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Screening Peas for Winterhardiness Under Field and Laboratory Conditions[1]

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ABSTRACT

Winter injury of the winter pea (*Pisum sativum* subsp. *arvense* (L.) Poir) crop of northern Idaho causes severe losses. The development of cultivars with higher levels of winterhardiness would increase both the seed yield and area of adaptation of this crop. To evaluate techniques for determining winterhardiness in peas, 14 genotypes of winter-hardy peas, and six genotypes of spring peas (*P. sativum* L.), were screened in the field and laboratory. Trials were planted on three dates at Moscow, Idaho, and a single date at Bozeman, Mont., during the fall of 1976 and 1977. Seedlings were counted in both the fall and spring to determine the percent survival. At all planting dates and locations, the spring pea cultivars failed to survive the winter, indicating that spring types can be eliminated from segregating populations by fall planting. Three lines, 'Romack', ID 89-1, and C8-M-23, appeared to be intermediate in winterhardiness and could be used as controls in future trials. Screening at −9 C in a programmable freezer eliminated all spring cultivars and produced differential survival among the winter-hardy lines. Correlation coefficients between percent survival of the 13 winter-hardy lines in the eight field environments and three test temperatures indicated that screening in the laboratory at −9 C successfully identified those lines with intermediate levels of winterhardiness. Fourteen lines of the *P. sativum* and four lines of the *P. arvense* USDA plant introduction collection had usable levels of winterhardiness in field trials at Moscow.

***Additional index words:* *Pisum sativum* subsp. *arvense* (L.) Poir, *Pisum sativum* L., Selection, Plant introductions.**

AUSTRIAN winter peas (*Pisum sativum* subsp. *arvense* (L.) Poir) were introduced into northern Idaho in 1936 and are currently grown on approximately 20,000 hectares annually (6). This crop is usually seeded in September and harvested the following August as dried seed. During severe winters, stands of peas may be reduced or lost due to winter injury. The development of Austrian winter pea cultivars with increased winterhardiness would reduce winter losses and improve seed yield.

Because the winter survival of peas is dependent upon cultural and environmental factors such as seeding date, plant population, snow cover, and soil drainage; screening for winterhardiness in the field is difficult (1,2,3,4,5). However, in most cooler climates, spring peas (P. *sativum* L.) that lack winterhardiness fail to survive even moderate winters. Separation among winter-hardy lines under field conditions often requires replanting trials for several years before differential survival is detected. Recently a procedure was developed in which pea seedlings were evaluated for winterhardiness in a programmable freezer (7). This procedure allows rapid separation of hardy and nonhardy pea lines, but it has not been evaluated as a tool for comparing the relative winter hardiness of hardy lines.

The purposes of these studies were to 1) determine if changes in planting dates or test locations could improve field separation, 2) compare results from field and laboratory screening techniques, and 3) identify lines with differential winterhardiness for use in future studies. In addition, several hundred lines of the USDA *Pisum* germplasm collection were screened to identify additional sources of winterhardiness.

MATERIALS AND METHODS

Field Screening

Thirteen lines of winter-hardy peas and six cultivars of spring peas were evaluated in the field at Moscow, Idaho; and Bozeman, Mont. during the winters of 1976-1977 and 1977-1978, to determine their relative winterhardiness. At Moscow, the 19 lines were planted in mid-August, mid-September, and mid-October 1976 and 1977 in fields which had been summer fallowed. Fifty seeds of each line were planted 7.6 cm apart in rows spaced 50 cm apart. Each row was 3.8 m in length. A split-plot design with dates of planting as main plots and lines as subplots was used. There were three replications in each year of the study. An infestation of adult pea leaf weevils in early September 1976 was controlled with an application of phosmet [*N*-(mercaptomethyl) phthalimide S-ester *O,O*-dimethyl phosphorodithate] at the rate of 5.4 kg/ha. In 1977-1978 the field was sprinkler irrigated immediately after planting to insure uniform, rapid emergence. Thirty days after emergence, the seedlings within each planting date were counted to determine the number of established seedlings. Seedlings were counted on 8 Apr. 1977 and 28 Mar. 1978. Plants with succulent, green tissue were considered to have survived.

At Bozeman the fields were irrigated prior to being planted with a cone seeder on 15 Sept. 1976 and 27 Sept. 1977. Each line was planted in a 6.1-m row spaced 61 cm apart. Counts were made approximately 30 days after emergence to determine the number of established plants. Surviving seedlings were counted on 28 Apr. 1977 and 27 Apr. 1978. These studies were conducted as randomized complete-block designs with three replications.

The mean percentages of winter survival of lines at each planting date and location were analyzed by a separate analysis of variance. Means were separated by Duncan's new multiple range test where F-tests indicated significant differences.

Laboratory Screening

Seeds of the 19 lines were scarified by scratching the seed coat on one cotyledon and then soaking in aerated, distilled water for 16 hours. The imbibed seed of each line were transplanted into

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Table 1. Percent survival of 13 winter pea lines and 6 spring pea cultivars evaluated for winter hardiness in eight field environments and at three temperatures in the laboratory.

Line	Planting date—Moscow, Idaho						Planting date—Bozeman, Mont.		Laboratory test temperature		
	16 Aug. 1976	16 Sept. 1976	14 Oct. 1976	15 Aug. 1977	15 Sept. 1977	15 Oct. 1977	15 Sept. 1976	27 Sept. 1977	−3 C	−6 C	−9 C
	% survival										
Winter lines											
Melrose	92 a*	59 a*	49 bcd*	97 a*	97 ab*	84 ab*	64 ab*	95 ab*	100 a*	100 a*	60 ab*
Fenn	95 a	44 ab	58 abc	100 a	100 a	92 a	49 abc	96 ab	97 a	93 a	72 ab
Common	98 a	56 a	63 ab	97 a	99 a	81 abc	51 abc	98 a	96 a	100 a	72 ab
Romack	57 b	25 bc	45 bcd	76 b	100 a	68 cd	24 d	92 ab	100 a	96 a	42 bcd
EFB 33	96 a	44 ab	38 cd	99 a	99 a	86 ab	42 bcd	99 a	100 a	97 a	54 abc
ID-2	100 a	55 a	75 a	95 a	100 a	84 ab	58 ab	98 a	97 a	97 a	66 ab
ID 89-1	65 b	18 cd	30 d	91 ab	100 a	94 a	33 cd	100 a	93 a	81 ab	16 de
ID 113	94 a	48 ab	61 abc	94 ab	99 a	72 bcd	51 abc	97 ab	100 a	90 a	64 ab
ID 135-8	99 a	47 ab	56 abc	94 ab	98 a	86 ab	51 abc	86 b	100 a	100 a	62 ab
ID 138	97 a	44 ab	59 abc	95 a	99 a	66 d	63 ab	98 a	93 a	100 a	77 a
C8-M-23	94 a	56 a	46 bcd	89 ab	97 ab	65 d	51 abc	98 a	91 a	88 b	27 cde
C14-M-8	95 a	53 a	68 ab	91 ab	95 b	82 abc	68 a	96 ab	100 a	96 a	56 abe
C26-25-26	99 a	36 ac	59 abc	98 a	97 ab	81 abc	55 abc	100 a	96 a	95 a	80 a
Avg.	91	45	54	94	99	80	51	96	97	94	57
Spring cultivars											
Tracer	0 c	0 d	0 e	0 c	0 c	0 e	0 e	12 c	85 a	47 c	0 e
Dark Grey Sugar	0 c	0 d	0 e	0 c	0 c	0 e	1 e	4 cd	41 bc	61 bc	0 e
Dark Skin Perfection	0 c	0 d	0 e	0 c	0 c	0 e	0 e	0 d	23 c	7 d	0 e
Alaska	0 c	0 d	0 e	0 c	0 c	0 e	0 e	0 d	33 c	47 c	0 e
Latah	0 c	0 d	0 e	0 c	0 c	0 e	0 e	4 cd	67 ab	34 cd	0 e
Garfield	0 c	0 d	0 e	0 c	0 c	0 e	0 e	5 cd	30 c	8 d	0 e
Avg	0	0	0	0	0	0	0	4	47	34	0

* Means not followed by the same letter differ at the 0.05 level of probability by Duncan's New Multiple Range Test.

growth pouches (7). The growth pouches were filled with distilled water and supplemented with a complete Hoagland's solution. Seedlings were grown for 7 days at 25 C under continuous 40-W fluorescent light at 90 μ E m^{-2} sec^{-1}. When the seedlings had two fully expanded leaves, they were transferred to a growth chamber maintained at 4 C and supplied with a 9-hour photoperiod by 40-W fluorescent light at 53 μ E m^{-2} sec^{-1}. After the seedlings had hardened for 4 weeks in this environment, the growth pouches were transferred to styrofoam boxes.

The boxed seedlings were frozen in a programmable chest freezer using a procedure described by Swensen (7). Boxes of seedlings were placed in the freezer chamber at 0 C and cooled from 0 to −3 C at a rate of 2 C hour^{-1}. Air temperature in the freezing chamber was held at −3 C for 15 hours to ensure that nucleation occurred in the seedlings. Seedlings to be evaluated at −3 C were removed, and the air temperature was then lowered at a rate of 2 C hour^{-1} from −3 C to −6 C. An hour was allowed for temperature equilibration, and the seedlings to be evaluated at −6 C were removed. The temperature was then lowered to −9 C at the same rate, and after 1 hour of temperature equilibration the final group of seedlings was removed. Seedlings were moved directly from the freezer into a 4 C chamber and allowed to thaw for 15 hours. Thawed seedlings were transplanted into greenhouse flats containing a potting mix composed of equal proportions by volume of sand, peat, and perlite. The seedlings were placed on a light table at 25 C with a 16-hour photoperiod provided by 40-W fluorescent light at 90 μ E m^{-2} sec^{-1}. The number of surviving plants was counted 4 weeks following the freezing test with completely desiccated or decayed plants classified as dead. Percent survival was computed for each growth pouch.

This study was conducted as a randomized complete block design with the three replications staggered in time. Percent survival data of lines were analyzed for each test temperature, and means were separated by Duncan's New Multiple Range Test. Correlation coefficients were calculated between the percent survival in the eight field environments and three laboratory test temperatures using the values for all 19 lines and for only the 13 winter-hardy lines.

Screening Plant Introductions

On 17 Aug. 1977, 370 lines of the USDA collection of *P. sativum* L., a nonhardy pea cultivar ('Alaska'), and a winter-hardy cultivar ('Fenn') were planted at Moscow. Fifteen seeds of each line were planted 6.5 cm apart in single rows spaced 46 cm apart. The cultivars, Alaska and Fenn, were seeded between every 20 plant introduction lines for comparison of winter survival between lines. Each plant introduction line occurred only once. The field was sprinkler irrigated immediately after planting to insure uniform emergence. Counts of established plants were made 40 days after emergence. On 28 Mar. 1979 counts were made to determine the percentage of winter survival using the procedure previously described.

In May 1978 five seeds from each line of the USDA collection of *P. arvense* were planted in the greenhouse. Plants were indexed with *Chenopodium amaranticolor* L. to determine if they were infected with pea seed borne mosaic virus. Plants with positive virus readings were destroyed. Virus-free seeds were planted at Moscow on 24 Aug. 1978 in a field which was irrigated prior to planting to insure uniform emergence. Fifteen seeds of each entry were planted 6.4 cm apart in single rows spaced 46 cm apart. A total of 422 individual plant selections representing 99 plant introductions, four winter pea lines, and two spring pea lines were evaluated. The seedbed was sprayed with dinoseb amine (2-*sec*-butyl-4, 6-dinitrophenol) at the rate of 6.7 kg/ha immediately after planting to control broadleaf weeds. Counts of established plants were made 42 days after emergence. The trial was conducted as a randomized complete block design with two replications. On 20 Mar. 1978 counts were made to determine the percent winter survival for each line.

RESULTS AND DISCUSSION

Field and Laboratory Screening

Differential levels of survival were detected between the winter pea lines and the spring cultivars in all eight field

Table 2. Correlation coefficients (r) of percent survival of 13 winter pea lines and six spring pea cultivars evaluated for winter hardiness in eight field environments and at three temperatures in the laboratory.†

Test	Planting date—Moscow, Idaho						Planting date—Bozeman, Mont.		Laboratory test temperature		
	16 Aug. 1976	16 Sept. 1976	14 Oct. 1976	15 Aug. 1977	15 Sept. 1977	15 Oct. 1977	15 Sept. 1976	27 Sept. 1977	−3 C	−6 C	−9 C
Location and planting date	r value										
Moscow, Idaho											
16 Aug. 1976	--	0.96**	0.96**	0.98**	0.97**	0.95**	0.97**	0.97**	0.85**	0.91**	0.93**
16 Sept. 1977	0.78**	--	0.94**	0.92**	0.90**	0.88**	0.96**	0.90**	0.80**	0.86**	0.87**
14 Oct. 1976	0.62*	0.59*	--	0.94**	0.93**	0.91**	0.95**	0.93**	0.83**	0.88**	0.94**
15 Aug. 1977	0.76**	0.42	0.24	--	0.99**	0.99**	0.94**	0.99**	0.88**	0.92**	0.90**
15 Sept. 1977	0.37	0.46	0.24	0.06	--	0.98**	0.92**	0.99**	0.88**	0.92**	0.87**
15 Oct. 1977	0.03	0.14	0.13	0.51	0.07	--	0.90**	0.98**	0.87*	0.90**	0.85**
Bozeman, Mont.											
15 Sept. 1976	0.81**	0.76**	0.67*	0.54	0.62*	0.03	--	0.92**	0.82**	0.87**	0.91**
27 Sept. 1977	0.10	0.07	0.07	0.34	0.11	0.02	0.10	--	0.89**	0.91**	0.86**
Laboratory test temperatures											
−3 C	0.03	0.13	0.19	0.02	0.17	0.19	0.03	0.52	--	0.89**	0.78**
−6 C	0.47	0.48	0.50	0.22	0.20	0.13	0.43	0.39	0.50	--	0.85**
−9 C	0.68**	0.41	0.72**	0.57*	0.06	0.00	0.56*	0.03	0.32	0.73**	--

*,** Significant at the 0.05 and 0.01 level of probability, respectively.
† Upper diagonal has correlation coefficients of all 19 lines and lower diagonal has correlation coefficients of 13 winter hardy lines.

environments and at all three test temperatures in the laboratory (Table 1). None of the spring pea cultivars survived in the field at any planting date at Moscow. At Bozeman during 1977-1978, the pea seedlings were protected by a snow cover during most of the winter and several spring cultivars had a low percentage of survival. Under this snow cover, the winter pea lines averaged 96% survival which would have allowed the separation of winter and spring lines.

At −3 C in the laboratory, the spring cultivars 'Tracer' and 'Latah' survived as well as the winter pea lines (Table 1). At −6 C, most spring cultivars had a survival percentage lower than the winter hardy lines, but 61% of the seedlings of 'Dark Grey Sugar' survived. At −9 C all of the spring cultivars were completely eliminated. This indicates that segregating populations should be screened at −9 C in the laboratory to insure elimination of nonhardy types.

Differential survival was detected among the 13 winter pea lines in all eight field environments as well as at −6 and −9 C in the laboratory (Table 1). 'Romack' had poor survival in six of the field environments and at −9 C in the laboratory. ID 89-1 had poor survival in four field environments and at −9 C. The lines C8-M-23 and EFB 33 had poor survival in two field environments. The line C8-M-23 also survived poorly at both −6 and −9 C. The lines 'Melrose' ID 135-8, ID 113, ID 138, and C14-M-8 had poor survival in only one field environment, which may have been due to positional effects within the study and may not reflect true winterhardiness. However, the lines Romack, ID 89-1 and C8-M-23 probably do have a lower level of winterhardiness than the other hardy lines as suggested by screening data at −9 C under laboratory conditions.

Correlation coefficients calculated for the percent survival of all 19 pea lines between test environments ranged from 0.80** to 0.99** (*, ** significant at the 0.05 and 0.01 levels, respectively) (Table 2). These high values indicate that selection for winterhardiness in any field or laboratory test environment would probably identify nonhardy lines. Correlation coefficients calculated on the survival percentage of the 13 winter pea lines between test environments

Table 3. Winter survival of 14 PI lines of *Pisum sativum* L. and 15 single plant selections from four PI lines of *P. arvense* from the USDA collection evaluated at Moscow, Idaho.

Line	Origin	Flower color	Winter survival
			%
P. sativum 1977-1978:			
PI 102888	China	Purple	100
PI 125673	Austria	Purple	44
PI 163131	India	Purple	100
PI 167253	Turkey	Purple	93
PI 173054	Turkey	White	100
PI 173780	Turkey	White	100
PI 180867	Turkey	White	83
PI 180868	Turkey	Purple	100
PI 184128	Yugoslavia	White/Purple	57
PI 189168	Netherlands	White/Purple	79
PI 210641	Iowa	White	100
PI 220184	Afganistan	Purple	69
PI 251051	Czechoslovakia	Purple	100
PI 269818	England	Purple	93
Fenn†	--	Purple	100
Alaska	--	White	0
P. arvense 1978-1979:			
PI 358620	Ethiopia	White	
Selection A			0
Selection B			0
Selection D			0
Selection E			72
PI 377693	Arkansas	Purple	
Selection A			97
Selection B			100
Selection D			93
Selection E			89
PI 383730	Turkey	Purple	
Selection A			100
Selection B			91
Selection C			20
Selection D			73
PI 383731	Turkey	Purple	
Selection B			93
Selection C			62
Selection D			100
Garfield	--	White	0
ID 89-1	--	Purple	44
Romack	--	Purple	87
Fenn	--	Purple	91
Melrose	--	Purple	94

† Fenn—*P. arvense* control; Garfield—*P. sativum* control.

ranged from 0.00 to 0.81** (Table 2). Thirteen of 45 correlation coefficients were significant, indicating that separation of the relative levels of winterhardiness among winter pea lines was difficult. Screening at −9 C in the laboratory was significantly correlated with four of the eight field environments, but screening at −3 and −6 C in the laboratory was not correlated with any field environment.

These results indicate that screening in the laboratory at −9 C could be used either to screen segregating populations to eliminate nonhardy types or as a progeny test of advanced lines to identify those with only intermediate levels of hardiness. The laboratory procedure could greatly facilitate the development of new cultivars with high levels of winter hardiness.

Screening Plant Introductions

Of the 370 plant introduction (PI) lines of *P. sativum* seeded in the early fall of 1977, only 14 lines had seedlings survive the winter (Table 3). All of the nonhardy Alaska seedlings died while nearly 100% of the hardy Fenn seedlings survived. Only 3.8% of the PI lines of *P. sativum* had sufficient winterhardiness to survive even the relatively mild winter of 1977-1978. Seven of the lines had a survival percentage equivalent to Fenn. They were collected from such diverse locations as China, Austria, and Turkey. Two PI lines, PI 173054 and PI 173780, with white seed coats had 100% winter survival.

Only 4% of the 99 PI lines of *P. arvense* had single plant selections survive the winter of 1977-1978 (Table 3). The single plant selections from PI 377693 and PI 383731 appeared to survive the winter as well as the winter-hardy cultivars, of Fenn and Melrose. PI 377693 is a landrace of Austrian winter peas. PI 358620 and PI 383731 appeared to be heterogenous for winterhardiness based upon the differential survival of single plant selections made from these PI lines.

Because the winter-hardy PI lines come from diverse origins, their incorporation into crosses and subsequent selection could result in the development of cultivars with increased levels of winterhardiness. The use of these PI lines as sources of improved agronomic traits would also allow the improvement of winter pea cultivars without diluting the level of winterhardiness. The identification of winter hardiness in white-seeded lines may allow the utilization of winter-hardy peas for processing and dry edible pea crops. Such cultivars are currently used to extend the period during which fresh peas are processed (3) in Central Europe which has relatively mild winters.

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