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Determination of Optimum Cold Acclimation Period under Controlled Conditions and Winter Resistance of Some Winter Wheat Genotypes

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ABSTRACT

The fact that climate conditions that will cause winter damage do not occur every year in the locations where wheat breeding studies are carried out poses an obstacle to selection in terms of cold resistance in breeding material. The research was planned and carried out to carry out the selection in question in a short time and clearly. 135 different bread wheat genotypes were acclimated to cold for 0, 21, 35 and 49 days using Hoagland solution under controlled conditions, and at the end of these periods, genotypes with high survival rates were identified by testing them to temperatures of -3, -5, -7, -9, -11 and -13°C. At the same time, the survival rates of the genotypes under the shelter where snow cover was prevented were also determined. It was revealed that the temperatures between -3°C and -13°C and the cold acclimation periods linearly affected the survival rates of the genotypes and the winter hardiness increased as the cold acclimation period increased. In the winter resistance studies to be conducted using Hoagland solution in wheat, it was determined that a 49-day cold acclimation period was appropriate and the maximum test temperature was -13°C. As a result of the study, it was decided to eliminate 97 genotypes from a total of 135 genotypes, which had high yields but low winter hardiness, before they were taken to the next breeding stage. Thus, 60% savings were achieved in terms of labor, time and other costs in the studies.

Keywords: Cold acclimation, hoagland solution, wheat, winter hardiness

Introduction

Cool climate cereals, which are the product group with the largest cultivation area in the world among cereals, constitute approximately 90% of cereal planting areas in Türkiye and 78% in the Eastern Anatolia Region. The average yield from wheat in the region is 3.1 tons-ha, which is around the Turkish average (3.2 tons-ha) (TÜİK, 2023). However, there is more yield potential in the region due to the high total annual rainfall. One of the reasons for this low yield is the spring or freezing planting due to winter damage. It is necessary to develop winter-resistant, high-yielding wheat varieties and expand the planting areas in the region. In Türkiye, as in the world, winter crops have higher grain yields than spring crops in areas with annual rainfall below 600 mm (Kırtok, 1974; Akten, 1985). Fowler and Gusta (1979) and Brule-Babel and Fowler (1989) stated in their studies that if varieties that are not resistant to cold are used in regions with harsh winters, their yield will decrease significantly. Öztürk et al., (1998) emphasized that winter planting is necessary to obtain high yields from wheat in the Eastern Anatolia Region and therefore winter resistance should be emphasized in breeding studies.

In Türkiye, winter resistance selections in cereals have mostly been carried out under field conditions,

but with this study, winter resistance tests in breeding material have begun to be carried out under controlled conditions. Field trials are affected by climate factors that change over the years in natural conditions. With this study, as a result of wheat breeding studies carried out in our institute, the winter hardiness degrees of genotypes brought to advanced breeding stages and varieties adapted to the region have been determined and good selection opportunity has been achieved. In addition, genotypes with determined hardiness are used as parents in crossing studies and are contributed to variety development studies.

Materials and Methods

In the breeding studies carried out by our institute, 128 bread wheat lines brought to advanced breeding stages by crossing, selection and induction methods and 7 bread wheat varieties Bezostaja-1, Yıldırım, Daphan, Karahan-99, Kate A-1, Pehlivan and İntensivnaya) with high adaptation to the region constituted the material of the study. Yıldırım and Daphan varieties were developed for the Eastern Anatolia Region.

The research was carried out with a total of 135 bread wheat genotypes according to the split-split plot experimental design in randomized blocks with three replications (Yıldız and Bircan, 1991).

There were three factors in this study, the first factor was the acclimation periods to cold, the second factor was temperature degrees and the third factor was bread wheat genotypes.

1. For the snow-free environment study, the seeds of the material were planted by hand under the shelter in a 2 m x 1 row (Figures 1 and 2).

2. In determining the degree of winter hardiness, the method applied by Fowler et al., (1995) was used with modification as follows.

Seeds belonging to genotypes were placed in petri dishes, watered and kept at +4°C and in the dark for 2 days against the possibility of dormancy. Then, the petri dishes were kept in the growth chamber at 20-22°C for 2 days. Then, the petri dishes were kept in the climate chamber at 20-22°C for 2 days. The next day, the seeds (5 seeds x 6 degrees x 3 replication=90 seeds) of each genotype that were just beginning to germinate were placed on perforated trays (containing 90 holes) for each cold acclimation periods. These trays were placed on previously prepared pots (40x16x12.5 cm) containing Hoagland solution, and the solution was changed every 2 weeks. The pots were placed in a growth chamber with a temperature of 20°C and a light of approximately 300 m mol m^{-2} s^{-1} (16 000 lux).

16 hours of photoperiod application was made in the growth chamber and the plants were kept in the chamber until they had 3-4 leaves (approximately 2 weeks). When the plants had 3-4 leaves, they were taken to the chamber containing 4±0.2°C temperature and the same light environment for cold acclimation (Figure 3). Cold acclimation periods were applied as 0 (control), 21, 35 and 49 days. At the end of each cold acclimation period, the pots determined for that period were taken out of the growing cabinet, and the plants in the pots were cut 0.5 cm below the root crown and 3-4 cm above the stem (leaf) crown (so as not to damage the roots and growth crown) (Figure 4). This process was applied in one replication from each genotype for each degree (-3, -5, -7, -9, -11, -13°C) with 5 plants. After cutting the roots and stems, the plant parts containing live roots and shoots of each genotype were grouped according to their test degrees and placed separately in aluminum boxes filled with 5 cm moist sand, and 5 cm thick moist sand was added on top. Then, these boxes were closed and placed in the freezer (Figure 5).

The temperature of the freezer was set at -3±0.2°C and the materials were kept in the freezer for 12 hours. This process is necessary to ensure complete freezing of the intercellular substances. At the end of twelve hours, the boxes marked -3°C were taken out, and then the process was continued by decreasing the temperature by 2°C every hour. The containers corresponding to the temperature after each hour (5 plants from each genotype at each temperature) were taken out of the freezer. The containers taken from the freezer were kept in the climate room at +4°C for one day. The next day, they were transferred to pots containing specially prepared humus flower soil and placed in the growth chamber where room temperature and 16 hours of photoperiod were applied. The pots were watered by adding fertilizer containing microelements (1 ml per 1 liter of water as stated in the fertilizer's instructions) and the pot soil was completely saturated and the live plants were regenerated. (Figure 6).

l. After approximately 3 weeks, the regenerating plants were counted and the temperature degree at which at least 3 plants out of 5 survived was determined as the winter resistance degree.

Results and Discussion

Without snow cover studies under the shelter

The material was planted in an environment without snow cover in order to understand whether the winter damage that may occur when snow cover is prevented under natural conditions coincides with the winter damage tests to be carried out under controlled conditions. The study material was planted under the shed in 2 m x 1 rows and it was watered after planting and 4 times with one week for 100% germination before winter.

100% germination was achieved before winter. After winter, the plants were watered for regeneration. However, regeneration occurred in 13 genotypes, while 112 genotypes died completely due to winter damage. This shows that snow cover is a good protector in winter months. In winter wheat, a snow cover must be occur to prevent freezing and increase survival rate. There should be 5 cm of snow cover at -8°C, 7 cm at -10°C, 14 cm at -15°C, 20 cm at -20°C, and 27 cm at -25°C. As the thickness of the snow cover increases, the survival rate of plants will increase (Dilmurodov and Ziyadullaev, 2020; Shakirjanovich and Dilmurodovich, 2021).

The results of the without snow cover could not be evaluated statistically, the germination rates are given in Table 4. Line 32 showed 70%, line 90 45%, line 116 40%, line 5 20%, lines 19, 20, 56, 8 and 50 10%, lines 74, 71, 1 and 15 5% post-winter regeneration rate (Table 4). Although it was observed that the performance of these lines as a result of winter hardiness tests under controlled conditions was parallel to their resistance under shelter, this relationship was weak in other genotypes. No literature was found on this subject.

Winter hardiness studies in controlled conditions

Creating cold-resistant varieties in wheat breeding programs is one of the most difficult tasks because several genes are involved. The main difficulty in creating cold-resistant varieties in breeding is usually the relationship between high cold tolerance and low yield and late ripening. Therefore, the purpose of selection is not to maximize cold tolerance, but to create varieties that can withstand the minimum temperature for a particular area (Dilmurodovich et al., 2021). In order to develop winter-hardiness varieties, studies that can yield results in a short time are needed and the study was conducted for this purpose.

The differences between cold acclimation periods (days), temperature test degrees, and genotypes, as well as the interactions between day x temperature, day x genotype, temperature x genotype, and day x temperature x genotype were found to be statistically significant ($p<0.01$) (Table 1).

The variance analysis showing the effects of different cold acclimation periods and temperature applications on the winter hardiness performances of genotypes is presented in Table 1.

The analysis results of the performances of genotypes against low temperatures with different cold acclimation periods are presented in Table 2.

Winter wheats develop adaptation mechanisms during the cold acclimation process to increase winter resistance (Struthers and Greer, 2001). As shown in Table 2, all plants exposed to low temperatures without cold acclimation died. As the cold acclimation period increased, winter hardiness performance improved, and the highest number of plants was obtained from plants acclimated for 49 days (2,289 plants). While the number of living plants was 1,426 on the day 21, it was 2,065 on the day 35. The effects of different cold acclimation periods on the winter hardiness of genotypes are presented in Figure 7. Galiba et al., (2011) reported that cold hardiness mechanisms are activated during the cold acclimation process.

Plants that were not acclimated to cold could not withstand winter (0.000 units), winter hardiness started to increase in plants acclimated to cold for 35 days. The analysis results of the winter hardiness performances of genotypes with different temperature applications are presented in Table 3.

As the temperature decreased, decreasing hardiness performance was observed in the genotypes. While the maximum number of live plants (2,714) was obtained from the -3°C temperature application, the minimum number of live plants was obtained from the -13°C application (0.018). The temperatures at which the least resistance was obtained were determined as -9°C, -11°C, -13°C. A rapid decrease in resistance was recorded after -9°C. In a study where Homer et al., (2016) researched the winter resistance of local and registered pea genotypes under controlled conditions, they could not determine any genotypes that could withstand -12 and -16°C, while local and registered genotypes that could withstand -8°C were determined.

The effects of different test temperatures on the cold resistance performance abilities of the genotypes are presented in Figure 8.

-3°C, -5°C, -7°C, -9°C temperatures had similar effect whereas -11 and -13°C temperatures gave similar results.

Dendrogram of temperatures applied were presented in Figure 8. The groupings are similar according to both the dendrogram and the variance analysis results. In Figure 10, although the genotypes showed similar reactions at -3°C, -5°C, -7°C, and -9°C, they showed different reactions at -11 and -13°C. Therefore, they were placed in different groups in Figure 9. The fact that the genotype x temperature interaction was statistically very significant supports this result. The hardiness levels shown by the genotypes at -11 and -13°C temperatures formed the basis for the classification of the genotypes. Therefore, the cluster analyses in Figures 9 and 10 show similarity with the variance analysis table (Table 3). The winter hardiness

rates of the genotypes under controlled conditions and their regeneration rates after winter without snow cover are presented in Table 4.

As a result of the applied cold acclimation and low temperature applications, genotypes 90 and 74 were determined to be the hardiest genotypes (2,958 and 2,681 plants, respectively). While genotype 90 showed a 45% regeneration ratio after winter in without snow cover, genotype 74 showed a 5% regeneration ratio. There was no regeneration observed in genotypes 130 and 131 (Table 4). The effects of cold acclimation duration and low temperature applications on the hardiness of genotypes were mostly determined to be linear and quadratic. Among the registered varieties included in the study, the following number of live plants were obtained: 1,236 from Bezostaja-1, 0.625 from Yıldırım, 0.694 from Daphan, 0.806 from Kate A-1, 1,139 from Pehlivan, 1,667 from Karahan-99 and 1,014 from Intensivnaya. No winter survival rate was observed in the rows of these varieties under the shelter. These values were obtained as a result of the combined analysis of data from all temperatures between -3°C and -13°C and four cold acclimation periods. However, each genotype responded differently to each cold acclimation period and applied temperature degrees. In a study conducted by Yıldırım et al., (2003) using 24 wheat and barley genotypes under controlled conditions, it was found that the Kıraç 66 variety was the most sensitive wheat variety to winter (-3.5°C) while Bülbül 89 was the most sensitive barley variety (-2.7°C). As a result of this study, line no. 8 (NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12) was registered with the name AYYILDIZ, taking into account other agronomic features. As seen from Figure 7, 1, 5, 9, 7, 27, 34, 33, 40, 24, 22, 14, 15, 31, 44, 26, 25, 23, 41, 39, 35, 18, 48, 60, 113, 12, 2, 3, 6, 8, 11, 16, 20, 46, 51, 63, 117, 67, 57, 84, 85, 112, 106, 19, 62, 119, 32, 52 genotypes constituted of same group. The next group had 56, 71, 74, 77, 78, 79 86, 88, 90, 104, 122, 128, 134 genotypes. While 126, 127, 82, 93, 53, 87, 121, 68, 116, 125, 58, 72, 89, 105, 111, 129, 83, 95, 120, 135, 105, 55, 123, 59, 73, 99, 70, 105, 103, 115, 118 genotypes occurred in same group; the last group had 133, 114, 28, 38, 52, 54, 76, 61, 29, 124, 94, 108, 100, 30, 107, 132, 65, 17, 96, 21, 66, 43 64, 109, 69, 97, 102, 80, 98 91, 36, 13, 42, 92, 47, 45, 49, 10, 130, 131 and 37 genotypes.

The orthogonal partitioning table used to determine the effects of cold acclimation period and low temperature applications on the hardiness of genotypes is given in Table 5.

As indicated in Table 5, the effects of cold acclimation period and test degrees applications on the hardiness of genotypes were mostly determined to be linear and quadratic. The effects of cold acclimation period and low temperature applications on the winter resistance performance of genotypes and the effect type in terms of linear and quadratic effects are given in Table 6.

Through modeling calculations, the optimal cold acclimation period for maximum viability of genotypes was determined to be 58 days. In addition, cold acclimation periods after 40 days cause a relatively lower increase in plant vitality.

It has been determined that wheat genotypes acclimated to cold for a longer period can maintain their vitality at lower temperatures. The response to this acclimation period is not linear but quadratic. In other words, in plants acclimated to cold up to (58th day), maximum cold resistance is achieved although it varies according to the genotypic capacity. The winter resistance feature is not linear but quadratic although it varies according to the capacity of the genotypes. The maximum winter hardiness temperature has been identified as -16°C.

As a result, wheat genotypes subjected to a cold acclimation period of at least 40 days can survive winter without snow cover down to -16°C. Therefore, considering these results in applications to be made in breeding studies will increase success. It has been demonstrated that various temperature degrees between -3°C and -13°C and cold acclimation periods linearly affect the survival rates of the genotypes. Both cold acclimation periods and various temperature degrees have similar effects on the survival rates of the genotypes. The survival temperature limit for plants grown in Hoagland solution was determined to be -13°C in this study. Plants hadn't survived below this degree. In plants grown in a soilless environment, the presence of excessive water in plant cells caused more damage due to freeze-thaw cycles, reducing the survival rates of the plants. However, it is predicted that the hardiness levels of plants may decrease if winter hardiness studies are conducted under soil conditions. A method study was carried out by Küçüközdemir (2025) using soil and Hoagland solution media to determine the winter hardiness degrees of wheat under controlled conditions. In this study, stepwise and non-stepwise cold acclimation periods were applied in both media, and it was revealed that performing winter resistance tests in soil media by applying stepwise cold acclimation degrees gave the closest results to reality. For this reason, conducting newly planned winter hardiness test studies in soil conditions will be easier and data much closer to natural conditions will be obtained.

Conclusions

As the cold acclimation period increases, the winter hardiness of plants also increases. The maximum survival rate was determined at the end of the 49-day cold acclimation period. On the other hand, it was found that various temperature degrees between -3°C and -13°C linearly affect the survival rates of the genotypes.

With this study, all genotypes in advanced breeding stages were tested in terms of winter hardiness. From 135 genotypes, 97 genotypes with low winter hardiness levels were eliminated. This resulted in a 60% savings in terms of labor, time, and other expenses. While the breeding process of hardy genotypes continues, they have started to be used as parents in crossing studies.

Acknowledgements

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Figure 1. Genotypes planted in 2 m x 1 rows in a snow-free environment (Original).

Figure 2. Post-winter appearance in an environment without snow cover (Original).

Figure 3. Cold acclimated plants in Hoagland solution (Original).

Figure 4. Cutting of plants after cold acclimation period is completed (Original).

Figure 5. Plant parts placed in sand-filled containers for cold tests and placed in the freezer (Original).

Figure 6. Transferring the plant parts, whose test process has been completed, into soil pots to regeneration (Original).

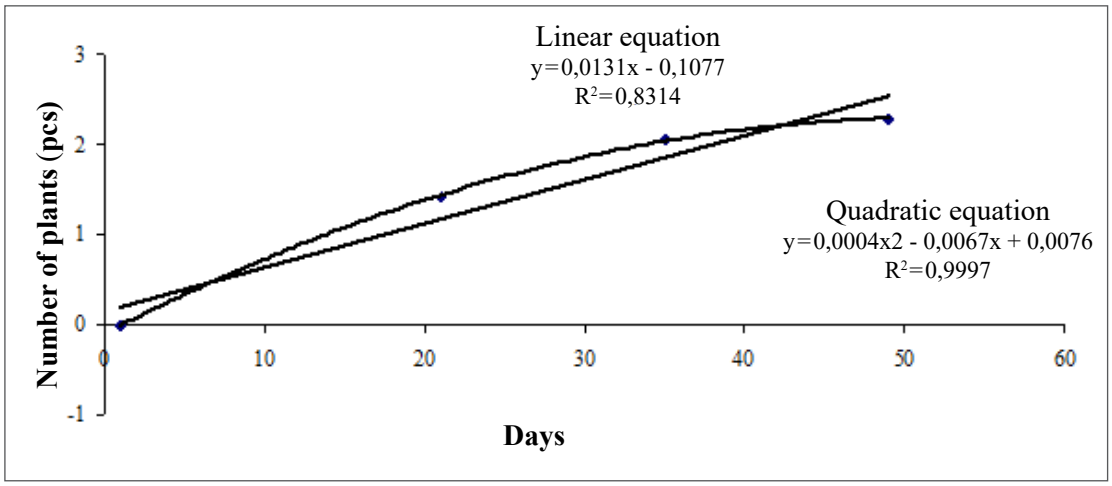

Figure 7. Effects of different cold acclimation periods on the winter hardiness performances of genotypes.

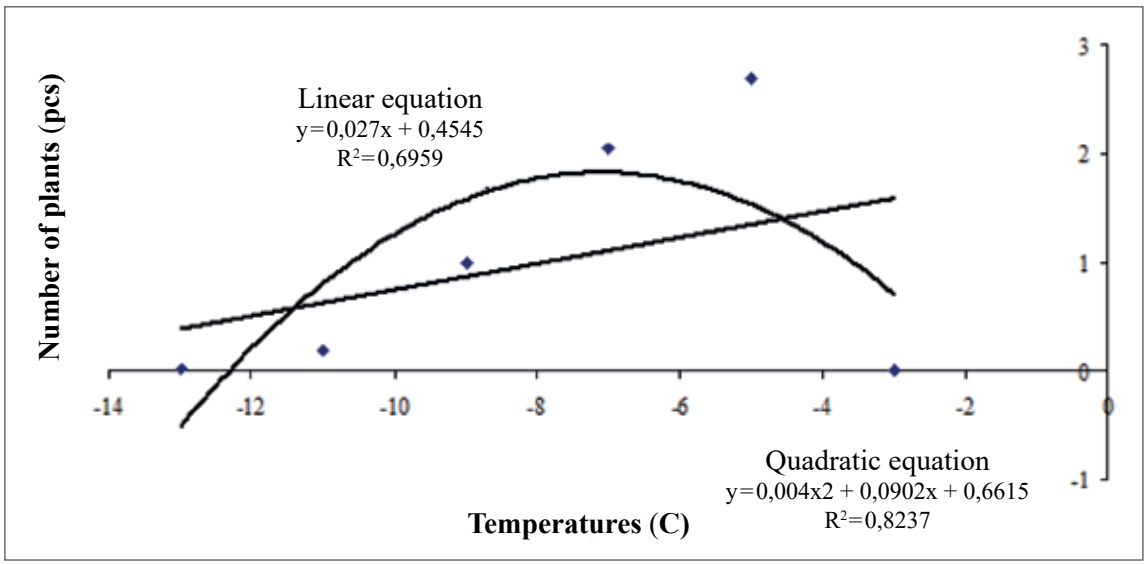

Figure 8. Effects of different temperatures on winter hardiness of genotypes.

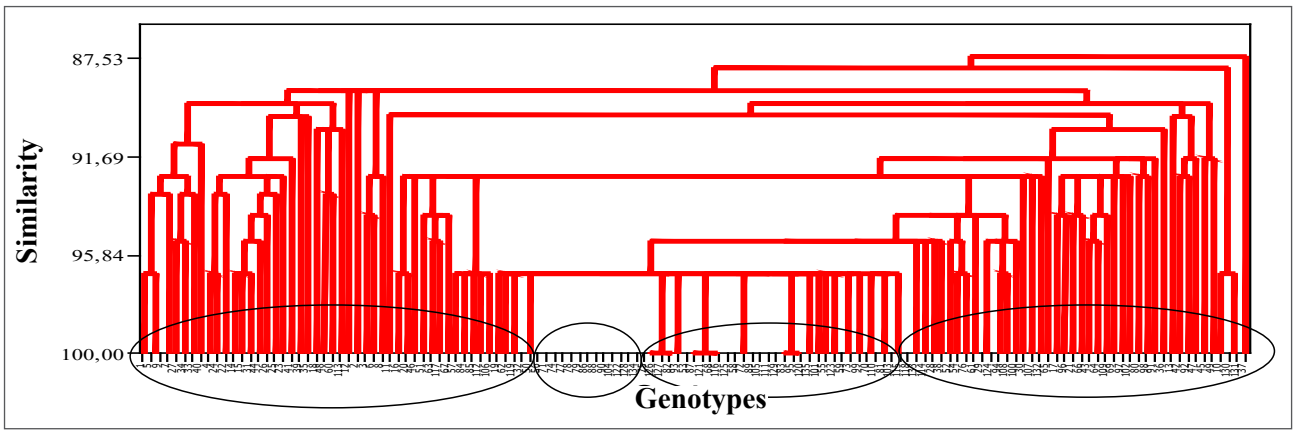

Figure 9. Dendrogram of genotypes.

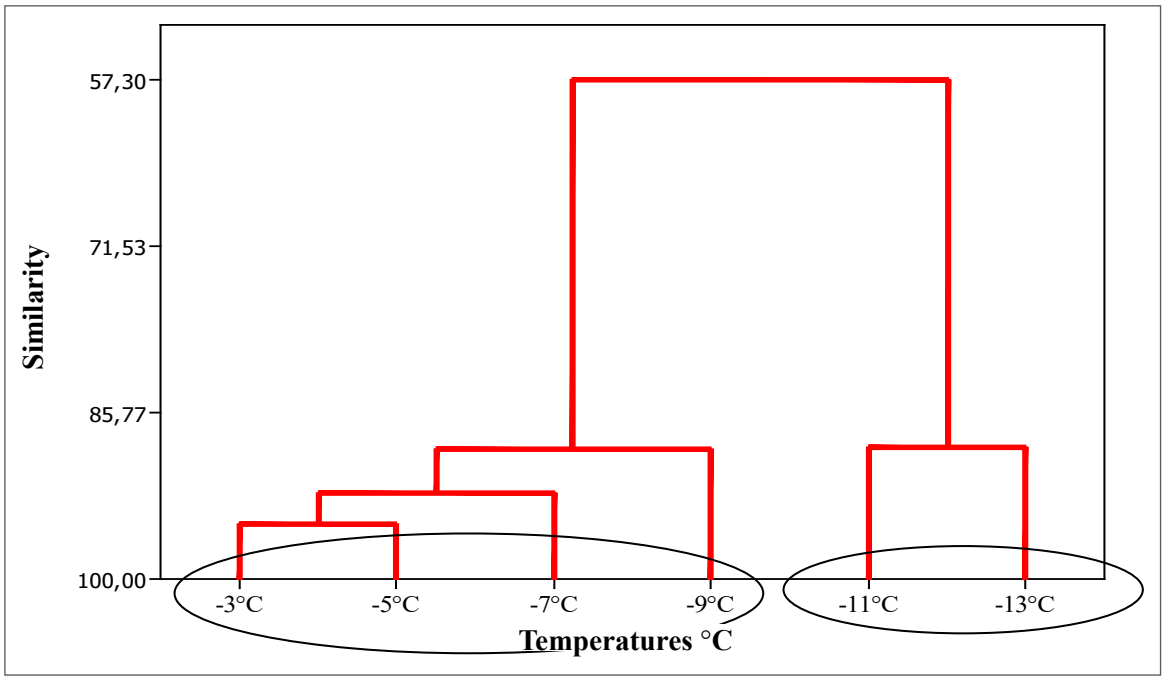

Figure 10. Dendrogram of temperatures applied as a factor.

Table 1. Variance Analysis of Winter Hardiness Performances of Genotypes with Different Cold Acclimation Periods and Temperature Applications.

Source of Variation	Degree of Freedom	Mean Squares	F Value
Replication	2	4.448	2.224*
Cold Acclimation Day	3	2579.019	234.915**
Temperature	5	2391.353	217.821**
Day x Temperature	15	294.571	26.832**
$Error_1$	48	10.978	
Genotype	134	36.832	73.840**
Day x Genotype	402	6.188	12.405**
Temperature x Genotype	670	4.979	9.981**
Day x Temp x Genotype	2010	1.720	3.449**
$Error_2$	6432	0.499	-
General	9719	4.328	-
C.V. (%): 32.04			

Table 2. Effects of Different Cold Acclimation Periods on Winter Hardiness Performances of Genotypes.

Cold Acclimation Days	Number of Live Plants
Control (0 day)	0.000 C
Day 21	1.426 B
Day 35	2.065 A
Day 49	2.289 A
Mean	1.445
L.S.D. (%): 0.255	

Table 3. Effects of Different Temperature Treatments on Winter Hardiness Performance of Genotypes.

Test degrees	Number of Live Plants
-3°C	2. 714 A
-5°C	2.702 A
-7°C	2.054 B
-9°C	0.992 C
-11°C	0.190 D
-13°C	0.018 D
Mean	1.912
L.S.D. (%): 0.313	

Table 4. Survival Rates of Genotypes After Winter Hardiness Tests and Post-Winter Regeneration Rates in Without Snow Cover under the Shelter.

No	[a]L.P (number)		[b]R (%)	No	[a]L.P (number)		[b]R (%)	No	[a]L.P (number)		[b]R (%)
90	2,96	A	45	36	1,85	I-W	-	18	0,96	`-h	-
74	2,68	AB	5	32	1,82	J-X	70	6	0,93	a-i	-
89	2,46	BC	-	81	1,82	J-X	-	11	0,93	b-j	-
93	2,46	BC	-	106	1,79	K-X	-	5	0,88	b-j	20
77	2,46	BC	-	119	1,78	L-Y	-	91	0,88	c-k	-
112	2,42	B-D	-	95	1,76	M-Y	-	30	0,88	c-k	-
71	2,40	B-E	5	19	1,75	M-Y	10	20	0,86	c-k	10
88	2,39	B-F	-	85	1,72	N-Y	-	64	0,86	c-k	-
116	2,39	B-F	40	84	1,72	N-Y	-	9	0,85	c-k	-
86	2,35	B-G	-	67	1,69	O-Z	-	97	0,83	c-l	-
87	2,35	B-G	-	117	1,69	O-Z	-	44 (Kate A-1)	0,81	c-m	-
72	2,33	B-G	-	115	1,69	O-Z	-	98	0,78	d-m	-
79	2,31	B-H	-	62	1,68	O-[	-	66	0,78	e-n	-
56	2,29	B-H	10	111	1,68	O-[	-	109	0,75	e-n	-
78	2,29	B-H	-	54	1,67	P-\	-	1	0,74	e-o	5
120	2,28	B-I	-	49 (Karahan-99)	1,67	P-\	-	29	0,72	e-o	-
129	2,28	B-I	-	3	1,67	P-\	-	2	0,71	e-o	-
8	2,26	B-I	10	124	1,67	P-\	-	12	0,71	e-p	-
126	2,26	B-I	-	76	1,65	Q-]	-	14	0,69	e-p	-
128	2,25	B-J	-	65	1,64	Q-]	-	40	0,69	f-p	-
134	2,25	B-J	-	99	1,64	Q-]	-	43 (Daphan)	0,69	f-p	-
101	2,24	C-J	-	61	1,61	R-]	-	48	0,64	g-q	-
127	2,24	C-J	-	38	1,61	R-]	-	34	0,63	g-q	-
125	2,22	C-K	-	110	1,61	R-]	-	42 (Yıldırım)	0,63	g-q	-
53	2,21	C-L	-	100	1,60	S-]	-	21	0,60	g-q	-
75	2,18	C-M	-	94	1,58	S-]	-	18	0,57	h-r	-
82	2,15	C-N	-	69	1,58	S-]	-	33	0,56	i-r	-
70	2,15	C-N	-	13	1,58	S-]	-	23	0,51	j-s	-
83	2,14	C-N	-	51	1,57	T-^	-	35	0,50	j-t	-
55	2,11	C-O	-	112	1,57	T-^	-	22	0,49	k-t	-
123	2,11	C-O	-	16	1,54	U-^	-	17	0,46	k-t	-
121	2,10	C-P	-	46	1,49	V-_	-	25	0,42	l-u	-
118	2,07	C-Q	-	102	1,43	W-`	-	96	0,42	l-u	-
59	2,07	C-Q	-	80	1,42	W-`	-	113	0,42	l-u	-
73	2,04	C-R	-	28	1,39	X-a	-	15	0,40	m-u	5
104	2,01	D-S	-	133	1,35	Y-b	-	31	0,36	n-u	-
50	2,01	D-S	10	108	1,26	Z-c	-	41	0,36	n-u	-
135	2,00	D-T	-	60	1,25	[-c	-	24	0,32	o-u	-
52	2,00	D-T	-	37 (Bezostaja-1)	1,24	\-d	-	4	0,28	p-u	-
105	1,97	E-U	-	132	1,22	]-d	-	27	0,24	q-u	-
103	1,97	E-U	-	45 (Pehlivan)	1,14	^-e	-	26	0,14	r-u	-
58	1,96	F-U	-	107	1,10	_-e	-	39	0,11	s-u	-
114	1,94	G-U	-	57	1,01	_-f	-	10	0,07	t-u	-
122	1,94	G-U	-	92 (Intensivnaya)	1,01	`-g	-	131	0,00	u	-
7	1,88	H-V	-	47	1,00	`-g	-	130	0,00	u	-

a: Number of live plants [Number of living plants resulting from the combined analysis of all temperatures (out of 5 plants)]

b: Regeneration (%): Percentage of plants surviving after winter in without snow cover under the shed)

Table 5. Orthogonal Partitioning Table Conducted to Determine the Effects of Cold Acclimation Period and Low Temperature Applications on the Resistance of Genotypes.

Source of Variation	D.F	Cold Acclimation Periods (day)		Test Degrees (°C)	
		Mean Squares	F-value	Mean Squares	F-value
Linear Effect	1	6844.879	13722.334**	11284.899	22623.504**
Quadratic Effect	1	875.400	1754.966**	38.571	77.325**
Cubic Effect	1	16.778	33.636**	628.431	1259.852**
Quadratic Effect	1	-	-	1.259	2.525ns
Error	6432	0.499	-	0.499	

**: Significant at the 1% level; ns: Non-significant.

Table 6. The Effect of Cold Acclimation Period and Low Temperature Applications on the Winter Hardiness Performance of Genotypes and the Effect Form in Terms of Linear and Quadratic Effect.

Effect Type	Cold Acclimation Period (days)		Test Degree (°C)	
	Formula	R^2	Formula	R^2
Linear Effect	y = 0,0131x - 0,1077	0.8314	y = 0.027x + 0.4545	0.6959
Quadratic Effect	$y = 0,0004x^2 - 0,0067x + 0,0076$	0.9997	$y = 0.004x^2 + 0.0902x + 0.6615$	0.8237

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