

Cold Hardiness in Near Isogenic Lines of Bread Wheat (*Triticum Aestivum* L. em. Thell.)

Abolfazl Rashidi Asl, Siroos Mahfoozi, and Mohammad Reza Bihamta

Abstract—Low temperature (LT) is one of the most abiotic stresses causing loss of yield in wheat (*T. aestivum*). Four major genes in wheat (*Triticum aestivum* L.) with the dominant alleles designated *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn4*, are known to have large effects on the vernalization response, but the effects on cold hardiness are ambiguous. Poor cold tolerance has restricted winter wheat production in regions of high winter stress [9]. It was known that nearly all wheat chromosomes [5] or at least 10 chromosomes of 21 chromosome pairs are important in winter hardiness [15]. The objective of present study was to clarify the role of each chromosome in cold tolerance. With this purpose we used 20 isogenic lines of wheat. In each one of these isogenic lines only a chromosome from 'Bezostaya' variety (a winter habit cultivar) was substituted to 'Capple desprez' variety. The plant materials were planted in controlled conditions with 20°C and 16 h day length in moderately cold areas of Iran at Karaj Agricultural Research Station in 2006-07 and the acclimation period was completed for about 4 weeks in a cold room with 4°C. The cold hardiness of these isogenic lines was measured by LT₅₀ (the temperature in which 50% of the plants are killed by freezing stress). The experimental design was completely randomized block design (RCBD) with three replicates. The results showed that chromosome 5A had a major effect on freezing tolerance, and then chromosomes 1A and 4A had less effect on this trait. Further studies are essential to understanding the importance of each chromosome in controlling cold hardiness in wheat.

Keywords—Cold hardiness, isogenic lines, LT₅₀, *Triticum*.

I. INTRODUCTION

WHEAT is grown across a wide range of environments and is considered to have the broadest adaptation of all cereal crop species [2]. This broad adaptation is due, to a large extent, to wheat's cold tolerance, i.e. the ability to withstand temperatures much lower than 1-4°C, considered the minimum temperature for growth.

In a general sense, cold tolerance in wheat should refer to performance at temperatures lower than the optimum for growth (about 20°C), and there are definitely differences in the growth rate of cultivars at low temperatures and, consequently, in their adaptation to cool climate.

Low temperature stress is a major factor limiting cereal production worldwide. Winter soil temperatures at crown

A. Rashidi Asl is with the Department of Agriculture, Islamic Azad University, Shahre-Rey Branch, Shahre – Rey, Iran (corresponding author, e-mail: ab.rashidi@gmail.com).

S. Mahfoozi is with Physiology – Agronomy Unit of Department of Cereals Research, Seed and Plant Improvement Institute, P.O. Box 31585-4119, Karaj, Iran (e-mail: siroosmahfoozi@yahoo.com).

M. R. Bihamta is with Biotechnology Dept., Faculty of Agriculture, University of Tehran, Karaj, Iran (e-mail: mrghanad@ut.ac.ir).

depth normally fluctuate around 0 to -4°C in this region with the result that the plants are exposed to variable periods of temperatures in the vernalization range. Because cereals regulate their development through adaptive mechanisms that are responsive to low but non-freezing temperatures, the unpredictable nature of the over wintering period makes the selection of highly adapted genotypes for this region a difficult challenge.

Freezing tolerance is not a static condition, for it changes with time, temperature, soil and plant moisture, nutrition, and physiological age and status. It depends largely on the cold acclimation or hardening processes.

Indeed, differences in freezing tolerance of unhardened plants of different cultivars are negligible, while considerable differences can be detected after full hardening. The hardening process can be stopped, reversed, and restarted. Generally, under natural conditions, the dynamics of freezing tolerance are characterized by three stages [12]:

- a hardening period, in autumn, when cold tolerance is acquired;
- a period of tolerance maintenance, when the critical or lethal temperature varies, depending on temperature fluctuations in winter;
- a dehardening period, generally at the end of winter, when plants lose their cold tolerance.

II. MATERIALS AND METHODS

Plant materials were *Triticum aestivum* L. em Thell. Cultivars 'Bezostaya', 'Capple Desprez' and 20 substitution lines. In each one of these isogenic lines only a chromosome from 'Bezostaya' variety (a winter habit cultivar) was substituted to 'Capple desprez' variety. 'Bezostaya' is a winter habit variety with a long vernalization requirement which have been cultivated for several years in Russia and Iran, as a well yield and cold hardened variety. But because it is susceptible to yellow rust (*Puccinia striiformis*) the culture of this variety become limited in recent years. 'Capple Desprez' also is a winter variety in Western Europe which has a long vernalization requirement with good tolerance to cold stress, and is cultivated in west European countries. The cold hardiness of this variety is less than 'Bezostaya' [3,4].

Two varieties and 20 isogenic lines were planted on January in 2006-07. All plant materials were planted in controlled condition, with 20°C and 12h daylight. The seeds were germinated before planting on wet paper in petridishes (two days in 4°C and one day in 20°C). Germinated seeds were planted in pots with 2:2:1 (field soil: sand: pitmas) ratio.

After 2 weeks on February 4th when seedlings were emerged the pots transferred outdoor for acclimation. After 4 weeks in this conditions (from February 4th to March 4th) when the plants reached to 4-5 leaves stage we measured freezing resistance by LT_{50} (freezing temperature in which 50% of plants die). Since the vernalization requirements of 'Capple Desprez' and 'Bezostaya' are same so any increasing in cold hardiness in substitution lines specially about 5A chromosome from 'Bezostaya' to 'Capple Desprez' is due to other cold hardiness genes except vernalization genes.

III. RESULTS AND DISCUSSION

For freezing resistance trait the difference among varieties and substitution lines was significant ($F = 2.76^{**}$) (Table I).

TABLE I
THE ANOVA TABLE OF LT_{50} FOR VARIETIES AND SUBSTITUTION LINES

S.O.V.	df	MS
block	2	2.76n.s
treatment	21	5.02 ^{**}
error	42	

n.s : non significant

** : significant at $\alpha = 1\%$

According to Fig. 1 the LT_{50} of 'Capple Desprez' and 'Bezostaya' are -10°C and -12°C respectively. The freezing tolerance of isogenic line which had 5A chromosome increased up to -13°C . This increment is for this opinion that some other genetic factors (in addition to vernalization genes) which are related to cold hardiness are on the chromosome 5A. In contrast chromosome 5D had no significant effect in freezing tolerance. There are major quantitative trait loci (QTL) on 5A and minor quantitative trait loci on 1D chromosomes [1]. The QTL loci on 5A chromosome are located with 46 cM distance from vernalization gene (*Vrn-A1*) and discuss about 40% of variation in freezing resistance. The present of *vrn* allele is the reason for expression of resistant genes in winter wheat [10]. Pleiotropic effect of vernalization genes on cold resistance and growth habit is reported [10]. In this research 5A, 1A, 4A, 7D, 6B and 3D chromosomes cause increasing cold tolerance in 'Capple Desprez', although the effect of 5A is more significant (Fig. 1).

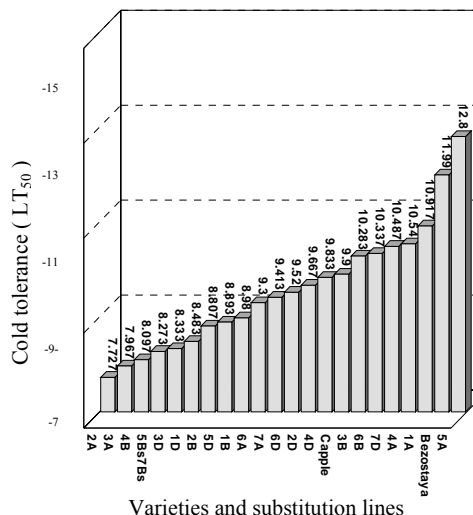


Fig. 1 The differences in cold tolerance among varieties and substitution lines

In other same research on substitution lines from 'Cheyenne' to 'Chinese spring' to clarify the relationship between cold hardiness and expression of *Wcs 120* gene family found that 5A, 7D, 3B, 6D, 5D, 2D, and 3B chromosomes cause increasing in cold hardiness in CS (Chinese spring) and also found that *Wcs 120* genes are located on chromosome 6 group that their expression is under control of vernalization genes. Cumulation of *Wcs 120* proteins in substitution lines with 1A, 3A, 5A, 6A, 7A, 3B, 5B and 7D were more than others [11]. In this study also 5A chromosome had important role in expression of cold tolerance, interaction between chromosomes and genes in some other substitution lines causes decrease in cold hardiness. 5A and then 5B chromosomes have important effect on cold hardiness [13]. The fact that several chromosomes (5A, 7D, 3B, . . .) play role in cold hardiness says that this trait is under control of many genes. Expression of cold hardiness and *Wcs 120* proteins are related to chromosomes in group 5 (5A) [3,6]. The most important QTL loci in barley which control winter survival, LT_{50} , growth habit and fructane content in crown is located on the long arm of 7 chromosome which is similar to 5 chromosome in bread wheat.

The chromosomes of group 5 in wheat contain vernalization and cold hardiness genes [7,11]. Genetic factors which influence cold hardiness are studying by monosomic and substitution lines. According to some reports about 15 chromosomes of 21 in wheat have a role in cold tolerance and also the role of 5A is more important [14] and then 7A, 4D and 5D chromosomes also play a role in cold hardiness [8].

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