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Improvement of Field Screening for Winter Hardiness of Barley

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ABSTRACT : Low temperature is one of the most severe abiotic stress factors limiting growth, productivity and distribution of winter cereals. Reliable field screening method, which can detect small differences in winter survival, is important for the effective selection and development of plants to identify superior cold tolerant winter cereal genotypes. This study was undertaken to provide improved screening method of winter hardiness in the field by increasing the accuracy in evaluating winter hardiness of barley (*Hordeum vulgare* L.). We introduced furrow in field screening of winter survival. By sowing the plants at the ridge and base, we could minimize the effect of topographic variation in a field by giving higher and lower level of stress at the same time. This method could be used by breeders to conduct accurate evaluation of winter hardiness by selecting the better treatment, which shows close to normal distribution, among the winter survival rate from the ridge, base and mean survival rate of the two in a screening field.

Keywords: winter hardiness, winter survival, field test, barley

One basic condition for the reliable production of winter cereals is to resist extreme winter climatic conditions. There are a number of factors that contribute to winterkill. The main factors causing winterkill (alone or in combination) are related to low temperature, inadequate hardening, due to late emergence in autumn or a sudden drop in temperature (Reynold *et al.*, 2001), long periods of cold-induced desiccation (Gusta *et al.*, 1997a), prolonged periods of low sub-zero temperatures, alternate freezing and thawing, which causes increased injury from ice crystal growth with each freeze (Olien, 1969), drought (Andrews & Pomeroy, 1977), the state of development of the plant (Grébner, 1929; Lelley & Mándy, 1963).

Freezing tolerance, the ability of plant to survive form freezing temperatures without damage, both as seedling and in the early stages of growth and development, is the most important of winter hardiness (Levitt, 1956; Olien, 1967; Steponkus, 1978; Fowler *et al.*, 1997). A large number of different methods have been used for studying the cold tolerance. To get precise genetic studies, experiments were carried out under artificial conditions, where the climatic factors of growth, hardening, freezing, and regrowth after freezing were controlled (Sutka, 1981; Tischner *et al.*, 1997). Controlled environment should allow rigid control of freezing conditions. Evaluating winter survival in artificial conditions is precise and reproducible, but these methods also have problems including limiting space. In addition, the main disadvantage of this approach is the lack of control over the hardening level The result of Jenkins & Roffey (1974) showed that there was a significant interaction between cultivar and hardening-duration in freezing response. Fowler *et al.* (1981, 1993) concluded that field trials usually provide more repeatable results and have lower experimental errors than direct freezing test.

Winter hardiness can be measured in field experiments. Field evaluation of winter hardiness allows large-scale, inexpensive characterization of breeding materials against the full range of factors affecting winter survival, whereas controlled freeze test measures only low temperature tolerance. For this reason, most breeding programs favor field-testing to measure winter survival (Fowler *et al.*, 1993). On the other hands, a major disadvantage of field-testing is the infrequent occurrence of conditions that result in different winterkill. Levitt (1972) defined a 'test winter', or 'differential winter', as a winter severe enough to kill the most tender plants and damage those of intermediate hardiness to various degrees. Winters with good differentiation among genotype for their winter hardiness are infrequent, even in areas that require a high level of cold tolerance (Levitt, 1956; Reynold *et al.*, 2001).

A simple and improved method for evaluation of winter hardiness is needed to cope with these problems. The objective of this study was to increase the accuracy in evaluating winter hardiness in the field by regulating winter stress in a given screening nursery. We introduced furrow to decrease the fluctuation in temperature and soil moisture by planting barley on the ridge and base of the furrow, simultaneously. While plants sowed at the ridge of the furrow might be more stressed by relatively low temperature, insufficient soil

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moisture, and soil erosion than the plants sowed at the base, plants on the base of furrow could decrease winterkill, from which soil moisture and snow depth could be maintained.

MATERIALS AND METHODS

Screening of winter survival

This study was conducted during the 2000/2001 winter season at two regions, Sangju (North latitude: 36.26°, Soil: Fine loamy, mixed, mesic family of Fluvaquentic Endoaquepts) and Daegu (North latitude : 35.53°, Soil: Fine, mixed, mesic family of Aquic Hapludalfs), in Republic of Korea. Seeds of 110 barley varieties, 61 six-rowed and 49 two-rowed, were tested. Ridge (60 cm wide by 3 m long and 25 cm high) was formed by Multi-Purpose Cultivator (Asia Agricultural Machinery Co., Korea). The side-slope degree of the ridge was about 40°. Barley seed was sown by hands, about 1cm below the soil surface, 10 cm apart in a row in the furrows of the base and ridge. A schematic diagram of the system with crop configuration is presented in Fig. 1.

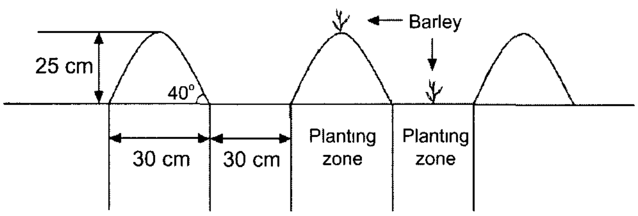

Fig. 1. Schematic diagram for field screening of winter hardiness.

The experiment was laid out in a randomized block design with three replications in two locations, Sangju and Daegu. Barley was sown on October 14 and October 20 at Sangju and Daegu, respectively. The winter survival rate of barley was measured as the difference between the emerged and final plant stand on March 14, 2001.

Statistical analysis

Experiment was conducted randomized complete block design. Statistical analysis was conducted by the PROC ANOVA procedure in Statistical Analysis System (SAS). Difference among treatment was evaluated for significance by the least significance difference (LSD, p=0.05).

RESULTS AND DISCUSSION

Air temperature of January is the most severe in the Korea. It is used as the main criteria in determining the barley production zone. Air temperature at Sangju and Daegu in Average, minimum and maximum temperatures of January in the two locations were presented in Table 1. Air temperatures of January in the two locations were slightly lower than those of the mean air temperatures at 5-year of return

Table 1. Average, minimum, and maximum temperature of January at two locations, Sangju and Daegu.

Year	Temperature (°C)					
	Sangju (°C)			Daegu (°C)		
	Average	Minimum	Maximum	Average	Minimum	Maximum
2001 (B)	-5.3	-10.0	-0.6	0.7	-2.9	4.7
1996-2000 (A)	-4.4	-9.8	1.4	1.0	3.3	5.9
Difference (A-B)	-0.9	-0.2	-2.0	-0.3	-0.4	-1.2

Table 2. Analysis of variance for winter survival of barley.

Source of variance	DF	Sums of square	Mean square	F Value†
Location (L)	1	27502.8	27502.8	169.40**
Replication	2	808.8	404.4	2.49^{ns}
Furrow condition (F)	1	1602403.0	801201.5	4934.86**
Variety (V)	109	279740.3	2566.4	15.81**
(L)×(F)	2	5766.7	2883.3	17.76**
(L)×(V)	109	43295.3	397.2	2.45**
(L)×(V)×(F)	436	112313.5	257.6	1.59**
Error	1318	213984.4	162.3	
Total	1979	2285815.1		

** : significant at P=0.01, ns : not significant.

Table 3. Effect of location and furrow condition on winter survival of barley.

Source of variance		Winter survival (%)†	SD
Location	Daegu (A)	60.1a	35.0
	Sangju (B)	52.6b	32.4
Furrow condition	Base (C)	91.2a	12.1
	Ridge (D)	21.6c	23.8
	Mean (E)	56.4b	15.6
Location×furrow condition	A×C	97.1a	7.8
	A×D	23.2e	26.1
	A×E	60.1c	14 6
	B×C	85.3b	15.0
	B×D	19.9f	23.4
	B×E	52.7d	15.6

†Values with different letter are significantly different at P=0.05.

period (1996 - 2000).

The results of analysis of variance for the winter survival of the 110 varieties are presented in Table 2. The effects of the source of the variance including location, furrow condition, variety were significantly different. There was no significant difference between replications. Interactions of location × furrow condition, location × variety, location × variety × furrow condition were significantly different.

The effects of the furrow condition and location to winter survival were presented in Table 3. Means of winter survival rate of the 110 barley varieties at Sangju and Daegu were 52.6% and 60.1%, respectively. When the varieties were planted at base and ridge of the furrow, the means of winter survival rate was 91.2%, and 21.6%, respectively. The mean survival rate between base and ridge of the furrow was 56.4%. When the varieties were tested at Sangju, average winter survival rates at base and ridge were 97.1 and 23.2%, respectively. When the varieties were tested at Daegu, average winter survival rates at base and ridge were 85.3 and 19.9%, respectively.

Results showed that winter survival rate of barley varieties tested at Sangju was significantly lower than that of the Daegu by the different environmental conditions including low temperature. Plants sowed on the base or ridge of furrow represented wide range of winter survival. Plants sowed on the ridge of furrow were significantly more stressed than those of the base. Plants on the base of furrow could be protected from low temperature desiccation by furrow and deep snow cover.

The effects of furrow in the two different locations were presented in Fig. 2. When seeds were sowed at ridge or base of the furrow, the winter survival rate of each variety showed biased distribution closed to zero and 100% of winter survival, respectively. Mean survival rate of ridge and

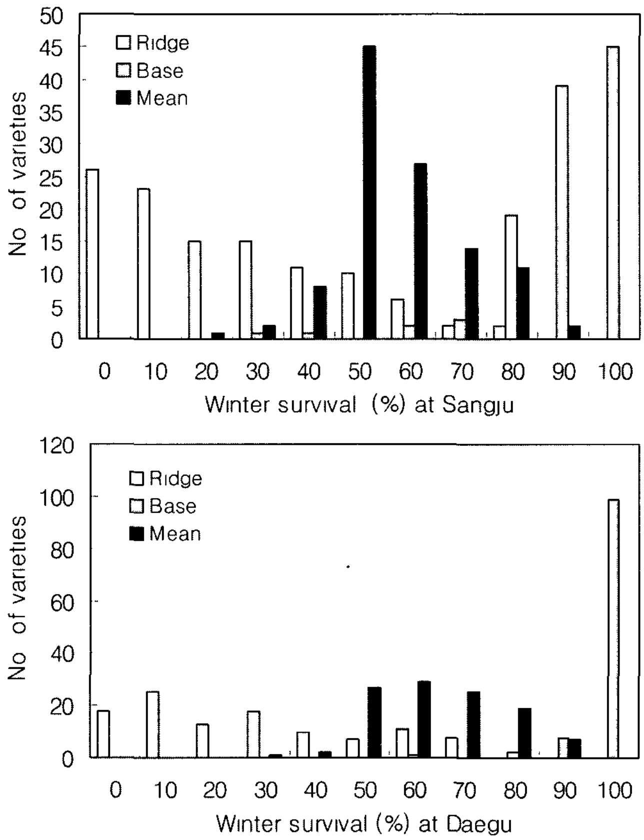

Fig. 2. Effects of location and furrow condition on winter survival of barley.

furrow showed close to the normal distribution. To optimize the winter survival rate for screening the barley winter hardiness, it was better to use the mean survival rate at base and ridge of the furrow. So, the mean of winter survival rate at base and ridge of the furrow was used as the data of the winter survival rate of the barley varieties. Though there showed

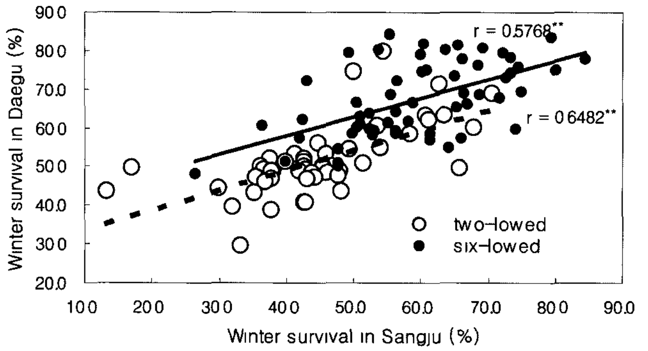

Fig. 3. Comparison of winter survival rate of two-rowed (O) and six-rowed (●) barley at different locations.

different winter survival rate in each combination of location and furrow conditions, we could see the similar changes of winter survival rate by different furrow conditions at the two locations. Even within a relatively small plot or field, large spatial differences in survival can occur. These differences can be related to topography and variation in environmental factors such as snow depth, soil moisture and temperature (Hayhoe & Andrews, 1999). By sowing the plants at the ridge and base, we could minimize the effect of topographic variation in a field by giving higher and lower level of stress at the same time.

The winter survival rates of forty-nine two-rowed and sixty-one six-rowed barley varieties at Sangju and Daegu were highly correlated with each other, as 0.6482** and 0.5768**, respectively (Fig. 3). Despite the minimum temperature of January of Sangju was 7.1°C lower than that of the Daegu, the winter survival rate between Sangju and Daegu was highly correlated with each other. By sowing the seeds at the ridge and base alternatively, we could choose the proper treatment among them. In a given screening field, we could select the best data, which shows close to normal distribution, among the winter survival rate from the ridge, base and mean survival rate of the two for accurate evaluation of winter hardiness. In our results, it was better to use the mean survival rate between base and ridge of the furrow.

Nam *et al.* (1982) demonstrated that planting wheat on ridge could enhance winter stress in the field, from which snow is usually blown out, leaving plants more exposed to low temperature desiccation. Planting seeds in wooden boxes placed above the ground or leaving plots without snow cover by gently removing newly fallen sow from the snow are also one of methods enhancing winter stress (Reynold *et al.*, 2001). However, methods that can reduce the winter stress are also needed to increase accuracy and get good differentiation among genotypes in screening winter hardiness. In this experiment, barley plants planted on the base of furrow could decrease winterkill, from which soil moisture and snow depth could be maintained. By planting barley on the ridge and base of the furrow, simultaneously, breeders could be choose the treatment close to normal distribution of winter survival among the data from ridge, base, and mean of the two in a given field. This method also could

Table 4. Comparison of winter survival rate between forty-nine two-rowed and sixty-one six-rowed barley varieties.

Location	Mean winter survival (%)†					
	Two-rowed			Six-rowed		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Sangju	13.2	70.6	44.8	26.4	84.5	59.0
Daegu	29.6	79.8	74.7	48 1	84.3	67.2
Average	30.3	74.7	50.4	43.8	82.6	65.5

†Mean between the winter survival rate of ridge and base of furrow.

Table 5. Winter survival rate of cold tolerant and susceptible varieties selected from 110 barley varieties.

Row type	Winter survival (%)							
	Cold tolerant variety				Cold sensitive variety			
	Variety	Sangju	Daegu	Mean	Variety	Sangju	Daegu	Mean
Six-rowed	Jeonnamjaerae	79.3	83 4	82.6	Daichi	47.8	50.3	49.8
	Reno	84.5	77.9	79.2	Sakigake	39.7	51.6	49.2
	Dongbori-1	69.3	80.7	78.4	Karl	26.4	48 1	43.8
Two-rowed	Malta	54.4	79.8	74.7	Franklin	32.0	39.9	38.3
	Viva	62.8	71.7	69.9	Schooner	13.2	43.9	37.7
	Diana	70.6	69.2	69.5	PI283398	33.2	29.6	30.3

be have some limitations including site of screening field. If the site is located on heavy soil with poor surface drainage, plants in the base of the furrow might be subjected to damage through flooding and ice formation. Also, If the site is located on sandy soil with frequent freezing, thawing climate, plants on the ridge of the furrow could be damaged by soil erosion.

Winter survival rates of the six-rowed barley varieties were higher than those of the two-rowed varieties (Table 4). Average winter survival rate between two locations about the six-rowed barley varieties was 65.5%, it was about 15.1% higher than that of the two-rowed varieties.

Among the varieties tested, a Korean native six-rowed variety, 'Jeonnamjaerae' has the highest value of 82.6% in the rate of winter survival (Table 5).

Jeonnamjaerae was more cold tolerant than American cold tolerant variety, Reno (CI6561) and Korean cold tolerant variety, Dongbori 1. The varieties such as Malta, Viva and Diana were comparatively cold tolerant among two-rowed varieties. These varieties will be used as genetic resources for breeding two-rowed barley varieties with cold tolerance. The most sensitive variety was PI283398. Winter survival rate of PI283398 was 33.2% in Sangju and 29.6% in Daegu.

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