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Genetic Analysis of Cold Hardiness in Peas (*Pisum sativum* L.)

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Abstract: Four pea lines as female parents (Carina, Sprinter, Cosmos, Manuel) were crossed with three male tester parents (B_1, B_6 and B_{12}) and 12 F_1 hybrids and 12 F_2 populations were evaluated in Konya, during 2000-2001 and 2001-2002 growing seasons. General and specific combiners suitable for use in breeding programmes were identified from a line×tester cross in peas. Although several genetic studies have been conducted, there is not a general consensus as the made gene action controlling the expression of winter hardiness in pea. The inheritance of cold hardiness was controlled mainly by non- additive gene action. Sprinter and B_{12} were good general combiners for cold hardiness. The best specific combiners were Sprinter×B_1 for cold hardiness. The average level of heterosis and heterobeltiosis for winter hardiness in F_2 population was low that in F_1 hybrids. Broad sense heritability was high due to the major role of environmental factors in expression of winter hardiness in pea. Thus, delay selection was suggested for breeding of winter resistance.

Key words: *Pisum sativum*, winter hardiness, selection

Introduction

Pea seeds are rich in protein and most mineral elements. Pea varieties are sown in the spring and their yields are very variable, being very dependent on the temperature and water supply at the end of their growth cycle. Autumn-sown varieties may have higher and more stable yields than spring-sown ones: they also enter the flowering period earlier and so avoid stresses due to the high temperatures and poor water supply which are frequently encountered in late spring. Hovewer, autumn sowing is only possible if the varieties are winter hard (Bourion *et al*., 2003). Winter hardiness is one of the major a biotic stresses in pea. Winter hardiness is a complex quantitative trait conditioned by the plant genotype and the environment in which is grown. Current varieties grown in World are considered as cold tolerant. Winterhardiness has been sought by North American and French scientists (Auld *et al*., 1983; Eteve, 1985) and although some level tolerance to cold has been obtained it remains a challenge (Murray and Swensen, 1991). The level of winter hardiness does not match that of the winter cereals.

Most winter pea breeding programs have used winter *Pisum fulvum* pea because of their good resistance to freezing: such genotypes can survive temperatures of -6°C (no damage to leaves; 100% survival) (Balackova *et al*., 1986). Although several genetic studies have been conducted, there is not a general consensus on the made of gene action controlling the expression of cold hardiness in pea. Genetic studies on winter hardiness in pea have rivaled that it is quantitative in nature and governed by intermediate dominance (Cousin *et al*., 1985) and additive genes (Auld *et al*., 1983). Liesenfeld *et al*. (1986) reported that as few as three or four genes or linkage groups could be responsible for winter hardiness. They also observed that winter survival is associated with genes which produce pigmented hilum and pigmented seed coat.

Cultivation of pea in Turkey is very limited mainly in Western Anatolia region, compared to beans, chickpea and lentils, which constitute approximately 95% of grain legumes production. This research aims to investigate to inheritance of cold hardiness in pea as a good source of plant protein. If winter tolerant or resistant varieties can be selected and grown successfully, pea can be involved in crop rotation in Turkey.

Materials and Methods

Seven pea lines, Carina, Sprinter, Cosmos, Manuel representing the main cultivars used in dried and canning pea breeding and B_1, B_6 and B_{12} (obtained by Dr. Ahmet TAMKOÇ) representing a wide range in winter survival ability were chosen as parental material. The four pea cultivars (lines) and tree lines (tester) were made by Line x Tester. The seven parents were established under the field condition during 2000 summer growing season and crossed in 12 combinations including Line×Tester. The 12 F_1 and F_2 populations with the seven parents were planted in a randomized complete blocks design with tree replications at the experimental site of Faculty of Agriculture, Selcuk University, in Konya, during 2000-2001 and 2001-2002 growing seasons. While the plots consist of one rows in F_1 populations, F_2 populations consist of three rows, each row containing five plants.

Cold hardiness was examined in F_1 and F_2 populations. All plant material tested for cold hardiness was grown under field conditions. F_1 populations were scored according to five categories (1= extremely cold sensitive, with 100% plants killed; 5 = most cold tolerant, with less than 25% plants killed) (Guye *et al*., 1987), as F_2 populations were survival on the each plot. Data were collected on plants in the each plot.

In both experimental years, monthly minimum, maximum and mean temperature values were higher than observed for 10-year mean (Table 1). Similarly, total rainfall values were higher than observed for the 10-year mean in both grown seasons. First grown season was lower than 10-year mean value of 60.4%. However, 10-year was higher than second grown seasons.

Regarding statistical analysis, parents and F_2 population data were analyzed together as suggested by Sing and Chaudhary (1979). The combining ability analysis was done following Kempthore (1957). Broad and narrow sense heritabilities were calculated for each character by using the Falconer's (1982) methods. Heterosis over mid-parent (HMP) or the better parent (HBP) of the observed characters were also calculated.

Results and Discussion

Growth Habit and Winter Hardiness

Cold hardiness was lower at F_2 populations than at F_1 hybrids. At a given sub-zero temperature in the ranged 0 to -16.8°C the freezining injury observed in the field. At below -10°C, damage first appears on the edge of the leaflets, later on the whole leaflets, the main stem. The young tissues are more resistant. The B_1, B_6 and B_{12} lines had not injury at -16.8°C on January in 2002. The F_1 and some F_2 populations had more resistant than the female. Frost occurring on January of relatively minimum temperatures destroyed in plants from F_2 populations and the female. However, a period of frost in 2000-2001 grown seasons of F_1 hybrids had not cause any injury. The air temperatures gradually drop during the winter plants starts to redevelop cold resistance. Very high levels of cold hardiness are obtained by B_1, B_6 and B_{12} lines among the parents. The highest level of cold hardiness was obtained by Sprinter×B_{12} crosses among the hybrids. This region, air minimum temperatures ranged from 0 to -25.0°C (Table 1) while short periods at -13.0°C air temperatures have

Table 1: Monthly minimum, maximum and mean temperature, relative moisture and total rainfall during the 2000, 2000-2001, 2001-2002 and 10- years average growing seasons

	Temperature (°C)											
	1990–1999			2000			2000-2001			2001-2002		
Months	Min.	Max	Mean	Min.	Max	Mean	Min.	Max	Mean	Min.	Max	Mean
October	-2.8	31.4	12.9	-----	-----	-----	-0.8	27.4	11.2	-3.2	29.0	12.8
November	-16.2	23.0	5.4	-----	-----	-----	-4.9	21.4	6.9	-10.4	20.1	5.9
December	-14.6	20.0	1.4	-----	-----	-----	-9.0	16.0	1.4	-13.6	13.8	2.4
January	-18.2	16.2	-0.9	-----	-----	-----	-12.2	16.8	2.4	-16.8	12.2	-5.9
February	-25.0	18.6	-0.3	-----	-----	-----	-12.2	17.6	2.5	-8.8	15.8	3.1
March	-11.8	25.2	4.2	-11.8	25.2	4.0	-4.3	28.9	11.0	-2.4	23.2	7.7
April	-8.6	30.0	10.3	-2.0	26.0	12.7	-0.2	28.6	12.7	-0.6	25.0	9.7
May	1.7	33.4	15.4	4.4	26.0	14.7	4.0	30.4	15.0	4.0	28.6	15.2
June	3.7	36.7	19.8	3.7	33.4	19.6	7.9	35.2	22.5	5.4	34.6	19.8
July	8.6	40.6	23.3	13.2	40.6	26.4	13.0	38.4	26.3	11.0	36.5	24.1
Mean	-25.0	40.6	9.2	-11.8	40.6	15.0	-12.2	38.4	11.2	-16.8	36.5	9.5

	Total rain (mm)				Relative moisture (%)			
Months	1990-99	2000	2000-01	2001-02	1990-99	2000	2000-01	2001-02
October	34.8	-----	32.3	1.9	55.0	-----	60.0	43.8
November	32.5	-----	26.2	50.1	66.5	-----	60.5	72.1
December	38.4	-----	22.1	118.4	75.8	-----	79.1	79.7
January	27.2	-----	1.0	27.8	74.6	-----	71.1	80.0
February	17.5	-----	10.6	12.9	69.8	-----	65.7	69.3
March	28.4	11.2	3.7	24.2	62.5	57.0	50.0	55.8
April	36.5	38.7	14.1	70.0	57.6	55.4	47.8	67.2
May	39.8	56.2	66.0	22.9	56.0	55.4	57.2	53.9
June	26.5	17.6	0.7	15.3	46.9	42.3	36.4	47.5
July	8.1	----	1.3	27.1	39.3	27.6	35.2	39.8
Mean	189.7	112.7	178.0	370.9	60.4	58.5	56.3	60.9

Table 2: Mean squares related to cold hardiness in line x tester analysis

Source	df	F_2 population
Replications	2	11.106
Hybrids	11	568.634**
Lines	3	481.766**
Testers	2	770.300**
Line×testers	6	544.848**
Error	22	83.827

**: p<0.01

Table 3: Estimates of components of genetic variance for cold hardiness in pea

Parameters	F_2 population
σ^2GCA	1.026
σ^2SCA	153.674
σ^2GCA/ v^2SCA	0.006
σ^2D	2.052
σ^2H	153.674
$(H/D)^{1/2}$	74.890
H^2	0.85
h^2	0.01

σ^2GCA: General Combining ability. σ^2SCA: Specific Combining ability. σ^2D: Additive variance. σ^2H: Dominant Variance. H^2: Broad sense heritability. h^2: Narrow sense heritability

little effect on cold hardiness pea hybrids, long period of -13.0°C cause severe damage. Balackova *et al*. (1986) obtained that among the *Pisum fulvum* cultivars, a temperature of -6°C caused no damage to leaves (100% survival). Resistance in the cultivars of *Pisum sativum* ranged 5 to 100%; it was highest in four conventional lines and five short-stemmed lines. In France, air temperatures -23°C and below are considered lethal to overwintering pea, while air temperatures of -6 to -14°C are not injurious to fully hardened pea (Eteve, 1985). Our some hybrids survived exposure to air temperatures of -16.8 ^{0}C without snow cover in field. The findings of this study agree with reported by above literatures.

The analysis of variance of F_2 populations indicated that variation among crosses was significant for winter hardiness (Table 2). Statically analysis of the results showed a highly significant difference ($p<0.01$) in winter hardiness of hybrids, lines, tester and line×tester in 2001-2002 grown seasons.

Table 4 presets the data from a screening analysis of parents, F_1 and F_2 population cold tolerance and score in the field. The survival percentage ranged from 6.62 to 100.00% among the parents and the survival percentage of hybrids was 100.00%. The percentage survival of F_2 populations which were found to be cold tolerant and of the most susceptible lines is given in Table 4. The most tolerant was B_1, B_6, B_{12} lines and F_1 hybrids. In the F_2 populations, the survival percentage ranged from 31.10 to 100.00%. The highest cold tolerant was obtained to Sprinter×B_{12} hybrids.

General Combining Ability (GCA) and Specific Combining Ability (SCA)

The estimations of the variances of general combining ability (σ^2GCA), specific combining ability (σ^2SCA) additive and dominance are presented in Table 3. The differences of σ^2GCA are mainly due to the additive variance in the population whilst the differences of σ^2SCA are attributable to the non-additive genetic variance (Falconer, 1982). The σ^2SCA variance was higher than σ^2GCA variance for winter hardiness. This results show that non additive gene effects were important in the inheritance for winter hardiness, it revealed over dominance effect on winter hardiness.

There has been some of the ambiguity in the literature with respect to the inheritance of winter hardiness. Cousion *et al*. (1985) observed intermediate dominance, while Auld *et al*. (1983) observed additive genes. In contrast, this result confirms nature of dominance and non-additive genes for winter hardiness. Liesenfeld *et al*. (1986) obtained three or four genes or linkage groups for winter hardiness. This study showed that the over-dominant effects and non-additive effects were important in the inheritance of winter hardiness.

General and specific combining ability for cold hardiness varied significantly among parents and hybrids (Table 4). Sprinter showed highly significant positive GCA for cold hardiness (7.591), while Carina had significant negative GCA for cold hardiness. B_{12} line had the largest significant positive GCA for cold hardiness at F_2 populations. B_1 line had significant negative GCA for cold hardiness. Considering SCA effects, Carina×B_{12} and Carina×B_1 had the highest (11.853**) and the lowest (-19.259**) SCA effects for cold hardiness at F_2 populations. Sprinter×B_1, Sprinter×B_{12}, Bolero×B_1, Manuel×B_6 and Carina×B_6 had positive SCA effects for cold hardiness; while others F_2 populations had negative SCA effects.

GCA for males was greater than for females in cold hardiness, reflecting the greater diversity among the male parents. Among the parents, Sprinter cultivar and B_6 line were chosen as source of cold hardiness for this breeding programme. In this study, two crosses showed significant positive SCA for cold hardiness and two crosses showed a significant negative SCA effect in F_2 population, indicating non-additive gene action. Of the 12 F_2 progenies analyzed, Sprinter×B_1, Sprinter×B_{12}, Manuel×B_6, Carina×B_6 and Carina×B_{12} confirmed their cold hardiness (Table 4). All selected progenies will be advanced in the next generation. The results obtained to in this breeding programme are encouraging. Selected cold hardiness breeding lines for grain yield is better than the best control used in the programme under the field (not show here).

Table 4: General and specific combining ability and heterosis (%) values over mid-parent (HMP) and better-parent (HBP) related to cold hardiness in pea

	F_1 population		F_2	
Lines/Testers	Survival (%)	Score	Survival (%)	GCA
Sprinter	20.00	1	12.22	7.591*
Bolero	14.00	1	6.67	4.630
Manuel	15.00	1	8.89	-4.260
Carina	18.00	1	8.89	-7.961**
B_1	100.00	5	100.00	-6.667**
B_6	100.00	5	100.00	-2.222
B_{12}	100.00	5	100.00	8.888**

	F_1 population		F_2 population		F_2 population	
Hybrids	Survival (%)	Score	Survival (%)	SCA	HMP (%)	HBP (%)
Sprinter×B_1	100.00	5	77.78	11.852*	38.61	-22.22**
SprinterxB_6	100.00	5	57.78	-12.593*	2.97	-42.22**
SprinterxB_{12}	100.00	5	82.22	0.741	46.53	-17.78*
BoleroxB_1	100.00	5	71.11	8.147	33.33	-28.89**
BoleroxB_6	100.00	5	64.45	-2.962	20.83	-35.55**
BoleroxB_{12}	100.00	5	73.33	-5.185	37.50	-26.67**
ManuelxB_1	100.00	5	53.33	-0.740	-2.04	-46.67**
ManuelxB_6	100.00	5	66.67	8.148	22.45	-33.33**
ManuellxB_{12}	100.00	5	62.22	-7.408	14.28	-37.78**
CarinaxB_1	100.00	5	31.11	-19.259**	-42.85	-68.89**
CarinaxB_6	100.00	5	62.22	7.406	14.29	-37.78**
CarinaxB_{12}	100.00	5	77.78	11.853*	42.86	-22.22**
Mean				19.06	-35.00	

*: $p<0.05$. ** : $p<0.01$

Heterosis over Mid-parent (HMP) or the Better Parent (HBP)

The average values of HMP (19.06%) were found positive in cold hardiness. In contrast, the average values of HBP (% -35.00) were found negative in cold hardiness. These values ranged from -42.88 and -68.89% (HMP and HBP, respectively), for Carina×B_1 cross, to 46.53 and -17.78%, for Sprinter×B_{12} cross. Most of the values of HMP for cold hardiness were positive and insignificant, while the values of HBP for cold hardiness were all negative and significant (Table 4).

Table 4 gives the values of broad and narrow sense heritability for the cold hardiness. Broad and narrow sense heritability of cold hardiness was 0.85 and 0.01. Cold hardiness had the greatest heritability for broad sense heritability. Broad sense heritability was high due to the major role of environmental factors in expression of this trait. The narrow sense heritability of cold hardiness was relatively high suggesting that selection of cold hardiness in early generations would not be possible.

In conclusion, these results show that pea hybrids will be used successfully to improve cold hardiness. From the results of the breeding programme, we were able to improve some cold hardiness in pea cultivars. The hybrids were characterized by higher cold hardiness than the parents. In addition, new germplasm for useful morphological and agronomic traits were improved which will be used for combining different desirable traits in breeding programmes.

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