eight winter and facultative barley cultivars, a spring barley and a winter wheat (*Triticum aestivum* L.) was measured in controlled freeze tests of field-grown material. The temperature lethal to 50% of each genotype population (LT_{50}) was calculated from survival data. The LT_{50} values for each genotype were determined at multiple sampling dates at two locations over a 2-yr period. Sampling dates were considered environments and these data were used to identify genotypes showing stable, or full-season, cold tolerance. Genotypes were grouped according to patterns of comparable LT_{50} response across the six environments. The significant genotype group × environment interaction detected in the analysis of groups and environments was due to changes in magnitude of response rather than changes of rank. Averaged across all environments. 'Norstar' winter wheat was significantly more cold tolerant than the barley germplasm, and there was little variation for cold tolerance among winter barley genotypes. Winter barley cultivars were more cold tolerant than facultative cultivars. Two joint regression stability parameters, the regression coefficient (b) and squared deviations from regression (s_d^2), revealed genotypic variation for full-season cold tolerance.

In many production environments, winter cereals must be able to withstand relatively large fluctuations in ambient air temperature, soil temperature, and snow cover. Winterhardiness is a complex character determined by a genotype's resistance to both biotic and abiotic stresses. Selection for winterhardiness is complicated not only by genotype × environment interaction, but also by the relative magnitude of stresses contributing to winter injury. A fundamental component of winterhardiness is cold tolerance (Olien, 1967).

Field survival may not be an optimum measure of cold tolerance because of the infrequent occurrence of test winters that allow for effective discrimination among genotypes (Fowler and Gusta, 1979). Of the various laboratory and controlled environment tests used to measure cold tolerance (Stushnoff et al., 1984), controlled freeze testing to determine the LT_{50} has proven to be the most repeatable and efficient (McIntyre et al., 1988). This technique has been used extensively to characterize genotypic variation for cold tolerance in triticale (× *Triticosecale* Wittm.) and wheat (Fowler and Gusta, 1979; McIntyre et al., 1988) and to study the genetics of cold tolerance in wheat (Brule-Babel and Fowler, 1988; Lazar et al., 1988; Sutka, 1981).

Many environmental factors and their interactions determine plant cold tolerance, and winter cereal genotypes are known to vary in their level and duration of cold tolerance (Chen et al., 1893). A desirable genotype would be one that maintains an acceptable level of cold tolerance despite fluctuations in environmental conditions. Such a genotype would show full-season, or stable, cold tolerance. The significant genotype × environment interaction found in analyses of LT_{50} data based on field-grown material sampled at various points during the winter growing season has been attributed to the complex genetic basis of cold tolerance traits (McKersie and Hunt, 1987). The complexity of genotype × environment interaction typically remains unresolved, and cold tolerance data are often interpreted in terms of average performance across environments.

Olien (1964) used barley as the model system for his pioneering work in cold stress physiology, and Livingston et al. (1989) recently described genotypic variation for cold tolerance among three barley

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Abbreviations: ANOVA, analysis of variance; LT_{50}, temperature lethal to 50% of a genotype population.

genotypes. There are no reports describing genotypic variation for cold tolerance in a diverse array of contemporary winter and facultative barley germplasm, nor has there been any attempt to characterize full-season cold tolerance in such material.

Our objectives were to (i) characterize genotypic variation for cold tolerance, as measured by average LT_{50}, in an array of winter and facultative barley germplasm grown in diverse field environments, (ii) determine the basis of genotype × environment interaction in the expression of cold tolerance in this germplasm, and (iii) characterize genotypic variation for full-season cold tolerance, as measured by the stability of cold tolerance expression in diverse field environments.

MATERIALS AND METHODS

Eight winter and two facultative barleys of diverse geographic origin, a spring barley, 'Morex', and a winter wheat, Norstar (Table 1), were hand planted in field plots at the Hyslop and Sherman Experiment Stations, at Corvallis and Moro, OR, respectively, in both the fall of 1987 and 1988. Planting dates, rates, and inputs were in accordance with recommended practices for each location (data available upon request).

Each two row, 3-m plot was separated by a single 3-m row of Norstar winter wheat. A three-replicate randomized complete-block design was used at each location. Data presented in this report were generated from plants sampled at each of three dates at Corvallis in 1987–1988: 20 Dec., 23 Jan., and 24 Feb. In 1987–1988, drought conditions led to the loss of all plants at the Moro location. In 1988–1989, plant material was sampled on 28 Jan. and 17 Feb. at Corvallis and on 16 Dec. and 19 Jan. at Moro.

At each of the seven sampling dates, plants were washed and trimmed to 4 to 5 cm above, and to 0.2 to 0.3 cm below, the base of the crown. Crowns from each replicate were bundled into groups of 10, placed in stainless steel containers, covered with wet sand, and maintained at 0 °C for 12 h. One container, the control, was maintained at 0 °C. Ice crystals were then added to each of the remaining containers to initiate freezing. After 24 h at −2 °C, these containers were placed in a programmable freezer and subjected to a 3 °C/h drop in temperature. The first season (1987–1988), containers with three replicates of the 12 genotypes (10 plants per genotype per replicate) were removed at seven temperatures: −3, −6, −9, −12, −15, −18, and −20 °C. The second season (1988–1989), containers with two replicates of the 12 genotypes (10 plants per genotype per replicate) were removed at −4, −8, −12, and −17 °C. After freezing, samples were thawed for 15 h at 4 °C.

Crowns were replanted into a 1:1 mixture of vermiculite and greenhouse soil mix and maintained in a greenhouse with a 16-h photoperiod and daily temperature varying between 16 and 18 °C. Regrowth was scored four to five weeks after transplanting. Plants showing vigorous shoot and root growth were considered to have survived the freezing stress. Percent survival data were transformed by arcsine, and the LT_{50} for each genotype at each sampling date was computed as described by Pomeroy and Fowler (1973). In the subsquent discussion, LT_{50}, as determined from survival following the freeze test, is used synonymously with cold tolerance.

Data were analyzed using the Statistical Analysis System (SAS Institute, 1987), using the General Linear Models option in cases of imbalance. Genotypes were grouped using a maximum likelihood estimation of normal distribution mixture models program (McLachlan and Basford, 1988). Subsequent analyses of variance were performed using group means. Two joint regression measures of stability (Eberhart and Russell, 1966) were employed to identify genotypes with full-season cold tolerance.

Table 1. Genotypic differences associated with cold tolerance in a winter wheat and an array of barley germplasm evaluated in six environments.

Genotype	Origin	Growth habit	LT_{50}	LT_{50}† group	Stability parameters‡	
					b	s_d^2
			°C			
Morex	USA	Spring	−1.3	1	0.36	1.18
Robur	France	Winter	−7.3	2	1.02	1.41
Steptoe	USA	Facultative	−6.9	2	1.17	2.50
Tokak	Turkey	Facultative	−5.5	2	1.83	2.76
(Group mean)			−6.6			
Dicktoo	USA	Winter	−9.2	3	1.56	1.25
Plaisant	France	Winter	−9.8	3	0.90	1.56
Scio	USA	Winter	−8.3	3	1.48	1.15
(Group mean)			−9.1			
Cyclone	USSR	Winter	−10.0	4	0.80	0.58
Kearney	USA	Winter	−10.1	4	0.65	0.68
(Group mean)			−10.1			
Novator	USSR	Winter	−10.5	5	0.71	0.94
Schuyler	USA	Winter	−10.3	5	0.60	0.72
(Group mean)			−10.4			
Norstar	Canada	Winter Wheat	−11.8	6	0.91	2.08

† Temperature lethal to 50% of a genotype population (LT_{50}). Genotypes were grouped according to patterns of comparable LT_{50} response across the six environments.

‡ Two joint regression stability parameters, the regression coefficient (b) and squared deviations from regression (s_d^2).

RESULTS AND DISCUSSION

Despite differences in soil temperature, air temperature, and snow cover across years, locations, and sampling dates (Table 2), the 100% survival of all winter and facultative genotypes in the field underscored the need for alternative procedures to evaluate cold tolerance. Despite the stresses associated with excavation, transport, and preparation of field-grown material, control plants (those maintained at 0 °C) showed complete survival. We are thus confident that differential regrowth is a measure of stresses imposed during the freeze test.

Initial analyses of variance of the LT_{50} data were computed separately for the first and second year data. Genotypes were considered a fixed effect, while sampling dates and locations were considered random effects. In view of our objective to understand the basis of genotype × environment interaction, we used the following ANOVA-guided rationale to identify six distinct environments.

In the 1987 to 1988 one-location analysis, genotype, sampling date, and genotype × sampling date effects were highly significant ($P = 0.01$). Therefore, each of the three sampling dates was considered representative of a unique combination of environmental effects causing differential expression of cold tolerance. These three sampling dates defined Environments 1, 2, and 3. In the 1988–1989 two-location analysis, genotype and genotype × location effects were significant ($P = 0.05$). Separate analyses of variance were then conducted for each location. At Corvallis, genotype, sampling date, and genotype × sampling date effects were again significant ($P = 0.05$), leading to the resolution of an additional two environments (Environments 4

Table 2. Ambient and soil temperatures at sampling and since planting at Moro and Corvallis, OR, during the winter periods of 1987–1988 and 1988–1989.

Sampling date	Location	Environment	LT_{50} Group						Temperature							Snow‡
									On sampling date				Since planting			
									Ambient air		Soil at 10 cm		Ambient air		Soil at 10 cm	
			1	2	3	4	5	6	°C							
									Max.	Max.	Max.	Min.	Max.	Min.	Max.	Min.
20 Dec. 87	Corvallis	1	−3.5	−10.0	−10.9	−11.2	−10.7	−13.0	1.7	−0.6	3.3	2.8	22.8	−1.1	16.1	1.7	1
23 Jan. 88	Corvallis	2	−0.8	−5.9	−7.9	−9.7	−9.1	−10.1	6.7	1.7	5.0	4.4	22.8	−5.0	16.1	1.1	3
24 Feb. 88	Corvallis	3	−0.5	−3.4	−10.2	−9.3	−10.8	−11.2	15.5	−1.7	8.9	3.9	22.8	−5.0	16.1	1.1	4
28 Jan. 89	Corvallis	4	−2.0	−9.7	−10.7	−10.6	−11.9	−11.0	8.9	−1.7	6.7	4.4	23.3	−5.0	19.4	0.6	5
17 Feb. 89	Corvallis	5	−1.0	−4.0	−4.4	−7.9	−8.4	−9.5	9.4	5.0	5.6	3.9	23.3	−13.9	19.4	0.6	5
16 Dec. 88	Moro	6§	−0.1	−6.7	−10.7	−11.7	−11.3	−16.2	1.7	−7.8	2.2	2.2	28.9	−8.3	22.8	2.2	0
19 Jan. 89	Moro	6§							12.8	−2.2	6.7	3.9	28.9	−10.0	22.8	0.6	5

† Temperature that was lethal to 50% of a genotype population.
‡ Total number of days since planting with measurable snow on the ground.
§ Environment 6 is comprised of the mean of the two sampling dates.

and 5). Finally, because there was no genotype × sampling date interaction at Moro, Environment 6 was derived from main-effect means of the two sampling dates at this location. Subsequent analyses were based on genotype performance in these six environments.

There was an overall trend for genotypes to show maximum cold tolerance in Environments 1, 4, and 6, and the least cold tolerance in Environments 2 and 5 (Table 2). Exceptions to this generalization led to significant genotype × environment interaction. McKersie and Hunt (1987) attributed significant genotype × environment interaction in the expression of three distinct cold tolerances in winter wheat to complex genetic mechanisms. While plausible, this hypothesis is of little assistance in designing cold tolerance selection criteria.

With the objective of identifying patterns of comparable LT_{50} response so that genotype × environment interaction could be studied on the basis of group responses to environments rather than the responses of individual genotypes, we used the mixture model analysis procedure of McLachlan and Bashford (1988). Based on log likelihood ratio testing (McLachlan and Basford, 1988), six groups were identified (Table 1).

In the subsequent analysis of variance of groups and environments, the group, environment, and group × environment effects were significant sources of variation. Crossover interactions were not significant, according to the test of Azzalini and Cox (1984). Therefore, the interaction of groups and environments was due to changes in magnitude of response rather than changes in rank. Primary sources of interaction were (i) the poor performance of Group 2 genotypes relative to Groups 3 to 6 in Environment 3, and (ii) the high level of cold tolerance of Norstar wheat in Environment 6 (Table 2). This grouping approach may be a useful in breeding for cold tolerance. First, a group of genotypes is identified that has acceptable cold tolerance across an array of environments. Subsequently, genotypes within the target group can be selected that meet other selection criteria.

Partitioning of the highly significant group sums of squares into mutually orthogonal contrasts revealed that, in terms of mean LT_{50}, the winter and facultative barley genotypes were less cold tolerant than Norstar winter wheat ($P = 0.05$). There is modest variation for cold tolerance in this diverse array of winter barley germplasm (Groups 3, 4, and 5). The facultative genotypes (Group 2) and the spring barley Morex were less cold tolerant than the true winter types. The exception to the last generalization was 'Robur', a winter barley, which was grouped with the facultative habit cultivars in Group 2.

In view of our objective of characterizing full-season cold tolerance, we employed stability parameters in an attempt to identify genotypes that achieved and maintained a level of cold tolerance in a diverse set of environments. Stability analyses were based on genotype, not group, means. We found that weather data (Table 2) was of little assistance in explaining genotypic variation and genotype × environment interaction for cold tolerance. We therefore relied on the average LT_{50} of all genotypes in an environment as the index of that environment.

Becker and Leon (1988) have made a useful distinction between static and dynamic concepts of stability: cold tolerance is an example of a trait where an ideal genotype exhibits static stability, possessing "an unchanged performance regardless of any variation of the environmental conditions." Finlay and Wilkinson (1963) defined such genotypes as having regression coefficients (b) <1.0 and described them as having "above average stability." Yield is an example of a trait where an ideal genotype exhibits dynamic stability. Such genotypes follow a predictable response to environments and have "no deviation from this response to environments" (Becker and Leon, 1988). Eberhart and Russell (1966) previously defined a stable genotype as having $b = 1.0$ and squared deviations from regression (s_d^2) of 0.

In terms of static stability, a genotype with full-season cold tolerance should have $b = 0$ and $s_d^2 = 0$. The most cold tolerant barley genotypes (Groups 4 and 5) came closest to meeting these criteria. The cultivars Kearney and Schuyler had the lowest slopes of the winter barleys, (0.60 and 0.65, respectively) and also had some of the lowest s_d^2 values (Table 1). Kearney was released in 1952 and Schuyler in 1968 (Baum et al., 1985). Although subsequent winter barley releases

exceed these cultivars in agronomic performance, Kearney and Schuyler may represent promising sources of full-season cold tolerance. 'Novator' (Group 5) and 'Cyclone' (Group 4) were developed at the Krasnodar Research Institute of Agriculture, Krasnodar, USSR, using controlled freeze testing screening procedures and are agronomically competitive both in the U.S. Pacific Northwest and in their area of adaptation in the Russian Republic of the USSR. Both regions are subject to irregular snow cover and fluctuating winter temperatures. Given the diverse origin and presumably diverse genetic backgrounds of the four winter barley genotypes, some progress from selection for cold tolerance among their cross progeny might be expected.

Of the Group 3 winter barleys, the performance of 'Dicktoo' is of particular interest. Dicktoo, with the highest slope (1.56) of the winter barleys, and a high s_d^2 compared with the Groups 4 and 5 genotypes, was very unstable in its expression of cold tolerance. Dicktoo, released simultaneously with Kearney, is a cold-tolerant check in the Uniform Barley Winterhardiness Nursery (UBWHN). In the 1989 UBWHN, Dicktoo had the highest survival (82.0%) of any winter barley (USDA-ARS, 1989). In our experiments, Dicktoo was grouped with 'Plaisant' and 'Scio'. Plaisant is characterized as moderately frost resistant in Europe (Pierce, 1990).

Cultivars Dicktoo, Scio, and the Group 3 facultative barleys Tokak and Steptoe seem unstable, by the static definition of stability, perhaps because they are responsive to conditions favoring cold tolerance expression. These genotypes may be sensitive to environmental fluctuations and do not achieve and maintain a consistent level of cold tolerance across a range of environmental conditions. High survival ratings in long term barley winter-hardiness trials may be a measure of Dicktoo's expression of cold tolerance in uniformly cold environments that favor trait expression. For example, in Environment 6, which had the lowest environmental index for LT_{50}, Dicktoo had the lowest LT_{50} of any barley genotype (−11.8 °C).

Of the Group 2 genotypes, Steptoe and Tokak are both fall- and spring-planted in the U.S. Pacific Northwest and the Anatolian Plateau of Turkey, their respective areas of adaptation, and are considered facultative genotypes. The overall LT_{50} values of these genotypes are low, but the fact that they were significantly more cold tolerant than the spring barley Morex provides further confirmation for the hypothesis that a vernalization requirement is not a prerequisite for a level of cold tolerance (Doll et al., 1989). Tokak was particularly notable for having the highest slope and the highest s_d^2 of any genotype.

The inclusion of Norstar provides a baseline, allowing for comparison of our data with previous work. McKersie and Hunt (1987) reported a LT_{50} of −12.8 °C for Norstar and noted that this variety was considerably less cold tolerant in their experiments in eastern Canada than in western Canada. Norstar was relatively unstable, with a slope near 1 and a s_d^2 exceeded only by the two facultative barleys.

The genetic and physiological basis of full-season cold tolerance merits further study under controlled environment conditions. Improved winter survival of barley may be achieved through the systematic intermating of genotypes with acceptable, stable levels of cold tolerance. For breeding purposes, screening of field-grown cross progeny in controlled freeze tests, followed by mixture model analysis grouping, may give a positive selection response. To this end, we have developed, and are currently characterizing, a winter barley doubled haploid recurrent selection population.

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