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Genetic control of cold hardiness using generation mean analysis in bread wheat (*Triticum aestivum* L.em Thell)

Abolfazl Rashidi Asl[1*], Siroos Mahfoozi[2], M. Reza Bihamta[3]

[1]*Department of agriculture Islamic Azad University Shahr-e- Rey Branch, Iran*

[2]*Physiology Agronomy Unit of Department of Cereals Research, Seed and Plant Improvement Institute, Iran*

[3]*Biotechnology Department , Faculty of Agriculture ,University of Tehran, Iran*

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Abstract

Low – temperature (LT) tolerance in wheat is a quantitative character that is regulated by a complex arrays of genes. Although several genetic studies have been conducted , there is not a general consensus on the mode of gene action. The objective of the present study is to determine the mode of gene action and inheritance of cold tolerance genes(LT_{50}) in winter × spring cross of bread wheat. In this study 'Mironovskaya 808808' as tolerant (winter habit) was crossed with 'Pishtaz' as sensitive parent (spring habit) and F_1 , F_2 , BC_1 (back crossed to spring parent) , BC_2 (back crossed to winter parent) and F_2 generations were evaluated for freezing test. In this study it clarified that cold tolerance in wheat is controlled by additive, dominant and also epistatic genes. Overall, the number of genes related to cold tolerance in wheat estimated between 1 to 6. According to different methods, the broad sense (h^2_{BS}) and narrow sense heritabilities (h^2_{NS}) were 0.89 and 0.61 respectively. The h^2_{BS} shows that transmission of cold tolerance trait from tolerant to sensitive cultivars in breeding programs is possible.

* **Corresponding Author:** Abolfazl Rashidi Asl ✉ ab.rashidi@gmail.com

Introduction

Cold tolerance is a quantitative trait which control with different genes. A clear understanding about the role of genetic control in this trait and also developmental genes such as vernalization which passes the plant from vegetative to generative stage can help in breeding wheat cultivars for cold regions. Cold tolerance is a quantitative trait which controlled by major and minor genes. Sutka *et al.* (1997) found that freezing tolerance is controlling by additive and dominance genes. Estimating of gene number in quantitative traits is favorite for more plant breeders (Pohlman and Sleper, 1996).

The most powerful way for recognition the number of segregating factors is aneuploidy studies. This method estimate number, position and also the rate of relative effect of factors. Of course this method needs aneuploidy

sources and cytogenetic studies which are often unavailable. An alternative method is quantitative genetics methods which can not recognize monogenes. In this manner we can estimate segregating or effective factors(units) not the number of genes (Mather K. and Jinks L., 1982). In this method we use P_1, P_2, F_1, F_2, BC_1 and BC_2 generations. Each of BC_1 or BC_2 are the progeny of crosses between F_1 with each one of parents.

In many crops specially cereals LT_{50} (Lethal Temperature) is a common way to compare genotypes of their cold tolerance. LT_{50} is a temperature below zero in which 50% of plants die(Storlie *et al.*, 1998 ; Fowler *et al.*, 1981). This method is a well-known way to assess the cold tolerance in laboratory or field conditions (Limin and Fowler, 1988 , Fowler *et al.*,1999 , Mahfoozi *et al.*, 2000 , 2006).

Materials and methods

To study the genetic control of cold tolerance in wheat, two cultivars as parents were crossed to develop different generations in the present study. The parents were "Mironovskaya 808808" (highly cold-resistant cultivar with LT_{50} = -19°C) and "Pishtaz" (an Iranian highly cold-susceptible cultivar with LT_{50} = -7°C). By crossing this parents different generations including P_1, P_2, F_1, F_2, BC_1 and BC_2 developed and were used in freezing test. The seeds of these generations after sterilization germinated in petri dish between paper for two days in 4°C and then for one day in 20°C in green house. Then the germinated seeds were sown in trays by 30 × 50 × 15 dimension which filled by mixture of field soil, sand and peat (1:2:2). The trays for 18 days were put in green house with 20°C and a12/10h (D:N) photoperiod. After this period(in 5 Jan.) the seedlings in three leaves stage replaced in outdoor condition for cold acclimation for 30 days. After this step the seedlings were used for measuring LT_{50} . The experimental design was randomized complete block with three replications. There was 60 seedlings in each replication. Cold tolerance was assessed according to Fowler et al. (1981) and Mahfoozi et al.(2001b)). A set of 11 test temperatures ranging from −3°C to −23°C, with increments of 2°C were used. For LT_{50} testing , the crowns were detached from the plants and covered with moist sand in aluminum cans and placed in a programmable freezer and kept at −3°C for 12 h. After this period, they were cooled at a rate of 2 °C/h to −17 °C and then cooled at a rate of 8 °C/h to a minimum of −23 °C (Mahfoozi et al., 2001b). Five crowns were removed for each of the tested temperatures in each generation. Samples were thawed overnight at 4 °C and replanted in controlled chambers at 20 °C with a 14/10 h (D:N) photoperiod. Plant recovery was rated after 3 weeks of regenerate and LT_{50} was calculated for each generation.

The means and variances of parental, F1, F2, F3, BC1,and BC2 generations were used to estimate the components of gene action by the weighted least squares method (Mather and Jinks, 1982). The epistatic model describing non-allelic interactions between pairs of loci was tested by following the statistical model described by Mather and Jinks (1982):

$$Y = m + \alpha [d] + \beta [h] + \alpha 2 [i] + 2\alpha\beta[j] + \beta 2[l]$$

where Y = generation mean, m = mean of all possible homozygous lines deriving from the cross, [d], [h], [i], [j], and [l] = net directional effects of loci contributing to additive, dominance, additive x additive, additive x dominance, and dominance x dominance components, respectively, and α and β = coefficient of genetic parameters. Due to the different sizes and variances of generations, the weighted least square method was used to predict the genetic parameters (Kearsey and Pooni, 1996). The genetic model that best fit the data was found by the mean of joint scaling test (Mather and Jinks, 1982), and the accuracy of the models was verified by chi-square test. Components within each model were evaluated for significance by t-test. Estimates of dominance ratio, broad-sense heritability (h2 B) (Kearsey and Pooni, 1996), narrow-sense heritability (h2 N) (Warner, 1952), standard errors of h2 B (Ehdaie and Weines, 1994), and h2 N (Ketata *et al.,* 1976) for LT50 were obtained using the following equations:

$$\text{Dominance ratio} = \sqrt{(4\sigma_D^2/2\sigma_A^2)}$$

$$h^2_{Bs} = (V_{F2} - V_E)/V_{F2})$$

$$h^2_{Ns} = [2V_{F2} - (V_{BC1} + V_{BC2})]/V_{F2}$$

Gene number was estimated by the following formulae (Chen and Line, 1995). Although each formula has its restrictions and assumptions, all assume equal gene effects.

1) $n = (\mu_{P2} - \mu_{P1})^2/[8(\sigma^2_{F2} - \sigma^2_{F1})]$

2) $n = (\mu_{P2} - \mu_{P1})^2/\{8[\sigma^2_{F2} - (0.5\sigma^2_{F1} + 0.25\sigma^2_{P1} + 0.25\sigma^2_{P2})]\}$

3) $n = (\mu_{P2} - \mu_{P1})^2/\{8[\sigma^2_{F2} - (\sigma^2_{BC1} + \sigma^2_{BC2})]\}$

4) $n = (\mu_{P2} - \mu_{P1})^2/\{8[(\sigma^2_{BC1} + \sigma^2_{BC2}) - (\sigma^2_{F1} + 0.5\sigma^2_{P1} + \sigma^2_{P2})]\}$

5) $n = (\mu_{F1} - \mu_{P1})^2/\{4[\sigma^2_{BC1} - 0.5(\sigma^2_{F1} + \sigma^2_{P1})]\}$

6) $n = (\mu_{P2} - \mu_{F1})^2/\{4[\sigma^2_{BC2} - 0.5(\sigma^2_{F1} + \sigma^2_{P2})]\}$

Where $\mu_{P1} < \mu_{P2}$

Results

For all six generations the weight analysis of variance and means grouping in duncan method carried out with SAS software. The results showed that there was a significant difference between P_1, P_2, F_1, F_2, BC_1 and BC_2 for cold tolerance (LT_{50}) (table 1).

Table 1. The ANOVA table for LT_{50} in six generations of this cross.

S.O.V	df	MS	F
block	2	0.733	0.29 n.s
treatment	5	49.417	19.41 **
error	10	2.545	-
total	17	-	-

** and * significant in α = 0.01 and α = 0.05 respectively; n.s. non significant

This difference has clearly shown in means grouping. Mironovskaya 808808 and Pishtaz have -19.13 and -7.23°C LT_{50}s respectively. The LT_{50} of F_1, F_2, BC_1 and BC_2 generations are -11.36, -14.2, -10.9 and -14.73 °C respectively. The F_1 with BC_1 was in a same group, and F_2 population with BC_2 in other class. Each of parents were in a separate cluster in this grouping (Fig. 1).

Table 2. Degree of dominance , estimating of broad sense and narrow sense heritability using different formulas and genetic advance for cold tolerance (LT_{50}) in Mironovskaya 808808× Pishtaz cross.

Cross	Degree of dominance (h/d)	h^2_{BS}§					h^2_{NS}Θ	GAΦ
		1	2	3	4	5		
Mironovskaya808 × Pishtaz	-0.307	0.895	0.896	0.897	0.896	0.897	0.612	3.51

§ broad sense heritability; Θ narrow sense heritability; Φ genetic advance

Estimating of broad sense heritabilities based on population variances were calculated with different formulas which with narrow sense heritability mentioned in Table 2.

As it shown in table 2 the dominance degree is negative (h/d<0) that shows the partial dominance to sensitive parent. The heritability for this trait is shown in table-2. Estimating of broad sense heritability with different formulas showed the same results. As it shown the broad sense and narrow sense heritabilities are 0.89 and 0.61 respectively. In this study the genetic advance (GA) was 3.51 (with this hypothesis that 10% of segregated plants with more tolerance were selected (Table 2).

Table 3. Estimating of segregating genes(effective factors) for cold tolerance (LT_{50}).

Cross	Formula					
	1	2	3	4	5	6
Mironovskaya808 × Pishtaz	3.94	3.85	2.55	3.00	1.25	6.03

The least number of genes or effective factors were estimated with different formulas (Lande, 1981) (table 3). Estimating the gene number in this study (table-3) showed a range between 2 to 6 genes. Although all of mentioned models in this study had goodness of fit that shows there was no linkage, triple interaction or genotype × environment interactions. Since in this study early generations was used so there is no linkage balance(Mather and Jinks, 1982).

In current study the 'm', [d] and [h] components were significant(table-4). Also all possible models including six parameter model fitted to observed means. In this study [i] (additive × additive) and [j] (additive × dominance) interactions were significant which shows epistatic effects. Brule – Bable and Fowler (1988) in a same study by using different generations of winter × spring habit wheats showed that there is at least one dominant gene which is related to cold tolerance and vernalization requirement. Fowler *et al.* (1999) divided the genetical systems of cold tolerance into three classes as follow:

- Master switch genes that control the development of plants such as vernalization genes
- The genes which discovered within conventional genetic studies
- The genes such as *Wcs*120 and *Wcor*410 which are induced at molecular level in low temperatures

The results showed that not only the duration but also the level of gene expression controls the plant tolerance. Developmental genes such as vernalization or final leaf number (FLN) are responsible for tolerance expression(Mahfoozi *et al.*, 2000,2001,2005,2006; Fowler *et al.*, 2001; Limin and Fowler, 2006). Because in spring habit cultivars the expression of tolerance genes reduce significantly by entrance to generative stage thus the genetic potential of these cultivars don't have enough time to expression(Mahfoozi *et al.*, 2000,2006; Fowler *et al.*, 2001; Danyluk *et al.*, 2003).

Table 4. Estimating the genetic components of cold tolerance (LT_{50}).

Cross	m	[d]	[h]	[i]	[j]	[l]	χ^2
Mironovskaya808 × Pishtaz	-16.56 ± 0.644**	5.94 ± 0.109**	5.24 ± 0.750**	3.39 ± 0.658**	-4.31 ± 1.071**	-	1.709 n.s

** and * significant in α = 0.01 and α = 0.05 respectively; n.s. non significant.

Discussion

Winter wheat is characterized by medium to strong vernalization response and in many cases, photoperiod sensitivity, depending on the latitude where selection was carried out(Davidson *et al.* 1985; Flood and Halloran, 1986). This allows the crop to delay vegetative growth until temperatures rise in spring. Cold tolerance per se also contributes to winter wheat survival. Plants with winter growth habit or 'transgresinely late segregants' can be selected from crosses between spring varieties provided that parents posses different dominant *Vrn* genes (Jedel, 1994).

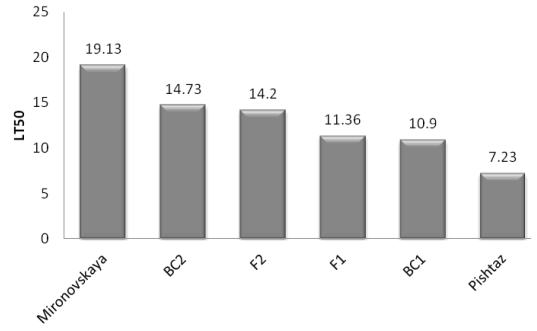

Fig. 1. Compare means of cold tolerance (LT_{50}) in different generations of Mironovskaya808 × Pishtaz.

The ability of plants to cold acclimate is a quantitative trait (Thomashow, 1990). Indeed, in wheat, there is evidence that nearly all chromosome pairs can contribute to freezing tolerance. Recent studies have identified a locus on chromosome 5A, the *Vrn1-Fr1* interval, that has a major effect on freezing tolerance(Galiba *et al.,* 1995). The *Vrn1-Fr1* interval contains the *Vrn1* gene, a major determinant of growth habit (Brule-Babel *et al.* 1988; Pugsley, 1971; Snape *et al.* 1976). Witer-type plants, which are sown in autumn, carry recessive *vrn1* alleles. Such plants require a period of vernalization(exposure to low temperature) to promote floral development. The vernalization requirement is thought to have evolved to insure that overwintering plants do not flower before the warm growing season. In contrast, spring-type plants can be sown in spring as they carry dominant *Vrn1* alleles that allow floral development without vernalization. Significantly, winter-type plants carrying *vrn1* alleles are almost exclusively more freezing tolerant than spring-type plants carrying *Vrn1* alleles, which indicates that either *Vrn1* itself is a freezing tolerance gene(s) or that it is tightly linked to a freezing tolerance gene(s).

The mechanism whereby the *Vrn1-Fr1* interval affects freezing tolerance remains to be determined. Limin *et al.* (1997) have shown that cold-induced expression of the *wcs120* genes, which are located on chromosomes 6A, 6B and 6C, is higher in a winter-type than in a spring type. It's possible that the *Vrn1-Fr1* interval encodes a protein(s) involved in regulating the expression of cold-inducible genes that have roles in freezing tolerance.

Based on the results of current study, it estimated that the number of genes affected the cold tolerance in wheat are 1 to 6. According to different methods, the broad sense (h^2_{BS}) and narrow sense heritabilities (h^2_{NS}) were 0.89 and 0.61 respectively. The broad sense heritability value shows that transmission of cold tolerance trait from tolerant to sensitive cultivars in breeding programs is possible. Thus development of cold tolerance cultivars of wheat is possible.

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