ABSTRACT

Available winter hardy lentil (Lens culinaris Medik.) germplasm has prompted interest in the development and use of cultivars that can be fall planted in cold highland areas. This change in production of lentil from normally spring sown to fall sown is environmentally sound and increases yield potential. Understanding the mode of inheritance of winter hardiness in lentil would assist breeding efforts. The objectives of this study were to determine the inheritance and heritability of winter hardiness in lentil. Ten F2 derived recombinant inbred line (RIL) populations from crosses of winter hardy germplasm lines with nonhardy germplasm were planted in a randomized complete block design with three replications at Haymana, and Sivas, Turkey, and at Pullman, WA, USA, between 1997 and 2001. Meaningful data for an analysis of the inheritance of winter hardiness were available only at Haymana in 1997-1998 and at Pullman in 1998-1999, indicating infrequent occurrences of sufficiently cold winters to provide differential killing. Percent survival was calculated on the basis of plant stand counts recorded after establishment in the fall and regrowth in the spring. Parental line WA8649041 was the most winter hardy followed by WA8649090, ILL-1878, and ILL-669. General combining ability (GCA) and specific combining ability (SCA) effects were significant at both locations. Heritability estimates among the 10 RIL populations ranged from 15.9 to 90.7%. Inheritance patterns of winter hardness appeared to be quantitative on the basis of frequency distributions and the lack of discrete segregation classes. Since winter hardiness in lentil appears to be a quantitative trait, accumulation of genes responsible for winter survival will probably require stringent field testing or marker assisted selection.

CROP BREEDING, GENETICS & CYTOLOGY

Genetics of Winter Hardiness in 10 Lentil Recombinant Inbred Line Populations

A. Kahraman, I. Kusmenoglu, N. Aydin, A. Aydogan, W. Erskine and F. J. Muehlbauer*

Lentil is an annual self-pollinated diploid (2n = 14 chromosomes) species, and a highly valued food legume grown extensively in the Middle East, North Africa, North America, Australia, and South Asia. In the Palouse region of the U.S. Pacific Northwest, the crop is usually sown in early spring and harvested in late July or early August. However, winter hardy germplasm is available and may be used to develop higher yielding types that can be planted in the fall. With fall or early winter planting, lentil yields can be increased up to 50% (Sakar et al., 1988) by making more efficient use of available moisture and by avoiding heat stress. Fall planting is desirable because drier soil conditions allow for planting the crop without the excessive soil compaction that is common with spring planting in cold wet soils. Spring planted lentil crops often experience heat stress and terminal drought in the latter part of the growing season that reduces yields. Crops planted in the fall may develop and mature sufficiently early to avoid the most severe heat and drought stresses.

In cold highland areas, winter lentils are not grown because cultivars with sufficient winter hardiness and acceptable quality traits are not available. Much of the work on lentil winter hardiness has been related to agronomic, physiologic, and germplasm screening (Kusmenoglu and Aydin, 1995; Eyupoglu et al., 1995; Summerfield et al., 1985; Erskine et al., 1981). On the basis of available germplasm, however, it should be possible to develop lentil cultivars with improved winter hardiness and acceptable quality (Erskine et al., 1981; Spaeth and Muehlbauer, 1991).

Genetic studies of winter hardness in lentil are limited. A recent study (Ali and Johnson, 2000) indicated that winter hardness had low to moderate heritability (32–71%). Genetic studies of winter hardness in other food legumes have been investigated in more detail. Winter hardness in pea (Pisum sativum L.) is controlled by dominant (Cousin et al., 1985) and additive genes (Auld et al., 1983) and by as many as three or four genes (Liesenfeld et al., 1986). Cold tolerance in chickpea (Cicer arietinum L.) is controlled by at least five genes with tolerance dominant over susceptibility (Malhotra and Singh, 1990).

Breeding for winter hardness is considered a long-term objective because field tests required for differential killing of segregating material are unpredictable and infrequent, occurring perhaps only once every 8 to 10 yr (Lewitt, 1980). Winter hardness is affected not only by tolerance to cold but by tolerance to factors such as frost heaving, water logging, freeze–thaw cycles, and diseases as well (Steponkus, 1978). Cultural practices including planting date, plant density, and depth of planting, also affect winter survival (Kusmenoglu and Aydin, 1995). However, among all these factors, cold temperature tolerance is the major component of winter hardness. Selection for winter hardiness on the basis of artificial cold tolerance tests can be considered an indirect selection criterion. However, associations between winter hardness and cold tolerance are not stable and vary among experiments, perhaps because of experimental design and artificial conditions (Gullord et al.,
in 1997-1998 and at three locations (Pullman, Haymana, and Sivas) in 1998-1999. Field evaluation experiments at Pullman were sown in a conventionally tilled field on 15 Oct. 1997 and in minimally tilled fields with barley stubble on 5 Oct. 1998. At Haymana and Sivas locations, planting was performed in conventionally tilled fields. Planting dates for Haymana were 25 and 18 Oct. 1997 and 1998, respectively, and for Sivas was 25 Oct. 1998. Soil characteristics were fine silty, mixed mesic Pachic Ultic Haploxerolls at Pullman and silty-clay at Haymana. The 10 sets of RILs and parental lines were planted in a randomized complete block design with three replications. Plots were single rows 1 m long and spaced 0.3 m apart with an average of 30 to 40 plants in each row. A non-winter hardy check, 'Brewer', and a winter hardy check, WA8649090, were included after every 20 plots to compare with the RILs.

Winter survival of the RILs at each location was determined on the basis of plant stand counts recorded after complete emergence of seedlings in the fall and after regrowth in the spring. Daily air and soil temperatures were recorded hourly with data loggers from October to May at the Pullman location. Daily air and soil temperatures for Haymana location could not be recorded because of malfunction of the data loggers. Instead, monthly maximum, minimum, and average temperatures were obtained from Haymana Experiment Station, Turkey. The data loggers for recording air and soil temperatures were placed 250 mm above ground and 50 mm below ground, respectively.

Data from each recombinant inbred line population were analyzed separately by SAS software 6.12 (1996) PROC MIXED and PROC GLM models. Since the survival data were based on percentages, they were transformed by arcsine square root before analysis of variance. When no difference occurred between raw and transformed data, the raw data were used in statistical analyses. Adjusted least square means of RILs (i.e., average values of the genotypes adjusted for block effects) were used to determine the frequency distributions of the RILs for winter survival.

The heritability estimates for winter hardiness were calculated as the ratio of the genetic to the phenotypic variance from plot means (Fehr, 1987). Inheritance of winter hardiness was determined on the basis of frequency distributions for winter survival in each population. Since F2 derived RILs are expected to be nearly homozygous, discrete segregation of 1 to 1 would be expected for single gene inheritance. A continuous distribution pattern for winter survival would indicate more than one gene conferred winter hardness with probable environmental effects. General combining ability and specific combining abilities were calculated from mean survival of RIL populations on the basis of Griffing’s half diallel analysis (Griffing, 1956). The program Dial 95 (Ukai, 1989) was used for diallel analysis.

**RESULTS**

Average monthly air and soil temperatures at Pullman from October to May 1997-1998 ranged from -1.7
to 10.8°C and from −0.9 to 13.1°C, respectively. Temperatures below zero were observed from October to May. The lowest average daily air and soil temperatures −19.5°C and −11.8°C, respectively, were recorded in December at Pullman in 1998-1999 (Fig. 1). At Haymana in 1997-1998, average monthly air temperatures from October to May ranged from −0.5 to 14.6°C and lowest air temperature recorded was −12.5°C. Temperatures below zero were observed from October to April. At Pullman, plants experienced low temperatures without snow cover while there was about 200 mm of snow cover in the winters at Haymana.

No winter killing was observed at Pullman in 1997-1998 while there was substantial winter killing during the winter of 1998-1999 (Table 3 and 4). At Haymana, there was moderate winter killing in the winter of 1997-1998 while no winter killing was present during the winter of 1998-1999. At Sivas, there was complete killing in the winter of 1998-1999.

Mean survival of the five parental lines ranged from 37 to 95% at Haymana in 1997-1998 and 0 to 76% at Pullman in 1998-1999 (Table 3). Survival of the parental lines was different at both environments, but their survival rank did not change suggesting no genotype × environment interactions (G×E). The hardest parent was WA8649041, followed by WA8649090, ILL-669, and Precoz (Table 3). Percent survival of the hardest parent, WA8649041, was significantly greater than the other parental lines after the severe winter conditions at Pullman in 1998-1999.

Mean survival of the 10 RIL populations ranged from 47 to 86% at Haymana and 0 to 75% at Pullman (Table 4). Analysis of variance results were significant for all populations at both locations (Table 5). Survival at both locations was lowest for the nonhardy × hardy crosses, while average survival was the highest for the hardy × intermediate hardy crosses at Haymana and the hardy × hardy cross at Pullman. One general observation from the Pullman field test was that as winter hardiness of the parents increased, mean survival of the RIL populations increased linearly in all crossing groups (Fig. 2). This general result suggested that the parental

Table 3. Mean winter survival (%) of parental lines at Haymana, Turkey, during the winter of 1997-1998 and at Pullman, WA, USA, during the winter of 1998-1999.

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Precoz</td>
<td>Nonhardy</td>
<td>37.0</td>
<td>25.0–54.2</td>
</tr>
<tr>
<td>ILL-669</td>
<td>Intermediate hardy</td>
<td>66.2</td>
<td>38.7–73.1</td>
</tr>
<tr>
<td>ILL-1878</td>
<td>Intermediate hardy</td>
<td>73.6</td>
<td>42.4–85.2</td>
</tr>
<tr>
<td>WA8649090</td>
<td>Hardy</td>
<td>77.5</td>
<td>61.5–86.3</td>
</tr>
<tr>
<td>WA8649041</td>
<td>Hardy</td>
<td>95.0</td>
<td>76.7–98.7</td>
</tr>
</tbody>
</table>
and moderately hardy groups and among RILs within mates were low such as for Populations 2 and 10. This may indicate a lack of genetic variation in those crosses.

Heritability estimates for the populations ranged from 15.9 to 63.7% at Haymana and from 35.5 to 90.7% at Pullman (Table 5). The heritability estimate was highest for Population 7 at both locations (63.7 and 90.7%, respectively), and similar heritabilities were estimated for Populations 2, 6, and 8 at both locations while heritability estimates were quite different for the other populations. The overall heritability estimate for winter survival at Haymana was 40.1% compared with 60.8% at Pullman (Table 5). When the difference between parents for winter hardiness was small, heritability estimates were low such as for Populations 2 and 10. This indicates that the mean survival of Population 10 (55.7%) (Table 4).
and gains from selection may be small because of difficulties in differentiating individual lines for winter hardiness. Heritability estimates were high when parents differed widely in winter hardness such as in Population 7, which was derived from a hardy × nonhardy cross.

Estimated variances for GCA and SCA were significant at both locations (Tables 6, 7, 8). The mean square for GCA was greater than the mean square for SCA and indicated that GCA was a major source of variation for winter survival. Mean survival of the Precoz/WA-8649041 cross combination with a SCA effect of 7.5% had better survival than expected at Haymana. Several crosses were significantly different from that expected on the basis of the GCA effects of the parents at Pullman, where winter killing was more severe. The cross ILL-1878/WA8649041 had high SCA effects, which accounted for an 8.2% increase in winter survival at Pullman. When survival of the RILs was evaluated for each cross, this same parental combination had the highest mean survival at both locations.
DISCUSSION

Winter hardiness of 10 RIL populations of lentil appeared to be under polygenic control with additive loci because the mean of the progeny resembled the average of its parents (i.e., midparent value), while the opposite is true when a major gene is segregating in an F2 population (Karlin et al., 1979). Frequency distributions for the 10 lentil populations were not consistent with a single gene model for winter hardiness either as continuous frequency distributions are considered an indicator of polygenic inheritance (Lynch and Walsh, 1998). However, if environmental variation is large, segregation of major genes can be continuous (Lynch and Walsh, 1998). Frequency distributions for the 10 lentil populations for winter survival at two locations were not consistent with a single gene model for winter hardiness. Our

Survival (%)

<table>
<thead>
<tr>
<th>Location</th>
<th>Precoz</th>
<th>ILL-669</th>
<th>ILL-1878</th>
<th>WA8649041</th>
<th>WA8649090</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haymana</td>
<td>-14.7</td>
<td>-1.14</td>
<td>3.67</td>
<td>13.78</td>
<td>-1.57</td>
</tr>
<tr>
<td>Pullman</td>
<td>-21.47</td>
<td>-8.27</td>
<td>0.62</td>
<td>30.73</td>
<td>-0.37</td>
</tr>
</tbody>
</table>

Table 7. Specific combining ability effects of cross combinations at Haymana, Turkey, in 1997-1998 (first rows) and at Pullman, WA, USA, in 1998-1999 (second rows).

<table>
<thead>
<tr>
<th>Parents</th>
<th>Precoz</th>
<th>ILL-669</th>
<th>ILL-1878</th>
<th>WA8649041</th>
<th>WA8649090</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precoz</td>
<td>0.51</td>
<td>-7.83</td>
<td>7.47</td>
<td>-0.15</td>
<td></td>
</tr>
<tr>
<td>ILL-669</td>
<td>5.12</td>
<td>0.46</td>
<td>-7.17</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>ILL-1878</td>
<td>1.56</td>
<td>-4.23</td>
<td>2.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WA8649041</td>
<td>-5.77</td>
<td>-0.84</td>
<td>1.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.52</td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.19</td>
<td>-2.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-5.77</td>
<td>-0.18</td>
<td></td>
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</tr>
</tbody>
</table>

results are in general agreement with reports by Thom-ashow (1990) on wheat (*Triticum aestivum* L.), Brouwer et al. (2000) on alfalfa (*Medicago sativa* L.), and Liesenfeld et al. (1986) on pea (*Pisum sativum* L.) that several genes control winter hardiness.

As the winter hardness level of the parent increased, average survival of the populations increased as well (e.g., Populations 1, 4, 5, and 6). Liesenfeld et al. (1986) reported similar results in pea indicating that increasing dosage of the hardier parent increased the recovery of winter hardy lines from the segregating populations. Mean survival in crosses with the hardiest parent (WA8649041) was always higher than mean survival of the other crosses (Fig. 2); thus, to improve winter hardness in development of future commercial winter hardy lentils, we suggest that WA8649041 should be used as the source of winter hardiness.

Although field tests are the ultimate measure of winter hardiness, major disadvantages include infrequent occurrence of winters with differential winter killing. In our case, at Pullman, there was differential winter killing in only 1 out of 5 yr of field tests. McIntyre et al. (1988) reported similar results for winter wheat indicating that differential winter killing occurred in only 1 out of 5 yr. We observed that, localized variations in soil temperatures, snow cover, water logging, and stubble distribution contributed to variable results. Experimental error associated with field tests is usually high which precludes detection of small differences in winter hardiness among progeny lines from hardy × hardy crosses.

General combining ability effects were significant and largely higher than SCA effects indicating that selection for improved winter hardiness should be effective. Significance of GCA indicates that parents that combine well with a number of other parents may provide opportunities for breeding improved winter hardness. The nonhardy parent Precoz had the largest negative GCA effects (−14.7 at Haymana and −21.5 at Pullman), while the hardiest parent WA8649041 had the largest positive GCA effects (13.8 and at Haymana and 30.7 at Pullman) for winter survival indicating that this parent would be the best choice to include in crosses. ILL-1878 was also very good combiner for improved winter hardness. Winter hardiness of Populations 7 and 9 showed good SCA and selection from Population 9 may produce lines with desired agronomic characters such as early flowering and large seed size (Table 1). Similar results indicating the importance of GCA and SCA were reported in pea (Auld et al., 1983), oat (*Avena sativa* L., Jenkins, 1969; Muchlbauer et al., 1970), and wheat (Brule-Babel and Fowler, 1988; Sutka, 1984).

Heritability estimates ranged from low to high for winter hardiness among the 10 RIL populations. Similarly, low to high heritability estimates ranging from 30.0 to 84.4% for winter hardiness were reported in diallel crosses of wheat (Orlyuk, 1985). Also, our estimates of heritability generally agreed with previously published results for winter hardiness (Ali and Johnson, 2000), with the exception of Population 7. The high heritability estimate for this population (90%) could be due to high genetic variance resulting from a wide range for winter hardiness between the two parents, or biased selection during generation advance of lines (Ukai, personal communication). Bias in selection during the development of RIL populations is unlikely because all RILs were developed in greenhouse conditions with no exposure to cold. Genetic constitution of the parents and over wintering conditions can also greatly influence heritability estimates. For example, relatively mild winters or very harsh winters may not differentiate among the progeny and genotypic variability may be comparatively low with low heritability estimates. Therefore, heritability estimates cannot be generalized and should be interpreted with regard to specific environments under which it was obtained. Overall results suggest that selection for winter hardiness should be effective at Pullman where a more differential winterkill occurred and the average heritability estimate for the 10 populations was higher.

In conclusion, inheritance and heritability studies of
winter hardiness under field conditions provided insight that winter survival is probably controlled by more than one gene and highly influenced by environment. To understand and characterize better the genetics of winter hardiness, we suggest that molecular markers be used to identify genomic regions involved in the expression of winter hardiness. Those markers may then be used in marker-assisted selection to minimize the need for field testing. Associations between winter hardiness and other plant traits also should be determined. Furthermore, cold tolerance studies under controlled environments should be performed to determine if selection for winter hardiness in lentil can be made on the basis of results of freezing tests. Freezing tests could be used to determine the importance of cold tolerance relative to other factors that determine winter survival.

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REFERENCES
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