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Variation in cold tolerance in F_6 durum wheat [*Triticum turgidum* (L.) Tell. convar. *durum* (Desf.) Mackey] RILs and the relationships of cold tolerance with some quality parameters and genetic markers

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Abstract

Low temperature is one of the critical factors for determining agricultural production. Therefore, the main objective was to develop the durum wheat lines, which are tolerant to cold particularly in Central Anatolia. The recombinant inbred lines (RILs) (141) derived from F_6 progeny of durum wheat, developed from Kunduru-1149 (female parent) and Cham-1 (male parent) cross, were characterized in terms of their cold tolerance both in the field and under controlled (in growth chamber) conditions. A 0–9 scale was used for the visual evaluation of cold damage under field conditions, and a quantitative measure of photochemical efficiency (Fv/Fm = variable fluorescence/maximum fluorescence) method was used for the evaluation of cold damage under controlled conditions. Seventeen RILs displayed the scores within the range of 1–2.5 in 0–9 scale evaluation, while 41 RILs showed the scores equal to or higher than 0.70 approaching to optimal (~0.83) in Fv/Fm evaluation. Some quality parameters tested and some gliadin fragments and random amplified polymorphic DNA loci showed meaningful correlations with the data of 0–9 scale and Fv/Fm ratios from lower to the medium level. The RILs considered as cold resistant; might have the potential to be used as parental lines to develop cold-resistant durum wheat varieties in the future durum wheat-breeding programmes.

Introduction

Durum wheat [*Triticum turgidum* (L.) Tell. convar. *durum* (Desf.) Mackey] is the main modern tetraploid wheat with genome formula *AABB* and seven homeologous groups ($2n = 4x = 28$). It is mainly grown in relatively dry regions in the Mediterranean basin, in India, the former USSR and low rainfall areas of the great plains of the United States and Canada (Feldman *et al.*, 1995). *Triticum durum* Desf. is the second widespread Triticum species containing 10–11% of the world wheat crop and covering about 8% of the total wheat production (Ganeva *et al.*, 2010). It has large and very hard grains, produce low gluten flour, which is appropriate for macaroni and semolina products (Feldman *et al.*, 1995). It is less well suited for bread production than hexaploid bread wheat (*Triticum aestivum* L., *AABBDD*). Wheat gluten proteins predominantly determine the rheological properties of dough and thus confer the unique viscoelastic properties on the dough (Li *et al.*, 2012).

Although, durum wheat is grown in many areas of the world, a major production area is located in West Asia and North Africa (WANA) (about 9 million ha) where the climate is characterized with low to moderate rainfall (less than 350 mm annual rainfall) and moderate cold to cold temperature in winter. In the WANA zone, including Turkey, low temperature (cold) causes considerable damages to durum wheat at various plant development stages, especially at the seedling and tillering stages. In this zone, the temperature can drop as low as −25 °C during the winter months of the year, causing considerable yield loss.

Low temperature is one of the critical factors for determining agricultural production. When environmental temperature, at which maximum growth and development occur in plants, is low, physiological, biochemical, metabolic and molecular changes are observed within plants. The reduced temperature induces stress tolerance in plants, and this process is called chilling and/or cold acclimation (CA). Chilling tolerance is the capability of a plant to tolerate low temperatures (0–15 °C) with having no injury or damage (Yadav, 2010). CA

is the process that hardy plants develop advanced essential tolerance for cold stress survival through which the plants have several changes at different levels of biochemical and cellular biology in plants (Heidarvand and Amiri, 2010).

In plant physiology, chlorophyll fluorescence is one of the efficient techniques for measuring the activity of photosystem II (PSII) (Murchie and Lawson, 2013). The ratio of the variable fluorescence to maximum fluorescence (Fv/Fm) is a common parameter to measure some physiological stress such as light intensity, water deficit, etc. in plants. The value of the Fv/Fm ratio is optimal ~0.83 under unstressed conditions for plant leaves and refers to the maximum quantum yield of photosynthesis (Demmig and Björkman, 1987; Murchie and Lawson, 2013). If the value of the Fv/Fm ratio tends to decrease, that is attributed to the presence of any type of 'stress' that causes inactivation damage of PSII (Long *et al.*, 1994; Murchie and Lawson, 2013).

The studies of the inheritance of frost resistance and winter hardiness started in the last century. Nilsson-Ehle (1912) was the first, who crossed two winter varieties to investigate winter hardiness and he came to the conclusion that winter hardiness is controlled by polygenes as well as other quantitative characters. Similar findings were also reported by several scientists (Hayes and Aamodt, 1927; Worzella, 1935: cited in Sutka, 2001). A gene for frost tolerance located on the chromosome 5A of wheat and the locus assigned as *Fr*1 was completely linked to locus *Vrn*1 controlling vernalization requirement, which might have a pleiotropic action or a close genetic linkage with *Fr*1 (Sutka and Snape, 1989). The winter hardiness of winter wheat positively correlated with plant height and grain yield (Ruzgas and Liutkevičius, 2001). Mekhlouf *et al.* (2006) assessed genetic variability for resistance to low temperatures using a programmable freezer to test their influence and examined the relationships between the results of cold tests and the agronomic performance and stability of a field grown set of durum wheat genotypes showed significant genetic variability and differential responses to the growth conditions experienced. A more accurate estimation of frost resistance in wheat varieties should be performed under natural and artificial conditions, and several years of testing is needed (Chipilski and Uhr, 2014).

The winter hardiness and frost tolerance are complex traits that can be improved not only by phenotypic selection, but also by the genotypic selection, which is based on quantitative trait locus (QTL) mapping and other genomic studies such as marker-assisted selection. However, genomic approaches hold potential for a knowledge-based improvement of those important traits in elite Triticale germplasm and three major QTLs on chromosomes 5A, 1B and 5R identified (Liu *et al.*, 2014*a*). It was suggested that the frost tolerance in winter durum wheat was mainly controlled by copy number variation of *CBF*-A14 at the *Fr*-A2 locus and it could almost be considered as monogenic with the major QTL *Fr*-A2 explaining about 90% of the genotypic variance while the second identified locus *Fr*-B2 explained a comparable negligible proportion of around 4% (Sieber *et al.*, 2016).

The economy depends mostly on agriculture in Turkey, and most of the farmers are not big and rich. The harvested cereal crops are partitioned by small farmers as a seedling for next crop season, for home use and for selling to merchants to cover their living expenses and for farming expenses such as fertilizers, gas oil, etc., and similar expenses in large scale necessary as well for big and rich farmers. The yield loss due to low temperatures leads to some problems in the economy and society. Therefore, the identification and development of germplasm sources of cold tolerance in durum wheat are very important in the success of future durum wheat-breeding programmes in this region.

This study is a part of the project 'WANADDIN (The West Asia and North Africa Dryland Durum Improvement Network), funded by International Fund for Agricultural Development (IFAD), was implemented in the period 1996–1998 with the objective of achieving sustainable improvement in the productivity and production of durum wheat in the dryland environments of the WANA (West Asia and North Africa) region through the establishment of a formal network involving the joint ICARDA/CIMMYT durum wheat research program and the National Agricultural Research Systems (NARS) of Algeria, Morocco, Syria, Tunisia and Turkey. It aimed to continue the development of improved germplasm by incorporating new sources of tolerance and/or resistance against the major abiotic and biotic stresses…' (ICARDA, 2005). The recombinant inbred lines (RILs), which are the F_6 progeny of Kunduru-1149 (female parent) and Cham-1 (male parent) crosses, were developed in line with the objectives of the WANADDIN project. The crosses were made to develop the RILs, which have tolerance to cold, boron toxicity and yellow rust.

The objectives of the current study are (i) to characterize 141 F_6 progeny of RILs in terms of their cold tolerance both in the field and under controlled (in growth chamber) conditions; (ii) to determine the correlations between cold tolerance and some quality parameters and (iii) to determine the correlations between cold tolerance and biochemical (gliadins and high molecular weight (HMW)-glutenins) as well as DNA (random amplified polymorphic DNA (RAPD)) markers in Central Research Institute for Field Crops (CRIFC) as one of the group member in Turkey, which is one of the partnerships of WNADDIN project.

Materials and methods

Plant material

F_6 progeny of Kunduru-1149 × Cham-1 cross, 141 RILs, developed by the International Centre for Agricultural Research in the Dry Areas (ICARDA) durum wheat-breeding programme in Aleppo, Syria, was analysed. Kunduru-1149 as the female parent had cold tolerance, high quality, low yield and disease susceptible genetic characters, while Cham-1 as the male parent had cold sensitive, low quality, high yield and disease resistance genetic characters.

Screening for cold tolerance in the field

Twenty-five seeds for each one of the 141 RILs from F_6 progeny were sown as three replications in three different locations, Sivas-Ulaş Directorate of Agricultural Management Research Field (COLDU), concrete bed in the research field of CRIFC in Ankara-Haymana (COLDH) and research field of CRIFC in Ankara-Yenimahalle (COLDY) (Fig. 1). The locations with their abbreviations used throughout the text, the coordinates and altitudes of these three locations, and the minimum temperatures recorded during growing seasons at these locations are indicated in Table S1.

The lines, which had uneven and inadequate emergences, were omitted from the study in all test locations. The evaluation of cold damage (CD %) was performed according to the 0–9 evaluation scale, which was adopted from Rizza *et al.* (1994) and routinely used in the cold-breeding studies of CRIFC. This scale depends on the visual evaluation of the percent of individual leaf area showing cold damage symptoms (0: <5, 1: 5–15, 2: 15–25, 3:

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Fig. 1. A map of Turkey showing the locations, where the cold tests carried out (Ankara-Haymana (COLDH), Sivas-Ulaş (COLDU) and Ankara-Yenimahalle (COLDY) test locations).

25–35, 4: 35–45, 5: 45–55, 6: 55–65, 7: 65–75, 8: 75–85 and 9: >85%; 0 = the most tolerant to cold; 9 = the least tolerant to cold).

Soil analysis

Soil samples from the locations, where the tests were carried out were analysed for soil pH, EC (mmhos/cm), $CaCO_3$, organic material, sand, clay, silt and important microelements (Table 1). Although the soil characteristics of these three test sites were different from each other, they were all accepted as useable in the analysis.

Meteorological data

The information related to main climate parameters (maximum and minimum temperatures, % humidity daily) was taken for the period of sowing and evaluating dates from the meteorology centres nearest to the locations where the tests performed (Göçmen, 2001). Only the minimum temperatures detected in these test locations are given in Table S1.

Screening for cold tolerance under controlled conditions

These tests were carried out in two SANYO-MIR 552 type incubators (SANYO Electric Co., Ltd., Japan) which operate within the range of −15 and +52 °C. The 56 cm × 36 cm × 14 cm boxes were filled with an equal amount of soil, and each box was fertilized with 3.6 g (=18 kg/da) diammonium phosphate. Soil from the research field of the Central Research Institute for Field Crops (CRIFC) in Ankara-Yenimahalle was utilized (Table 1). Seeds of 141 RILs were sown in a box, and the test was performed as three replications. The seeds of all lines were sown in the same depth of 3.0 cm in each replication. The number of sown seeds per line was initially 14, but after germination, each line was arranged as ten plants. The time schedule of activities in this procedure is described in Table S2.

Test materials were evaluated at +10, −5, −10 and −15 °C with an Opti-sciences OS1-FL Modulated Chlorofluorometer to determine the cold tolerance of lines. The parameter Fv/Fm, which is the quantitative measure of photochemical efficiency (Fisker *et al.*, 1995), was recorded on randomly selected four plants out of ten plants present for each line. The average of four plants' data was used for statistical analyses.

Table 1. Constituents of soil from three different test locations

Ingredients	COLDU	COLDH	COLDY
pH	7.97	7.39	7.87
EC (mmhos/cm)	0.16	0.28	0.24
% $CaCO_3$	9.69	25.44	8.72
% Organic material	1.21	2.18	1.07
% Sand	62.49	10.98	47.61
% Clay	7.77	50.01	29.76
% Silt	29.74	39.01	22.63
Zn (ppm)	0.20	1.35	1.20
B (ppm)	0.46	0.55	0.60

Sivas-Ulaş Directorate of Agricultural Management Research Field (COLDU), concrete bed in the research field of Central Research Institute for Field Crops (CRIFC) in Ankara-Haymana (COLDH) and research field of CRIFC in Ankara-Yenimahalle (COLDY).

Screening for quality parameters

Each of 141 RILs was tested in terms of their thousand kernel weight (dry-TKW and wet-TKW = g/1000 kernels), moisture contents (MC as percentage of wet TKW), vitreous kernel count (VKC) to determine the percentages of vitreous kernels (VK), opaque kernels (OK) and partly opaque kernels (POK), protein content (PC), hardness (particle size index = PSI), colour (yellow pigment = YP) and SDS sedimentation as quality parameters. These tests were carried out in the Quality Laboratory of CRIFC in Ankara by using standardized protocols. Details of each protocol are given in Göçmen (2001).

Analysis of gliadins

Analysis of gliadins was performed using an A-PAGE method previously described by Bushuk and Zillman (1978) with some modifications done by Cereal Quality Laboratory in ICARDA (Göçmen, 2001).

Analysis of HMW-glutenin subunits

Analysis of glutenins was performed using an SDS-PAGE method previously described by Ng and Bushuk (1987) with some

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Table 2. Operon random ten-mer primers, which were the most polymorphic and produced scorable bands, their sequence information and the fragment size range observed in the study

	Primer name	Sequence	Fragment size range (bp)
1	OPK-09	CCCTACCGAC	500–1750
2	OPJ-09	TGAGCCTCAC	1050–1650
3	OPC-07	GTCCCGACGA	1450–1800
4	OPE-09	CTTCACCCGA	900–1600
5	OPL-03	CCAGCAGCTT	500–2300
6	OPM-18	CACCATCCGT	900–1800
7	OPA-11	CAATCGCCGT	300–350
8	OPB-11	GTAGACCCGT	510–730
9	OPE-13	CCCGATTCGG	900–1100
10	OPE-17	CTACTGCCGT	340–700
11	OPE-08	TCACCACGGT	500–620
12	OPG-11	TGCCCGTCGT	500

modifications done by Cereal Quality Laboratory in ICARDA (Göçmen, 2001).

Analysis of RAPD loci

Genomic DNA was extracted from a whole seed using the extraction protocol of Plaschke *et al.* (1995). Thirteen ten-base oligonucleotide primers from Operon Technologies (Alameda, California) were used to screen the DNAs of each 141 RILs by using polymerase chain reaction (PCR) conditions described in Göçmen (2001) and Göçmen *et al.* (2003). The primer names, sequences and fragment sizes observed in the study are given in Table 2.

Statistical analysis

Analysis of variance

Analysis of variance (ANOVA) was carried out for determining differences among 141 RILs in terms of their response to low temperatures both in the field tests and the tests performed in controlled environments. ANOVA was calculated based on RILs' means and analysis was carried out using the generalized least squares procedure of SAS (SAS, Statistical User's Guide, 1988) because this program gives unbiased estimates of all mean squares when a data set has missing value. The following statistical model was used in the data analysis:

$$Z_{ik} = \mu + R_k + G_i + e_{ik}$$

where Z_{ik} is the mean performance of the ith RIL in the kth replication, μ is the experimental mean, R_k is the effects of replication, G_i is the effects of genetic types and e_{ik} is the experimental error (Kaya *et al.*, 1989).

Heritabilities

Heritability (h^2) was estimated from the components of variance as described by Kaya and Temerit (1994) and standard errors for heritability estimates were computed according to Becker (1992).

Correlations between cold scores and quality parameters

The phenotypic correlation between traits x and y was calculated from RILs mean squares and mean cross products for the traits according to Kaya *et al.* (1989), while the genotypic correlation between two traits was calculated according to Falconer (1989).

Correlations between cold scores and genetic markers

Gliadin and HMW-glutenin protein fragments and 33 segregating RAPD loci obtained in the analysis were correlated with the cold responses of RILs according to Spearman correlation *rho* (r) by using IBM SPSS version 22.

Results

Cold tolerance evaluation in field and under controlled conditions

Genetic variation

The differences among RILs in terms of their cold tolerance were statistically significant in the tests carried out in Haymana (COLDH) and Yenimahalle (COLDY) (at $P < 0.01$ level) and in Sivas-Ulaş (COLDU) (at $P < 0.05$ level) according to ANOVA. The component of variance due to RILs was moderately high for all tested sites; however, when the component of total variance between three tested sites compared, COLDY (CV = 47.2%) had a higher percentage than in COLDH and COLDU (CV = 31.3% and CV = 19.1% respectively) (Table 3, panel A).

Table 3. Mean squares, component of variance as a % of total variance (CV) and heritabilities

	Traits	Replication	CV (%)	Genotypes	CV (%)	Error	CV (%)	Heritability (h^2_g)
A	COLDH	5.664	6.9	0.805**	31.3	0.320	61.8	0.60
	COLDU	0.084	0.0	1.277*	19.1	0.868	80.9	0.32
	COLDY	7.125	8.4	0.998**	47.2	0.238	44.4	0.76
B	Fv/Fm (+10 °C)	0.005	8.2	2.45×10^{-4} ns	00.0	3.267×10^{-4}	91.8	–[a]
	Fv/Fm (−5 °C)	0.087	21.5	0.002*	6.8	0.002	71.7	0.22
	Fv/Fm (−10 °C)	0.007	0.0	0.012*	7.5	0.009	92.5	0.20
	Fv/Fm (−15 °C)	0.001	0.0	0.004*	12.7	0.003	87.3	0.30

Panel A is for the cold tests carried out in three locations: COLDH, COLDU and COLDY. Panel B is for the Fv/Fm ratio determined at four different temperatures (+10, −5, −10 and −15 °C) in the controlled environment.
Fv/Fm, ratio of the variable fluorescence to maximum fluorescence; COLDU, Sivas-Ulaş Directorate of Agricultural Management Research Field; COLDH, concrete bed in the research field of Central Research Institute for Field Crops (CRIFC) in Ankara-Haymana; COLDY, research field of CRIFC in Ankara-Yenimahalle; ns: not significant.
*Significant at $P < 0.05$ level; **significant at $P < 0.01$ level.

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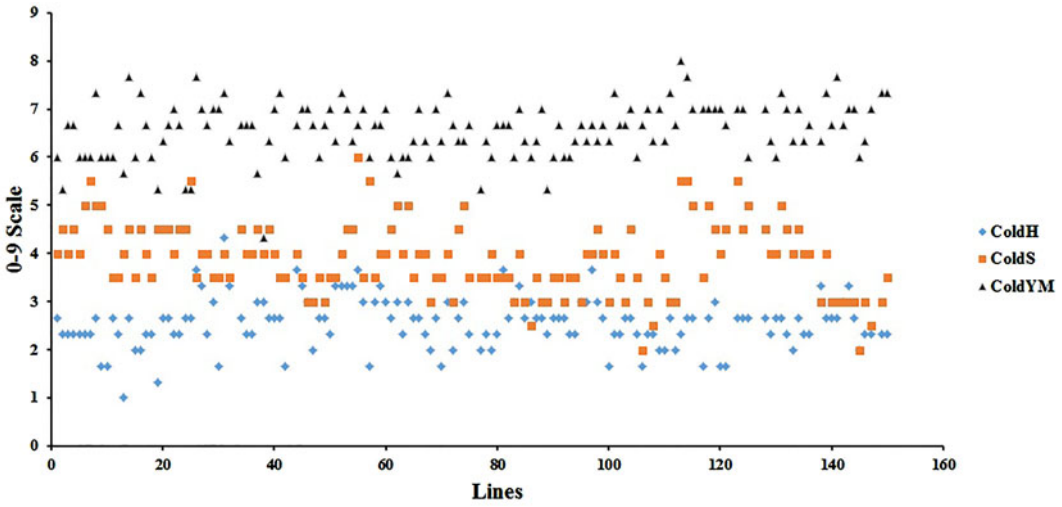

Fig. 2. (Colour online) Scattered diagram of 141 RILs derived from Kunduru-1149 × Cham-1 cross in 0–9 scale evaluation of cold tolerance; in Ankara-Haymana (COLDH), Sivas-Ulaş (COLDU) and Ankara-Yenimahalle (COLDY) test locations.

Because of the differences in the lowest temperatures recorded in the three test locations, response ranges of the RILs in the 0–9 scale have also shown differences among the locations. According to the mean values of three replications in the test fields, RILs responded within the ranges of 1.00–4.33, 2.00–6.00 and 4.33–8.00 scores in COLDH, COLDU and COLDY, respectively (Fig. 2).

The RILs displayed higher resistant scores in COLDH, where 124 lines (87.94%) responded to the cold with the scores between 1 and 3, while the rest of the lines (17) (12.06%) responded with the scores higher than 3. In COLDU, 29 lines (20.57%) scattered between the scores 2 and 3, while the rest of the lines (112) (79.43%) showed the scores higher than 3. In COLDY, 36 lines (25.53%) responded to the cold with the scores between 4.33 and 6, and the rest of the lines (105) (74.47%) responded with the scores higher than 6 (Fig. 2).

The estimated mean values of Fv/Fm ratio were in the ranges of 0.62–0.76, 0.25–0.53 and 0.16–0.37 for −5, −10 and −15 °C, respectively (data not given). The Fv/Fm ratio values of all the RILs at +10 °C varied within the range of only 0.72 and 0.78 (Fig. 3). For Fv/Fm ratio values, 70.92% of the RILs (100) scattered between 0.62 and 0.69, while 29.08% of the RILs (41) scattered between 0.70 and 0.76 at −5 °C; 87.94% of the RILs (124) responded within the range of 0.25 and 0.49 at −10 °C, while 12.06% of the RILs (17) responded within the range of 0.50 and 0.55; 81.94% of the RILs (132) have taken values between 0.16 and 0.29, whereas 6.38% of the RILs (9) have taken between 0.30 and 0.37 at −15 °C (Fig. 3).

The component of total variance for the Fv/Fm ratio attributable to RILs was calculated as 6.8, 7.5 and 12.7% at −5, −10 and −15 °C, respectively, while for +10 °C, which was the good temperature for growing all the RILs, was not significant (Table 3, panel B).

Heritabilities

Heritabilities (h^2) for cold tolerance in durum wheat lines ranged from moderate (0.32) in the COLDU test site to high (0.76) in COLDY (Table 3, panel A). On the other hand, the heritabilities estimated for the Fv/Fm ratio at low temperatures (−5, −10 °C and −15 °C) were low ranging from 0.20 in the Fv/Fm ratio (−10 °C) to 0.30 in the Fv/Fm ratio (−15 °C) (Table 3, panel B).

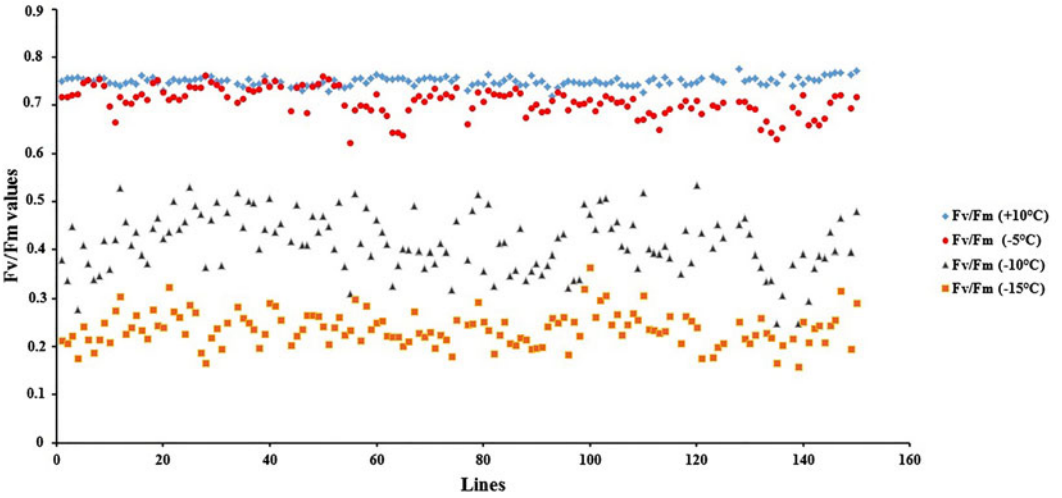

Fig. 3. (Colour online) Scattered diagram of 141 RILs derived from Kunduru-1149 × Cham-1 cross in cold tolerance evaluation by using Fv/Fm ratio at +10, −5, −10 and −15 °C.

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Table 4. The Spearman (*S*) correlation values between cold tolerance scores obtained by 0–9 evaluation scale and quality parameters' scores

Quality traits		COLDH	COLDU	COLDY	Quality traits		COLDH	COLDU	COLDY
Wet-TKW	r_S	0.072	**−0.245****	**−0.225****	OK	r_S	**−0.200***	−0.143	−0.103
	p	0.396	**0.003**	**0.007**		*p*	**0.018**	0.091	0.225
Dry-TKW	r_S	0.074	**−0.245****	**−0.225****	PC	r_S	0.056	0.105	0.123
	p	0.382	**0.003**	**0.007**		*p*	0.511	0.216	0.145
Moisture	r_S	**−0.203***	−0.030	0.007	PSI	r_S	−0.042	0.035	0.082
	p	**0.016**	0.722	0.937		*p*	0.621	0.682	0.332
VK	r_S	0.082	−0.103	0.054	YP	r_S	**0.235****	0.015	−0.159
	p	0.333	0.222	0.526		*p*	**0.005**	0.858	0.060
POK	r_S	0.018	**0.221****	0.004	SDS	r_S	0.090	0.015	−0.083
	p	0.829	**0.008**	0.964		*p*	0.287	0.859	0.330

COLDU, Sivas-Ulaş Directorate of Agricultural Management Research Field; COLDH, concrete bed in the research field of Central Research Institute for Field Crops (CRIFC) in Ankara-Haymana; COLDY, research field of CRIFC in Ankara-Yenimahalle; TKW, thousand kernel weight; VK, vitreous kernel; OK, opaque kernel; POK, partly opaque kernel; PC, protein content; PSI, particle size index; YP, yellow pigment; SDS: sodium dodecyl sulphate sedimentation.
*significant at $P < 0.05$ level; **significant at $P < 0.01$ level.

Correlations between cold scores and quality parameters

The results of analyses indicated that meaningful genetic correlations were observed between cold scores in COLDH, COLDU and COLDY and some quality parameters. The quality parameter MC, and OK% had negative correlation values with cold scores in COLDH as $r_S = -0.203$, $P = 0.016$ at $P < 0.05$ and $r_S = -0.200$, $P = 0.018$ at $P < 0.01$, while cold scores in COLDH had positive correlations with YP as $r_S = 0.248$, $P = 0.003$ at $P < 0.01$, respectively (Table 4). The quality traits wet-TKW and dry-TKW had negative and same correlation values ($r_S = -0.245$, $P = 0.003$ at

Table 5. The Spearman (*S*) correlation values between F_v/F_m ratios obtained at different temperatures and quality parameters' scores

Quality traits	*S*	Fv/Fm (+10 °C)	Fv/Fm (−5 °C)	Fv/Fm (−10 °C)	Fv/Fm (−15 °C)
Wet-TKW	r_P	**−0.231****	0.000	−0.063	0.015
	p	**0.006**	0.996	0.456	0.860
Dry-TKW	r_P	**−0.230****	0.000	−0.065	0.013
	p	**0.006**	0.999	0.442	0.879
Moisture	r_P	−0.099	0.030	**0.169***	**0.177***
	p	0.244	0.721	**0.045**	**0.035**
VK	r_P	0.000	−0.144	−0.056	0.042
	p	0.997	0.088	0.511	0.618
POK	r_P	0.014	**0.222****	0.146	−0.012
	p	0.872	**0.008**	0.083	0.885
OK	r_P	−0.004	−0.020	−0.077	−0.018
	p	0.962	0.812	0.362	0.833
PC	r_P	−0.022	**−0.296****	−0.050	−0.090
	p	0.800	**0.000**	0.557	0.288
PSI	r_P	−0.039	−0.099	−0.071	−0.014
	p	0.643	0.245	0.401	0.867
YP	r_P	0.040	**0.305****	**0.166***	−0.006
	p	0.642	**0.000**	**0.049**	0.947
SDS	r_P	0.061	0.132	0.097	0.104
	p	0.473	0.120	0.254	0.219
N		141	141	141	141

Fv/Fm, ratio of the variable fluorescence to maximum fluorescence; TKW, thousand kernel weight; VK, vitreous kernel; OK, opaque kernel; POK, partly opaque kernel; PC, protein content; PSI, particle size index; YP, yellow pigment; SDS, sodium dodecyl sulphate sedimentation.
*Significant at $P < 0.05$ level; **significant at $P < 0.01$ level.

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$P < 0.01$) for cold scores in COLDU, while they had negative and same correlation values ($r_S = -0.225$, $P = 0.007$ at $P < 0.01$) for cold scores in COLDY. In addition, cold scores in COLDU had positive correlation value with the quality parameter POK% ($r_S = 0.221$, $P = 0.008$ at $P < 0.01$) (Table 4). The cold scores in COLDH had positive correlation with YP as $r_S = 0.235$, $P = 0.005$ at $P < 0.01$.

The correlation values between Fv/Fm ratios and quality parameters indicated some interesting results (Table 5). Wet-TKW and dry-TKW parameters had negative correlations with Fv/Fm +10 °C (B-) and the correlation values are same as $r_S = -0.231$, $P = 0.006$ at $P < 0.01$. The highest correlation value was observed as $r_g = 0.305$, $P = 0.000$ at $P < 0.01$ between Fv/Fm −5 °C and YP. Fv/Fm −5 °C had negative correlation with protein content as $r_S = -0.296$, $P = 0.000$ at $P < 0.01$. Fv/Fm −10 °C and Fv/Fm −15 °C had positive correlation values with moisture content as $r_S = 0.169$, $P = 0.045$ at $P < 0.05$ and $r_S = 0.177$, $P = 0.035$ at $P < 0.05$, respectively.

Correlations between cold scores and gluten proteins

Correlations between gliadin and HMW-glutenin subunits (data not given), and cold scores of 141 RILs indicated that ω-gliadin fragment 5 had negative correlation ($r_S = -0.294$, $P = 0.00$ at $P < 0.01$) with cold scores in COLDH, while one of the γ-gliadin triplet (fragment 12) had negative correlation ($r_S = -0.167$, $P = 0.048$ at $P < 0.05$) with cold scores in COLDU (Table S3). In α-gliadin region, five gliadin fragments (25–29) had negative correlations with cold scores in COLDU as ($r_S = -0.203$, $P = 0.016$ at $P < 0.05$, $r_S = -0.203$, $P = 0.016$ at $P < 0.05$, $r_S = -0.199$, $P = 0.018$ at $P < 0.05$, $r_S = -0.199$, $P = 0.018$ at $P < 0.05$, $r_S = -0.199$, $P = 0.018$ at $P < 0.05$), respectively. HMW-glutenin subunits did not have any correlation with cold scores in three test locations. ω-Gliadin fragments 6 and 12 had negative correlations with the Fv/Fw ratio at −5 °C as $r_S = -0.167$, $P = 0.048$ at $P < 0.05$ and $r_S = -0.219$, $P = 0.009$ at $P < 0.05$, respectively. In α-gliadin region five fragments (25–29) displayed negative correlations ($r_S = -0.166$, $P = 0.048$ at $P < 0.05$, $r_S = -0.166$, $P = 0.048$ at $P < 0.05$, $r_S = -0.168$, $P = 0.047$ at $P < 0.05$, $r_S = -0.168$, $P = 0.047$ at $P < 0.05$, and $r_S = -0.168$, $P = 0.047$ at $P < 0.05$, respectively) with the Fv/Fm ratio (−5 °C). HMW-glutenin proteins did not have any correlation with Fv/Fw ratios in three test locations.

Correlations between cold scores and RAPD loci

The RAPD locus E-13/1100 had positive correlations with cold scores in COLDH, COLDU and COLDY ($r_S = 0.167$, $P = 0.047$ at $P < 0.05$, $r_S = 0.207$, $P = 0.014$ at $P < 0.05$, $r_S = 0.249$, $P = 0.003$ at $P < 0.01$, respectively) (Table S4). The RAPD locus E-13/900 showed positive correlations with cold scores in COLDH and COLDY ($r_S = 0.205$, $P = 0.015$ at $P < 0.05$, $r_S = 0.259$, $P = 0.001$ at $P < 0.01$, respectively), while the RAPD locus K-9/500 displayed positive correlation with cold scores in COLDU ($r_S = 0.193$, $P = 0.022$ at $P < 0.05$).

The RAPD loci B-11/730, B11/510, C-7/1800, C-7/1600, C-7/1450 and L-3/500 had positive correlations ($r_S = 0.176$, $P = 0.037$ at $P < 0.05$, $r_S = 0.240$, $P = 0.004$ at $P < 0.01$, $r_S = 0.244$, $P = 0.004$ at $P < 0.01$, $r_S = 0.193$, $P = 0.022$ at $P < 0.05$, $r_S = 0.185$, $P = 0.028$ at $P < 0.05$, $r_S = 0.284$, $P = 0.001$ at $P < 0.01$ and $r_S = 0.187$, $P = 0.026$ at $P < 0.05$, respectively) with cold scores in COLDY.

The correlations between RAPD loci and Fv/Fw ratios indicated that C-7/1600, C-7/1450 and L-3/500 had negative correlations ($r_S = -0.237$, $P = 0.005$ at $P < 0.01$, $r_S = -0.188$, $P = 0.025$ at $P < 0.05$, $r_S = -0.228$, $P = 0.007$ at $P < 0.01$, respectively) with Fv/Fw ratios at −15 °C.

Discussion

The results indicated that a considerable amount of genetic variation observed was consistent with the results of Mekhlouf *et al.* (2006). In addition, considerably higher components of variance due to genotypes and heritability estimates were determined for the tests carried out in three test locations. When the heritability results compared, it seemed that cold tolerance observations in the field indicated that the effect of genotype on the expression of phenotype was considerably high in COLDH and COLDU, whereas in COLDY, the effect of environment on the expression of phenotype was higher than genotype. Heritability results of cold tolerance in a controlled environment displayed that the effect of environment on the expression of phenotype was higher than genotypes. Therefore, studies for cold tolerance might be performed both in the field and in the controlled environment.

Many drawbacks of field tests were encountered as discussed in Sutka (1981), since survival is determined not only by cold damage to the tillering node of the plant, but also by other factors such as diseases, pushing out, water deficit, etc., or by interactions between these factors, it is very difficult to be certain on the cold tests carried out in the field. The use of a 0–9 scale as a qualitative evaluation, which is commonly used in cold-breeding programmes of many institutes is the other drawback of field tests performed as well in the current study. The drawbacks of the cold tests performed under the field conditions using a 0–9 scale were compensated by the tests carried out under controlled environmental conditions.

According to the 0–9 scale, score 4 represents 35–45% cold damage and the values up to score 4 can be considered as cold tolerant for RILs in the current study. In COLDH, lines 9, 10, 13, 19, 30, 42, 57, 70, 100, 117, 120 and 121 displayed the scores between 1 and 1.7, thus they can be considered as the most tolerant lines to cold. In COLDU, lines 106, 145, 86, 108 and 147 showed cold response scores between 2 and 2.5, while in COLDY, all the lines showed cold response scores higher than the score 4. These results are consistent with the lowest temperatures, −9.1, −12.8 and −15.2 °C observed in COLDH, COLDU and COLDY, respectively. In total, the 17 most tolerant RILs to cold are determined and these RILs could be managed as breeding material for further studies of crop improvement programmes.

The severe effects of cold damage upon a decrease in temperature could be seen clearly in the scattered diagram of Fv/Fm ratios at +10, −5, −10 and −15 °C in Fig. 3. The Fv/Fm ratio values displayed at −5 °C by some of the RILs might be considered as in the range of 0.79–0.84, which is the approximate optimal value for many plant species, with lowered values indicating plant stress (Kitajima and Butler, 1975; Demmig and Björkman, 1987; Maxwell and Johnson, 2000; Murchie and Lawson, 2013). If the RILs with equal or higher than the score 0.70 are considered as cold resistant, 41 RILs might have the potential for further durum wheat-breeding studies to develop new cold-resistant durum varieties.

According to the 0–9 scale cold damage and the evaluation values of Fv/Fm ratio, three test locations varied from each other at a considerably high level. Thus, the cold tolerance trait expressed differently in different test sites indicates that the test carried out in one site may not be adequate to select the cold-tolerant RILs. Therefore, besides the low temperature, other

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types of stress factors might also be considered and for trials, at least two locations might be recommended for the reliability of the results. Moreover, quantitative evaluation of chlorophyll damage due to low temperature created in the controlled environment of growth chamber by a chlorofluorometer was experienced as the quicker and more reliable method in cold studies (Clement and Van Hasselt, 1996).

The temperatures close to the lowest temperatures recorded in the field trials (−9.1 °C in COLDH and −15.2 °C in COLDY) were −10 and −15 °C, which were selected as test temperatures, and the damage at these temperatures in the growth chamber occurred more severely. This can be explained by the impossibility of mimicking the exact field conditions in the growth chamber in test boxes. However, −5 °C can be recommended as the best test temperature to determine the response of the material to cold in the growth chamber. In contrast, Rizza *et al.* (2001) reported that −12 °C was the most appropriate stress temperature to measure the differences among the genotypes in oat cultivars.

It was reported that some gliadin genes of wheat had linkage with frost tolerance genes (Šašek *et al.*, 1984, 2000; Sozinov, 1985; Černý *et al.*, 1990: cited in Prášil *et al.*, 2002). *Gli-1D5* and *Gli-6A3* were described as primary markers for frost tolerance, while *Gli-6D2*, *Gli-1B1* and *Gli-6A1* were described as secondary markers of frost tolerance. It was also confirmed that they had additive effects on frost tolerance and low frost tolerance linkage was reported with some gliadin alleles (*Gli-6A2*, *Gli-1B4* and *Gli-1B5*) (Šašek *et al.*, 2000: cited in Prášil *et al.*, 2002). Some correlations between cold tolerance and some gliadin fragments might be an indication of potential cold tolerance but, further detailed investigations needed to be performed. However, it was not observed any correlation between HMW-glutenin subunits and cold tolerance, while Witkowski *et al.* (2008) found that the SDS sedimentation value, resistance leaf blotch and frost tolerance (Naghavi *et al.*, 2010) had statistically significant association with the status of the *Glu-A1* locus, at which particularly null allele closely linked to the locus responsible for frost tolerance.

Molecular markers were used to determine their associations with traits such as cold resistance, drought tolerance, agronomic traits (grain yield, TKW and biomass yield, plant height, spike length, number of seeds per spike, etc.). The association between RAPDs and some traits was investigated in different plant species; such as drought tolerance in 20 bread wheat cultivars by Pakniyat and Tavakol (2007); the frost tolerance in winter faba bean (*Vicia faba* L.) by Sallam and Martsch (2016); some agronomic traits in barley by Cheghamirza *et al.* (2017). Some RAPD loci had positive correlations with cold evaluation values and variation fluorescence (Fv/Fm ratio) in three test locations as reported in previous studies, such as significant associations between freezing tolerance evaluation with field-laboratory method markers located on chromosome 5H in the region of gene *Fr-H2* (bin9–10: Xbmag812, Xmwg2230) and the region of gene *Fr-H1* (bin11: Xmwg514, HvBM5, Xmwg644) in barley reported by Rapacz *et al.* (2010). In addition, Rapacz *et al.* (2010) reported other significant associations with photochemical quenching of chlorophyll a fluorescence (qP) and PCR markers (Xpsr115 and Xmwg2062) and displayed the connection directly with the variation in the promoter region of *Vrn-H1* (HvBM5) (cited in Akar *et al.*, 2009) and freezing tolerance of plants partially de-acclimated in the field. Ganeva *et al.* (2013) used microsatellite loci to investigate their associations with frost tolerance and they found that the 176 bp allele at locus wmc327 tightly linked to *Fr-A2* in cvs Milena, Pobeda, Sadovo-1, Mironovskaya-808 and Bezostaya-1.

Investigation of tolerance, to abiotic stresses, is one of the main goals for the crop improvement studies, many genes are concerned with tolerance to stress. Revealing the molecular and genetic basis of stress tolerance will help to identify the functional marker and then increase the efficiency of selection for improved abiotic stress tolerance (Mishra and Varghese, 2012). Therefore, the RAPD loci, which had a positive correlation with cold evaluation values, might be used as candidate markers of cold tolerance for pre-selection of potential candidates for the development of new durum wheat varieties.

The low temperature negatively influences the traits OK, YP, wet-TKW and dry-TKW in the current study as in previous studies, a significant positive correlation between winter resistance and grain yield (Ruzgas and Liutkevičius, 2001) and influence of low temperature on starch damage and gliadin : glutenin ratios (Craven *et al.*, 2007) reported. Mohammadi *et al.* (2015) investigated the resistance to biotic and abiotic stress factors among 380 durum wheat collection. They identified that the landraces showed percentage reduction due to the stresses varied from 11.4% (yellow rust) to 21.6% (cold stress) for 1000-kernel weight and from 19.9% (yellow rust) to 91.9% (cold stress) for grain yield. It was also found that 0–9 scale cold evaluation values indicated that cold had a negative influence on some quality traits such as OK, moisture, wet-TKW and dry-TKW, while it had a positive effect on some traits such as POK and YP.

DNA markers such as a KASPar SNP assay is used for routine to screen *Rht-B1b* (*Rht-1*) or *Rht-D1b* (*Rht-2*), which control the semi-dwarf plant height in wheat in USDA Genotyping Labs (G. Brown-Guedria, 2013, unpublished: in Liu *et al.*, 2014*a*, 2014*b*); markers for photoperiod insensitive allele (*Ppd-D1a*) and the sensitive allele (*Ppd-D1b*) (Yang *et al.*, 2009: in Liu *et al.*, 2014*a*, 2014*b*); markers CDO708 and Vrn-D3F6/R8 were used to study *Vrn-A1* and *Vrn-D3* alleles for vernalization (Wang *et al.*, 2009; Chen *et al.*, 2010: in Liu *et al.*, 2014*a*, 2014*b*). Biochemical markers might have a close association with quantitative traits such as major quality traits of grain and flour protein and ash concentration, dough strength and extensibility, starch composition, grain hardness and end-use product colour are controlled by different genes, such as *Glu* and *Gli* loci, and some other genes (Liu *et al.*, 2014*a*, 2014*b*). Gluten proteins have large influence over both dough strength and extensibility (Payne, 1987; Luo *et al.*, 2001). Some of the gliadin and glutenin subunits proved to be good markers related to good bread-making and pasta quality (Payne *et al.*, 1980; Gupta and Shepherd, 1988; Pogna *et al.*, 1990). Gliadin γ-42 was found to be related to poor quality, and gliadin γ-45 to good quality. Both biotic and abiotic stresses can affect wheat end-use quality (Graybosch *et al.*, 1995). Therefore, DNA and biochemical markers, which have close association with quantitative traits could be used efficiently for preliminary research studies.

The current comparative results could be used efficiently in durum wheat-breeding programmes, which aim to develop cold tolerant varieties for the durum production areas suffering from severe cold damages. Especially the RILs, which were considered as cold tolerant, which selected at −5 °C in the growth chamber, could very confidently be used in cold tolerance improvement of durum wheat-breeding programmes.

Conclusion

It was concluded that the temperature at −5 °C in the growth chamber is the good temperature for pre-selection of cold-resistant

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lines. The RILs displayed great variation in expression of cold resistance in three different test locations at the same temperatures, thus not only temperature but also other associated factors also should be taken account to determine the cold responses of plants. DNA and biochemical markers might have a close association with quantitative traits could be used efficiently for preliminary research studies. For evaluation of cold tolerance, 0–9 scale and Fv/Fm ratios are reliable, practical and cost effective methods, which could be used efficiently.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0021859620000155.

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Ethical standards. This paper does not contain any studies involving animals or human participants performed by any of the authors.

References

Akar T, Francia E, Tondelli A, Rizza F, Stanca AM and Pecchioni N (2009) Marker-assisted characterization of frost tolerance in barley (*Hordeum vulgare* L.). *Plant Breeding* **128**, 381–386.

Becker WA (1992) *Manual of Quantitative Genetics*, 5th Edn. Pullman, WA: Academic Enterprises, p. 191.

Bushuk W and Zillman RR (1978) Wheat cultivar identification by gliadin electro-phoregrams I Apparatus, method, and nomenclature. *Canadian Journal of Plant Science* **58**, 505–515.

Černý J, Prášil I and Šašek A (1990) Values of gliadin markers of frost hardiness in common wheat: correlations with frost hardiness tests. *Genet a Šlecht* **26**, 83–89.

Cheghamirza K, Zarei L, Zebarjadi AR and Honarmand SJ (2017) A study of the association between ISSR and RAPD markers and some agronomic traits in barley using a multiple regression analysis. *BioTechnologia* **98**, 33–40.

Chen Y, Carver B, Wang S, Cao S and Yan L (2010) Genetic regulation of developmental phases in winter wheat. *Molecular Breeding* **26**, 573–582.

Chipilski R and Uhr ZL (2014) Study of frost resistance of common winter wheat varieties. *Trakia Journal of Sciences* **2**, 169–176.

Clement JMAM and Van Hasselt PR (1996) Chlorophyll fluorescence as a parameter for frost hardiness in winter wheat. A comparison with other hardiness parameters. *Phyton (Horn, Austria)* **36**, 29–41.

Craven M, Barnard A and Labuschagne MT (2007) The impact of cold temperatures during grain maturation on selected quality parameters of wheat. *Journal of Science, Food and Agriculture* **87**, 1783–1793.

Demmig B and Björkman O (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **170**, 489–504.

Falconer DS (1989) *Introduction to Quantitative Genetics*. Harlow, Essex, England: Longman Scientific & Technical.

Feldman M, Lupton FGH and Miller TE (1995) Wheats. In Smartt J and Simmonds NW (eds), *Evolution of Crop Plants*, 2nd Edn. London: Longman Scientific and Technical, pp. 184–192.

Fisker SE, Rose R and Haase DL (1995) Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1 + 1 Douglas-Fir seedlings. *Forest Science* **41**, 564–575.

Ganeva G, Korzun V, Landjeva S, Popova Z and Christov NK (2010) Genetic diversity assessment of Bulgarian durum wheat (Triticum durum Desf.) landraces and modern cultivars using microsatellite markers. *Genetic Resources and Crop Evolution* **57**, 273–285.

Ganeva G, Petrova T, Landjeva S, Todorovska E, Kolev S, Galiba G, Szira F and Bálint AF (2013) Frost tolerance in winter wheat cultivars: different effects of chromosome 5A and association with microsatellite alleles. *Biologia Plantarum* **57**, 184–188.

Göçmen B (2001) *Genetic Characterization of 150 F6-Inbred Durum Wheat Lines Derived from Kunduru-1149 × Cham-1 Cross Using Molecular Markers and Economically Important Traits* (PhD thesis) Middle East Technical University, Ankara, Turkey. Available at https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp.

Göçmen B, Keskin S, Kaya Z and Taşkın V (2003) Development of random amplified polymorphic DNA (RAPD) markers in 150 F6-inbred durum wheat (*Triticum durum*) lines derived from Kunduru-1149 × Cham-1. *Israel Journal of Plant Sciences* **51**, 245–249.

Graybosch RA, Peterson CJ, Baenziger PS and Shelton DR (1995) Environmental modification of hard red winter wheat flour protein composition. *Journal of Cereal Science* **22**, 45–51.

Gupta RB and Shepherd KW (1988) Low-molecular weight glutenin subunits in wheat; their variation inheritance and association with bread-making quality. In Miller TE and Koebner RMD (eds), *Proceedings of the 7th International Wheat Genetics Symposium*, Institute of Plant Science Research (IPSR), Cambridge, UK, pp. 943–949.

Hayes HK and Aamodt OS (1927) Inheritance of winter hardiness and growth habit in crosses of 'Marquis' with 'Minhardi' and 'Miturki' wheats. *Journal of Agricultural Research* **35**, 223–236.

Heidarvand L and Amiri RM (2010) What happens in plant molecular responses to cold stress? *Acta Physiologiae Plantarum* **32**, 419–431.

ICARDA (International Center for Agricultural Research in the Dry Areas) (2005) Turkey and ICARDA: Collaboration in Agricultural Research Since 1977. Ties that Bind, No. 23. Aleppo, Syria: ICARDA, vi+26 pp. En.

Kaya Z and Temerit A (1994) Genetic structure of marginally located *Pinus nigra* var. *pallasiana* Populations in Central Turkey. *Silvae Genetica* **43**, 272–277.

Kaya Z, Campbell RK and Adams WT (1989) Correlated responses of height increment and components of increment in 2-year-old Douglas-fir seedlings. *Canadian Journal of Forest Research* **19**, 1124–1130.

Kitajima M and Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta* **376**, 105–115.

Li Y, Wang Q, Li X, Xiao X, Sun F, Wang C, Hu W, Feng Z, Chang J, Chen M, Wang Y, Li K, Yang G and He G (2012) Coexpression of the high molecular weight glutenin subunit 1A×1 and puroindoline improves dough mixing properties in durum wheat (*Triticum turgidum* L. ssp. *durum*). *PLoS ONE* **7**, e50057.

Liu W, Maurer HP, Li G, Tucker MR, Gowda M, Weissmann EA, Hahn V and Würschum T (2014*a*) Genetic architecture of winter hardiness and frost tolerance in Triticale. *PLoS ONE* **9**, e99848.

Liu S, Rudd JC, Bai G, Haley SD, Ibrahim AMH, Xue Q, Hays DB, Graybosch RA, Devkota RN and Amand PST (2014*b*) Molecular markers linked to important genes in hard winter wheat. *Crop Science* **54**, 1304–1321.

Long SP, Humphries S and Falkowski PG (1994) Photoinhibition of photosynthesis in nature. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**, 633–662.

Luo C, Griffin WB, Branlard G and McNeil DL (2001) Comparison of low- and high molecular-weight wheat glutenin allele effects on flour quality. *Theoretical Applied Genetics* **102**, 1088–1098.

Maxwell K and Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* **51**, 659–668.

Mekhlouf A, Dehbi F, Bouzerzour H, Hannchi A, Benmahammed A and Adjabi A (2006) Relationships between cold tolerance, grain yield performance and stability of durum wheat (*Triticum durum* Dest.) genotypes grown at high elevation area of eastern Algeria. *Asian Journal of Plant Science Research* **5**, 700–708.

Mishra SC and Varghese P (2012) Breeding for heat tolerance in wheat. In Singh SS, Hanchinal RR, Singh G, Sharma RK, Tyagi BS, Saharan MS and Sharma I (eds), *Wheat: Productivity Enhancement Under Changing Climate*. New Delhi: Narosa Publishing House, p. 398.

Mohammadi R, Sadeghzadeh B, Ahmadi H, Bahrami N and Amri A (2015) Field evaluation of durum wheat landraces for prevailing abiotic and biotic stresses in highland rainfed regions of Iran. *Crop Journal* **3**, 423–433.

Murchie EH and Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany* **64**, 3983–3998.

Naghavi A, Sofalian O, Asghari A and Sedghi M (2010) Relation between freezing tolerance and seed storage proteins in winter bread wheat (*Triticum aestivum* L.). *Turkish Journal of Field Crops* **15**, 154–158.

Ng PKW and Bushuk W (1987) Glutenin of marquis wheat as a reference for estimating molecular weights of glutenin subunits by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *Cereal Chemistry* **64**, 324–327.

Nilsson-Ehle H (1912) Zur Kenntnis der Erblichkeitverhältnisse der Eigenschaft Winterfestigkeit beim Weizen. *Zeitschrift fur Pflanzenzüchtung* **1**, 3–12.

Pakniyat H and Tavakol E (2007) RAPD Markers associated with drought tolerance in bread wheat (*Triticum aestivum* L.). *Pakistan Journal of Biological Sciences* **10**, 3237–3239.

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. *Annual Review of Plant Physiology* **38**, 141–153.

Payne PI, Law CN and Mudd EE (1980) Control by homoeologous group-1 chromosomes of the high molecular weight subunits of glutenin a major protein of wheat endosperm. *Theoretical Applied Genetics* **58**, 113–120.

Plaschke J, Ganal MW and Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theoretical Applied Genetics* **91**, 1001–1007.

Pogna NE, Autran JC, Mellini F, Lafiandra D and Feillet P (1990) Chromosome 1B-encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. *Journal of Cereal Science* **11**, 15–34.

Prášil IT, Prášilová P, Šašek A and Černý J (2002) Assessment of frost tolerance of wheat doubled haploids by gliadin electrophoresis. *Czech Journal of Genetics and Plant Breeding* **38**, 104–108.

Rapacz M, Tyrka M, Gut M and Mikulski W (2010) Associations of PCR markers with freezing tolerance and photosynthetic acclimation to cold in winter barley. *Euphytica* **175**, 293–301.

Rizza F, Crosatt C, Stanca AM and Cattivelli L (1994) Studies for assessing the influence of hardening on cold tolerance of barley genotypes. *Euphytica* **75**, 131–138.

Rizza F, Pagani D, Stanca AM and Cattivelli L (2001) Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. *Plant Breeding* **120**, 389–396.

Ruzgas V and Liutkevičius G (2001) Investigation of winter wheat cold tolerance in Lithuania for breeding purposes. *Icelandic Agricultural Science* **14**, 29–34.

Sallam A and Martsch R (2016) Validation of RAPD markers associated with frost tolerance in winter faba bean (*Vicia faba* L.). *Turkish Journal of Botany* **40**, 488–495.

Šašek A, Černý J and Hanišová A (1984) Gliadinové bloky – markery mrazuvzdornosti u pšenice obecné. *Genet a Šlecht* **20**, 199–206.

Šašek A, Prášilová P, Černý J, Prášil I and Skupinová S (2000) Predikce zimovzdornosti pšenice seté pomocí gliadinových genetických markerů. *Rostl Výr* **46**, 245–254.

SAS Inst. Inc. (1988) SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC, p. 1028.

Sieber A-N, Longin CFH, Leiser WL and Würschum T (2016) Copy number variation of Cbf-A14 at the Fr-A2 locus determines frost tolerance in winter durum wheat. *Theoretical Applied Genetics* **129**, 1087–1097.

Sozinov AA (1985) Polimorfizm belkov i jego značenije v genetike i selekcii. Moskva: Nauka.

Sutka J (1981) Genetic studies of frost resistance in wheat. *Theoretical Applied Genetics* **59**, 145–152.

Sutka J (2001) Genes for frost resistance in wheat. *Euphytica* **119**, 167–172.

Sutka J and Snape JW (1989) Location of a gene for frost resistance on chromosome 5A of wheat. *Euphytica* **42**, 41–44.

Wang S, Carver B and Yan L (2009) Genetic loci in the photoperiod pathway interactively modulate reproductive development of winter wheat. *Theoretical Applied Genetics* **118**, 1339–1349.

Witkowski E, Waga J, Witkowska K, Rapacz M, Gut M, Bielawska A, Luber H and Lukaszewski AJ (2008) Association between frost tolerance and the alleles of high molecular weight glutenin subunits present in Polish winter wheats. *Euphytica* **159**, 377–384.

Worzella WW (1935) Inheritance of cold resistance in winter wheat with preliminary studies on the technique of artificial freezing test. *Journal of Agricultural Research* **50**, 625–635.

Yadav SK (2010) Cold stress tolerance mechanisms in plants. A review. *Agronomy for Sustainable Development* **30**, 515–527.

Yang F, Zhang X, Xia X, Laurie D, Yang W and He Z (2009) Distribution of the photoperiod insensitive Ppd-D1a allele in Chinese wheat cultivars. *Euphytica* **165**, 445–452.