

## Evaluation of Pea (*Pisum sativum* L.) Germplasm for Winter Hardiness in Central Anatolia, Turkey, Using Field and Controlled Environment

AZIZE HOMER<sup>1</sup>, MEVLÜT ŞAHİN<sup>1</sup> and ÜMRAN KÜÇÜKÖZDEMİR<sup>2</sup>

<sup>1</sup>Central Research Institute for Field Crops, Ankara, Turkey; <sup>2</sup>Eastern Anatolia Agricultural Research Institute, Erzurum, Turkey

### Abstract

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Winter pea can be grown in rotation with cereal crops in Central Anatolia, Turkey. However, winterkill can occur during harsh winters. The objective of this study was to screen pea accessions for winter survival, and identify genotypes with differential winter hardiness for future crop development. The plant material consisted of 58 accessions including local landraces, elite winter cultivars, selected lines and several checks. Twenty-five of them were evaluated under both field and laboratory conditions. The rest of the genotypes were tested under field conditions. Field trials were planted in Haymana, Ankara, and in Ulaş, Sivas, Turkey during the autumn of 2014. Winter hardiness was evaluated as the percentage of surviving plants. Differential survival of genotypes was observed at both locations. On average, the survival rate was lower in Ulaş (54.8%) than in Haymana (67.8%), and ranged between 1.5 and 100%. Turkish landraces TR 79404 (88.6%), TR 79407 (88.5%) and TR 80194 (84.8%) had survival percentages comparable with the three winter-hardy checks (Turkish cvs. Taşkent (90.0%) and Özkaynak (85.0%), US cv. Melrose (94.7%)). Twelve single plants were selected from these populations for future cultivar development. The European and US accessions, included in the trials for their previously reported winter hardiness, showed high levels of winter hardiness, and could be used in breeding programs. In the laboratory, no plants survived at –12°C and –16°C three weeks following the freezing test. Screening at –8°C generated differential survival among winter genotypes. Significant positive correlations ( $r = 0.67–0.87$ ,  $P < 0.001$ ) were found between the test environments for the percent survival.

**Keywords:** freezing tolerance; landrace; winter pea; winter survival

A winter wheat-fallow based cropping system remains common practice in Central Anatolia, Turkey, due to low precipitation which averages about 400 mm per year. However, fallow combined with tillage reduces soil organic matter (SOM), increases wind and water erosion, thus reducing soil productivity (BOLTON 1974). It has been shown that summer fallow is not very efficient in storing soil moisture, and can conserve only 25% of the precipitation (MCGEE *et al.* 1997; NIELSEN & VIGIL 2005). Adding annual legumes to the crop rotation can reduce the fallow period, improve soil physical conditions, and reduce soil erodibility as SOM is increased (TISDALL & OADES 1982; POWER 1990).

Winter pea, a nitrogen-fixing cool season annual legume, can be grown in rotation with cereals in Central Anatolia for forage or grain, and would provide quality feed for the livestock in the region. This crop is seeded in autumn in the coastal and southeastern regions of Turkey (AÇIKGÖZ *et al.* 1985). Using pea as a winter crop in cold highlands of Central Anatolia (>1000 m) is restricted due to below freezing temperatures. The area of winter pea adaptation might be increased by improvement of winter hardiness. Previously, two cold tolerant cultivars adapted to the region were released. However, winterkill is still a problem during harsh winters observed in some

years. The objective of this study was to evaluate the winter hardiness of pea accessions available from genebanks, and identify genotypes with differential winter hardiness for use in future studies.

## MATERIAL AND METHODS

**Plant material.** A total of 58 pea germplasm accessions (mostly field pea, *ssp. arvense* but several garden peas, *ssp. sativum* included) were obtained from genebanks and local universities (Table 1). The majority of the accessions were Turkish landraces or local cultivars, and were chosen according to the altitude of the collection site which ranged from 1000 m to an elevation of 2127 m a.s.l. There is no prior knowledge regarding the cold tolerance of these landraces. Some of the other accessions were winter pea cultivars/lines registered in Europe and in the US known to have a good level of winter hardiness. Several elite Turkish winter cultivars were included to compare hardiness with that of genebank germplasm. Two spring-type materials were also tested. More details on the PI lines from USDA can be found in the USDA Germplasm Resources Information Network (GRIN) (<http://www.ars-grin.gov/npgs/searchgrin.html>). Twenty-five of these accessions were screened under both field and laboratory conditions (from here on referred to as Experiment I). The rest of the materials were tested under the field conditions only due to seed and space limitation (from here on referred to as Experiment II).

### Experiment I

**Field screening.** Twenty-five accessions were evaluated in two field trials in Ulaş, Sivas (39°26'N, 37°2'E, elevation 1540 m a.s.l.), and Haymana, Ankara (39°43'N, 32°50'E, elevation 1055 m a.s.l.) in Central Anatolia of Turkey during the winter of 2014–2015 to assess their relative winter hardiness.

Thirty seeds of each genotype were planted on 13 Oct 2014 in Ulaş, Sivas and on 21 Oct 2014 in Haymana, Ankara in conventionally tilled soil. The trials were arranged as a randomized complete block design (RCBD) with three replications. Seeds of each genotype were sown 8 cm apart in single row plots spaced 60 and 50 cm apart at Haymana and Ulaş, respectively. Each row was 2.4 m in length. No fertilizer was applied. The infestation by cowpea aphid in late November in Haymana was controlled by an application of Malathion (1.2 l a.i. per ha).

Soil types at both locations are characterized as clay loam.

Rainfall received in October was 58 and 95 mm at Ulaş and Sivas locations, respectively, and provided rapid emergence and good stand. The seedlings within each plot were counted on 18 Nov 2014 at Ulaş, and on 5 Dec 2014 at Haymana, to determine the number of established seedlings. Surviving seedlings were counted on 4 May 2015 at Ulaş, and 23 Apr 2015 at the Haymana location. Winter hardiness was assessed as the percentage of survived plants, i.e. the number of plants which survived the winter divided by the number of plants established in autumn, expressed as a percentage.

**Laboratory screening.** Seeds of 25 genotypes were scarified by nicking the seed coat and planted in plastic rectangular pots (40 × 25 × 7 cm) containing a potting mix composed of equal proportions of sand and field soil. Twelve seeds of each genotype were sown in rows and subsequently thinned to eight seedlings. Plants were grown at 22/17°C day/night under 16 000 lux ( $\approx 230 \mu\text{mol}/\text{m}^2/\text{s}$ ) and 16 h photoperiod until the 2–3 leaf stage in a controlled growth chamber. Plants were then held at  $4 \pm 0.2^\circ\text{C}$  with a 9-h photoperiod. After the seedlings had cold acclimated for 4 weeks in this environment, they were subjected to four freezing temperatures ( $-4$ ,  $-8$ ,  $-12$  and  $-16^\circ\text{C}$ ) as described by AULD *et al.* (1983a) with some modifications. Plants were fertilized once with a complete Hoagland solution during the first growing period, and watered as required during the growing and acclimation period.

Pots were placed in a freezing chamber at  $2 \pm 0.2^\circ\text{C}$ , and the temperature was reduced to  $-2^\circ\text{C}$  at a rate of  $2^\circ\text{C}/\text{h}$ , and held at  $-2 \pm 0.2^\circ\text{C}$  for 16 h, to ensure that nucleation occurred in plants. The temperature was subsequently reduced from  $-2^\circ\text{C}$  to each freezing temperature ( $-4$ ,  $-8$ ,  $-12$  and  $-16^\circ\text{C}$ ) at a rate of  $2^\circ\text{C}/\text{h}$ . During this treatment, the seedlings to be evaluated at each test temperature were removed from the freezer after having been subjected to each treatment for an hour. Seedlings were moved directly from the freezer into a  $4^\circ\text{C}$  chamber and allowed to thaw for 24 h. Plants then were placed under initial growth conditions. The number of surviving plants was counted 3 weeks after the freeze test. A thermometer was used to monitor the temperatures in the freezing chamber. The study was conducted as a completely randomized design (CRD) with three replications using eight plants per replication.

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Table 1. *Pisum sativum* accessions screened for winter hardiness in two experiments in two field environments and in laboratory

Accession code/name	Status	Country of origin	Source	Elevation (m a.s.l.)
TR 37330	landrace	Kastamonu, TR	Genebank, TR	1200
TR 79404	landrace	Bayburt, TR	Genebank, TR	1741
TR 79415	landrace	Kars, TR	Genebank, TR	1849
TR 79416	landrace	Kars,TR	Genebank, TR	1914
TR 79422	landrace	Ardahan, TR	Genebank, TR	1890
TR 79425	landrace	Ardahan, TR	Genebank, TR	1860
TR 79427	landrace	Ardahan, TR	Genebank, TR	1831
TR 79428	landrace	Ardahan, TR	Genebank, TR	2127
TR 79431	landrace	Ardahan, TR	Genebank, TR	1903
TR 79434	landrace	Ardahan, TR	Genebank, TR	2058
TR 79435	landrace	Ardahan, TR	Genebank, TR	1973
TR 79439	landrace	Ardahan, TR	Genebank, TR	2058
TR 79441	landrace	Ardahan, TR	Genebank, TR	1776
TR 80194	landrace	Burdur, TR	Genebank, TR	1000
TR 79406	landrace	Bayburt, TR	Genebank, TR	1565
TR 79407	landrace	Bayburt, TR	Genebank, TR	1525
TR 79409	landrace	Bayburt, TR	Genebank, TR	1498
TR 79412	landrace	Erzurum, TR	Genebank, TR	1591
TR 79418	landrace	Kars, TR	Genebank, TR	2097
TR 79419	landrace	Ardahan, TR	Genebank, TR	1813
TR 79421	landrace	Ardahan, TR	Genebank, TR	1825
TR 79424	landrace	Ardahan, TR	Genebank, TR	1825
TR 79426	landrace	Ardahan, TR	Genebank, TR	2137
TR 79429	landrace	Ardahan, TR	Genebank, TR	1845
TR 79430	landrace	Ardahan, TR	Genebank, TR	2010
TR 79432	landrace	Ardahan, TR	Genebank, TR	1903
TR 79433	landrace	Ardahan, TR	Genebank, TR	2058
TR 79437	landrace	Ardahan, TR	Genebank, TR	2058
TR 79440	landrace	Ardahan, TR	Genebank, TR	1776
TR 79442	landrace	Ardahan, TR	Genebank, TR	1957
809	selection, winter type	UK	John Innes Center (JIC), UK	–
810	selection, winter type	UK	John Innes Center (JIC), UK	–
PI 272216	accession, winter type	Bulgaria	USDA-GRIN	–
PI 392018	accession, winter type	Bulgaria	USDA-GRIN	–
PI 392019	accession, winter type	former Soviet Union	USDA-GRIN	–
PI 517923	accession, Austrian winter pea	former Soviet Union	USDA-GRIN	–
PI 618628 (Melrose)	cultivar, winter type	USA	USDA-GRIN	–
Özkaynak	cultivar, winter type	Konya, TR	Selçuk University, TR	–

Table 1 to be continued

Accession code/name	Status	Country of origin	Source	Elevation (m a.s.l.)
Taşkent	cultivar, winter type	Konya, TR	Selçuk University, TR	–
B-8	pure line, intermediate hardy	Konya, TR	Selçuk University, TR	–
Kirazlı	cultivar, winter type	Bursa, TR	Uludağ University, Turkey	–
358 (Rafale)	cultivar, winter type	UK	John Innes Center (JIC), UK	–
711 (Austrian Winter)	cultivar, winter type	UK	John Innes Center (JIC), UK	–
808 (Winter Hardy)	selection, winter type	UK	John Innes Center (JIC), UK	–
1016 (Frimas)	cultivar, winter type	France	John Innes Center (JIC), UK	–
2276 (49 C)	cultivar, winter type	Bulgaria	John Innes Center (JIC), UK	–
2280 (CK-8)	cultivar, winter type	Bulgaria	John Innes Center (JIC), UK	–
2282 (N1 56)	cultivar, winter type	Bulgaria	John Innes Center (JIC), UK	–
2283 (Kalifer)	cultivar, winter type	Bulgaria	John Innes Center (JIC), UK	–
2289 (Champagne)	cultivar, winter type	France	John Innes Center (JIC), UK	–
3134 (Amac)	cultivar, winter type	UK	John Innes Center (JIC), UK	–
PI 339887 (Karagöz)	old local cultivar, winterhardy	TR	USDA-GRIN	–
PI 517924 (D59-5-6-3Y)	line, cold tolerant	USA	USDA-GRIN	–
PI517925 (D-60-2-6-1Y)	line, cold tolerant	USA	USDA-GRIN	–
PI 619084 (Oregon Sugar Pod)	cultivar, garden pea	USA	USDA-GRIN	–
PI 574505 (Common)	landrace, winter pea	USA	USDA-GRIN	–
US 647868 (Windham )	cultivar, winter pea	USA	USDA-GRIN	–
PI 628276 (Lifter)	cultivar, spring pea	USA	USDA-GRIN	–

TR – Turkey

## Experiment II

**Field screening.** Thirty-six accessions were screened in two field trials at Ulas, Sivas and Haymana, Ankara in Central Anatolia of Turkey during the winter 2014–2015 for their winter hardiness. Planting dates and all the other management applications were the same as previously described for Experiment I. The exception being that twenty-five seeds of each accession were planted 6 cm apart in a 1.5 m single row in an augmented design with four blocks.

**Data analysis.** For each field and laboratory test environment, a separate analysis of variance was performed on percent survival data. For field experiments, the percentages of winter survival of genotypes were transformed by the arcsine square root before analysis of variance. When there was no

difference in *F*-test significance between original and transformed data analyses, the original data were used. In laboratory, percent survival data at  $-8^{\circ}\text{C}$  was log transformed prior to analysis, and reported in an original scale.

Means were compared by Fisher's protected LSD where *F*-tests indicated significant differences. The least squares means (LS means) procedure of SAS (Ver. 9.3, 2008) was used for the mean separation. Pearson correlation coefficients were calculated between the mean percent survival in the two field environments and two laboratory test temperatures.

## RESULTS AND DISCUSSIONS

**Climate.** Monthly minimum air and soil surface temperatures for both experimental locations during the winter 2014–2015 growing season are presented

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in Table 2. There was harsh cold, and temperatures below zero were recorded between October and April at both locations. In general, Ulaş is much colder than Haymana due to the higher elevation. In Ulaş the absolute minimum air temperatures and soil surface temperatures were  $-20.3^{\circ}\text{C}$  and  $-16.1^{\circ}\text{C}$  and they occurred in January and December, respectively. At the Haymana location, the absolute minimum air and soil surface temperatures were  $-16.1^{\circ}\text{C}$  and  $-10.2^{\circ}\text{C}$  and occurred in December and March 2015, respectively. There was snow cover at both locations during the coldest period.

**Field Screening.** There was a variation in winter hardiness among the accessions at both locations in both experiments (Tables 3 and 4). Differential levels of winter survival were observed between the winter-type germplasm and local landraces as well as within the landraces. On average, winterkill was greater in Ulaş, Sivas than in Haymana, Ankara. Over two field environments the mean winter survival ranged from 1.5 to 100% (Tables 3 and 4). Local landraces derived from the genebank, which are typically unimproved cultivars, had a lower survival rate relative to the winter-type germplasm. In Ulaş, there was snow cover during most of the winter. Under this snow cover local landraces averaged 25% survival while the hardy checks averaged 90% survival, which would allow the separation of less hardy lines. Three of the landraces, TR 79404 (Bayburt, 1741 m), TR 79407 (Bayburt, 1525 m) and TR 80194 (Burdur, 1000 m) had a high survival rate similar to the winter checks (Tables 3 and 4), and expressed similar hardiness at both locations. Single plants were selected from heterogeneous landrace populations at the Ulaş location. These single plant lines can be further tested and evaluated for cold tolerance as well as disease

resistance, forage and seed yield for cultivar development. The consistent low survival of most of the landraces at both locations indicates that selecting the germplasm based on a high elevation does not necessarily provide winter-hardy lines. Most of these Turkish landraces were from the same province but different elevations (Table 1). Selection of landraces over wide geographical areas would be a better approach to find more winter-hardy genotypes.

Winter-hardy check cultivars Melrose, Taşkent and Özkaynak as well as other winter-type germplasm exhibited higher survival consistently within and between trials at both locations (Table 3). These genotypes were the result of breeding efforts for winter hardiness. Winter-hardy cultivar Özkaynak and intermediate hardy line B-8 had different survival rates in two experiments within the same location (Tables 3 and 4). This may have been due to positional effects (i.e. slight topographic variations) within the study.

In Experiment I intermediate hardy Turkish elite line B-8 had 60.7 and 77.3% survival while US spring line Lifter exhibited 18.6 and 64.6% survival at the Haymana and Ulaş locations, respectively (Table 3). In Experiment II, UK winter cultivar Rafale had lower mean survival (49.1%) than the rest of the European winter-type germplasm (86.3%). US garden pea cultivar Oregon Sugar Pod had the lowest survival rate (33.2%) across two field environments.

At the Haymana location, there was a period without snow cover during winter months. Winter injury by freezing temperatures damaged the leaves of some genotypes, causing a reduction in leaf area and delayed growth. The visual high injury score (data not shown) in this field environment was not always related to poor winter survival. For example, intermediate hardy line B-8 with the highest winter

Table 2. The number of days with freezing temperatures, and monthly minimum air and soil surface temperatures in the winter 2014–2015 at Ulaş and Haymana, Turkey

Months	Ulaş			Haymana		
	minimal air temperature ( $^{\circ}\text{C}$ )	minimal soil surface temperature ( $^{\circ}\text{C}$ )	frost days	minimal air temperature ( $^{\circ}\text{C}$ )	minimal soil surface temperature ( $^{\circ}\text{C}$ )	frost days
October	-2.8	-6.0	3	-1.7	-3.4	2
November	-7.0	-10.1	17	-2.8	-5.8	22
December	-15.0	-16.1	12	-7.8	-8.0	13
January	-20.3	-14.3	28	-16.6	-8.0	–
February	-11.0	-11.3	19	-12.9	-4.9	19
March	-6.0	-8.2	15	-7.0	-10.2	17
April	-6.1	-8.5	15	-5.1	-7.9	12



Table 3. Mean percent survival of 25 pea genotypes evaluated for winter hardiness in two field environments and at two temperatures in the laboratory

Genotype	Country of origin	Planting date		Laboratory test temperatures (°C)	
		Haymana, Ankara 21 Oct 2014	Ulaş, Sivas 13 Oct 2014	–4	–8
TR 37330	Kastamonu, TR	57.1 <sup>def</sup>	36.0 <sup>d</sup>	79.2 <sup>bcd</sup>	0 <sup>d</sup>
TR 79404	Bayburt, TR	94.5 <sup>a</sup>	88.6 <sup>ab</sup>	83.3 <sup>abc</sup>	0 <sup>d</sup>
TR 79415	Kars, TR	45.9 <sup>defg</sup>	16.6 <sup>e</sup>	66.7 <sup>cdef</sup>	0 <sup>d</sup>
TR 79416	Kars, TR	64.4 <sup>bcd</sup>	18.7 <sup>de</sup>	62.5 <sup>def</sup>	0 <sup>d</sup>
TR 79422	Ardahan, TR	36.7 <sup>efgh</sup>	20.1 <sup>de</sup>	57.1 <sup>f</sup>	0 <sup>d</sup>
TR 79425	Ardahan, TR	54.0 <sup>defg</sup>	22.5 <sup>de</sup>	70.8 <sup>bcdef</sup>	0 <sup>d</sup>
TR 79427	Ardahan, TR	44.7 <sup>defg</sup>	30.1 <sup>de</sup>	75.0 <sup>bcdef</sup>	0 <sup>d</sup>
TR 79428	Ardahan, TR	50.3 <sup>defg</sup>	27.0 <sup>de</sup>	75.0 <sup>bcdef</sup>	0 <sup>d</sup>
TR 79431	Ardahan, TR	54.7 <sup>def</sup>	20.0 <sup>de</sup>	83.3 <sup>abc</sup>	0 <sup>d</sup>
TR 79434	Ardahan, TR	34.1 <sup>fgh</sup>	23.0 <sup>de</sup>	58.3 <sup>ef</sup>	0 <sup>d</sup>
TR 79435	Ardahan, TR	39.2 <sup>defgh</sup>	12.4 <sup>e</sup>	79.2 <sup>bcd</sup>	0 <sup>d</sup>
TR 79439	Ardahan, TR	29.4 <sup>gh</sup>	29.5 <sup>de</sup>	61.3 <sup>def</sup>	0 <sup>d</sup>
TR 79441	Ardahan, TR	37.0 <sup>efg</sup>	25.5 <sup>de</sup>	70.8 <sup>bcdef</sup>	0 <sup>d</sup>
TR 80194	Burdur, TR	87.2 <sup>ab</sup>	84.9 <sup>ab</sup>	87.5 <sup>ab</sup>	0 <sup>d</sup>
GRU 809	UK	98.7 <sup>a</sup>	97.6 <sup>a</sup>	80.8 <sup>abcd</sup>	25.0 <sup>c</sup>
GRU 810	UK	87.7 <sup>ab</sup>	85.6 <sup>ab</sup>	78.6 <sup>bcde</sup>	0 <sup>d</sup>
PI 272216	Bulgaria	84.1 <sup>abc</sup>	83.0 <sup>ab</sup>	79.2 <sup>bcd</sup>	0 <sup>d</sup>
PI 392018	Bulgaria	90.8 <sup>a</sup>	95.6 <sup>a</sup>	87.5 <sup>ab</sup>	41.7 <sup>bc</sup>
PI 392019	former Soviet Union	97.6 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>	45.8 <sup>ab</sup>
PI 517923	former Soviet Union	100.0 <sup>a</sup>	98.8 <sup>a</sup>	100.0 <sup>a</sup>	50.0 <sup>ab</sup>
Melrose	USA	100.0 <sup>a</sup>	94.7 <sup>ab</sup>	100.0 <sup>a</sup>	62.5 <sup>a</sup>
Özkaynak	TR	100.0 <sup>a</sup>	90.9 <sup>ab</sup>	100.0 <sup>a</sup>	53.3 <sup>ab</sup>
Taşkent	TR	100.0 <sup>a</sup>	90.7 <sup>ab</sup>	100.0 <sup>a</sup>	50.0 <sup>ab</sup>
B-8	TR	60.7 <sup>cde</sup>	77.3 <sup>bc</sup>	65.3 <sup>cdef</sup>	0 <sup>d</sup>
Lifter	USA	18.6 <sup>h</sup>	64.6 <sup>c</sup>	55.9 <sup>f</sup>	0 <sup>d</sup>
Mean		66.7	57.3	78.2	13.1

TR – Turkey; values followed by the same letter in a column are not significantly different ( $P = 0.05$ ) based on LSD

injury scores had good survival due to its strong regrowth ability from the lower nodes of the stem. Large variability in spring vigour was observed among accessions (personal observation). Several winter lines exhibited poor spring regrowth and vigour, and performed poorly at the Haymana location. The US spring cultivar Lifter exhibited a high level of freeze injury and the lowest survival percentage of all lines at this location. Most of the survived plants of Lifter were weakened by cold, and were not vigorous.

It was observed that the winter-hardy germplasm including hardy checks exhibited a high level of sur-

vival while the accessions grown on adjacent plots had very low survival at the Ulaş location. This suggests that most of the observed differences in winter survival may be attributed to genotypic differences while the microsite (e.g. snow cover, soil moisture and water drainage) may also partially explain the variation in winter survival.

**Laboratory screening.** In laboratory, all cultivars survived well at  $-4^{\circ}\text{C}$ , and survival percentages ranged from 56% to 100% (Table 3). At  $-8^{\circ}\text{C}$ , both spring lines and local landraces were killed. Seven of nine winter lines including checks exhibited a differential

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Table 4. Mean percent survival of 36 pea genotypes evaluated for winterhardiness in two field environments in an augmented design

Genotype	Country of origin	Planting date	
		Haymana, Ankara, 21 Oct 2014	Ulaş, Sivas, 13 Oct 2014
TR 79406	Bayburt, TR	63.6 <sup>g</sup>	7.7 <sup>gh</sup>
TR 79407	Bayburt, TR	95.2 <sup>ab</sup>	88.5 <sup>abc</sup>
TR 79409	Bayburt, TR	83.1 <sup>de</sup>	33.5 <sup>f</sup>
TR 79412	Erzurum, TR	79.1 <sup>e</sup>	38.5 <sup>ef</sup>
TR 79418	Kars, TR	27.8 <sup>l</sup>	18.1 <sup>fgh</sup>
TR 79419	Ardahan, TR	12.1 <sup>m</sup>	28.5 <sup>fg</sup>
TR 79421	Ardahan, TR	34.1 <sup>ijkl</sup>	1.5 <sup>h</sup>
TR 79424	Ardahan, TR	35.9 <sup>ijk</sup>	3.5 <sup>gh</sup>
TR 79426	Ardahan, TR	17.7 <sup>m</sup>	3.5 <sup>gh</sup>
TR 79429	Ardahan, TR	36.7 <sup>ij</sup>	6.2 <sup>gh</sup>
TR 79430	Ardahan, TR	29.1 <sup>ijkl</sup>	15.3 <sup>fgh</sup>
TR 79432	Ardahan, TR	35.6 <sup>ijk</sup>	4.6 <sup>gh</sup>
TR 79433	Ardahan, TR	45.1 <sup>h</sup>	15.5 <sup>fgh</sup>
TR 79437	Ardahan, TR	40.7 <sup>hi</sup>	17.6 <sup>fgh</sup>
TR 79440	Ardahan, TR	46.9 <sup>h</sup>	16.0 <sup>fgh</sup>
TR 79442	Ardahan, TR	44.7 <sup>h</sup>	5.5 <sup>gh</sup>
Kirazlı	Bursa, TR	99.1 <sup>ab</sup>	98.5 <sup>a</sup>
Rafale	UK	70.5 <sup>fg</sup>	27.7 <sup>fg</sup>
711 (Austrian Winter)	UK	99.1 <sup>ab</sup>	71.3 <sup>cd</sup>
808 (Winter Hardy)	UK	76.9 <sup>ef</sup>	80.8 <sup>abcd</sup>
1016 (Frimas)	France	78.4 <sup>e</sup>	90.5 <sup>abc</sup>
2276 (49 C)	Bulgaria	100.0 <sup>a</sup>	83.8 <sup>abcd</sup>
2280 (CK-8)	Bulgaria	92.3 <sup>bc</sup>	97.5 <sup>ab</sup>
2282 (N1 56)	Bulgaria	93.1 <sup>abc</sup>	86.1 <sup>abcd</sup>
2283 (Kalifer)	Bulgaria	99.2 <sup>ab</sup>	89.9 <sup>abc</sup>
2289 (Champagne)	France	99.2 <sup>ab</sup>	78.2 <sup>bcd</sup>
GRU 3134 (Amac)	UK	64.7 <sup>g</sup>	72.5 <sup>cd</sup>
PI 339887 (Karagöz)	Northern TR	75.7 <sup>ef</sup>	61.5 <sup>de</sup>
PI 517924 (D59-5-6-3Y)	USA	86.7 <sup>cd</sup>	95.9 <sup>abc</sup>
PI517925 (D-60-2-6-1Y)	USA	100.0 <sup>a</sup>	97.5 <sup>ab</sup>
PI 619084 (Oregon Sugar Pod)	USA	28.4 <sup>kl</sup>	38.0 <sup>ef</sup>
PI 574505 (Common)	USA	100.0 <sup>a</sup>	93.3 <sup>abc</sup>
US 647868 (Windham)	USA	100.0 <sup>a</sup>	93.6 <sup>abc</sup>
Taşkent	Konya, TR	100.0 <sup>a</sup>	89.3 <sup>abc</sup>
Özkaynak	Konya, TR	100.0 <sup>a</sup>	78.8 <sup>bcd</sup>
B-8	Konya, TR	79.3 <sup>e</sup>	77.6 <sup>cd</sup>
Mean		76.6	62.7

TR – Turkey; values followed by the same letter in a column are not significantly different ( $P = 0.05$ ) based on LSD

Table 5. Correlation coefficients of the mean percent survival of pea genotypes between the test environments in two experiments

Field	Field		Laboratory (°C)			
	Haymana		–4		–8	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
<b>Experiment I</b>						
Ulas	0.846	< 0.0001	0.683	0.0002	0.678	0.0002
Haymana			0.845	< 0.0001	0.713	< 0.0001
<b>Experiment II</b>						
Ulaş	0.868	< 0.0001				

level of survival at  $-8^{\circ}\text{C}$  (Table 3). The most winter-hardy US cultivar Melrose had the mean survival of 63%, which is similar to the survival level of 60% at  $-9^{\circ}\text{C}$  reported by AULD *et al.* (1983a). Although three winter lines/cultivars including Russian (former Soviet Union) line PI 392019, US cv. Melrose and Turkish elite winter cv. Özkaynak exhibited partial survival at  $-12^{\circ}\text{C}$  two weeks after freezing, they did not survive the third week when the survival count was done. No plants survived at  $-12^{\circ}\text{C}$  and  $-16^{\circ}\text{C}$  three weeks after freezing.

FIEBELCORN (2013) reported that the test temperature of  $-12^{\circ}\text{C}$  was too harsh because of the nearly complete mortality across all winter lines tested including the most winter-hardy cv. Melrose, and suggested that freezing at  $-8^{\circ}\text{C}$  resulted in good differential survival. An optimum screening protocol for an artificial freezing test for the better prediction of winter hardiness would speed up the screening process.

The variation in survival of local landraces in the laboratory was less distinct than in the field. While none of the landraces survived at  $-8^{\circ}\text{C}$  in the laboratory, they had relatively low survival percentages in the field. This could be due to the fact that landraces are heterogonous populations, thus showing less consistent responses between the test environments. AULD *et al.* (1983b) reported similar observations in  $F_2$  populations in pea. Under the field environment winter survival of peas is affected by various environmental factors, e.g. snow cover, wind, soil moisture, soil drainage (AULD *et al.* 1983b). Furthermore, in the field, soil temperatures are warmer than air temperatures, and roots and crowns of plants are protected by soil. Controlled environments permit the more rigid control of freezing conditions, and plants reach the target temperatures (ANDERSON *et al.* 2007).

In this study correlation coefficients for the mean survival (%) of genotypes between test environments ranged from 0.68 to 0.87 ( $P < 0.001$ ) (Table 5). This suggests that identifying non-hardy genotypes could be possible under both field and laboratory environments. However, final evaluation for winter hardiness should be done under field conditions (AULD *et al.* 1983b). Most of the winter-type germplasm tested in this study had high winter survival. They can be used as parents in crosses for future breeding programs to develop winter-hardy cultivars with improved agronomic traits (i.e. high forage and seed yield).

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*Corresponding author:*

Dr. AZIZE HOMER, Ph.D., Central Research Institute for Field Crops, Ankara, Turkey; e-mail: ademirbas@hotmail.com

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