ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 100, No. 4 (2013), p. 417–424
DOI 10.13080/z-a.2013.100.053

Evaluation of freezing tolerance of winter wheat (*Triticum aestivum* L.) under controlled conditions and in the field

Rita ARMONIENĖ, Žilvinas LIATUKAS, Gintaras BRAZAUSKAS

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: rita.armoniene@lzi.lt

Abstract

Freezing tolerance of winter wheat (*Triticum aestivum* L.) is one of the main factors governing winter survival. To avoid winterkill it is very important to choose freezing tolerant wheat genotypes. Experiments were carried out to evaluate freezing tolerance of winter wheat varieties and breeding lines under field and controlled conditions with cold acclimation and with no cold acclimation stage. Field experiments were done for two years during the winters of 2010–2011 and 2011–2012. The evaluation of winter hardiness was conducted in April of 2011 and 2012 by visual scoring on a 1–9 score basis (score 1 – all plants were killed, score 9 – all plants survived). Wheat genotypes under investigation experienced very different climatic conditions in the field during the two winters. Consequently, no correlation between winter hardiness scores of 2010–2011 and 2011–2012 winters was observed. Winter hardiness scores ranged from 1.0 (WW-8) to 7.6 ('Zentos') after 2010–2011 winter, whereas winter hardiness scores varied from 5.8 (WW-31) to 7.8 ('Kovas DS') after 2011–2012 winter. Freezing tolerance of winter wheat genotypes was also assessed in the controlled conditions by measuring chlorophyll fluorescence parameter Fv/Fm in leaves and percentage survival of plants after the freezing test. High correlation ($r = 0.88$, $p < 0.01$) between freezing test survival of non-acclimated wheat and Fv/Fm ratio showed that this method can speed up the selection for freezing tolerance by evaluating wheat genotypes immediately after the test. Results of our study showed low negative, but significant relationship ($r = -0.41$, $p < 0.05$) between 2010–2011 winter hardiness scores in the field and freezing test survival of non-acclimated plants in the laboratory experiment. Wheat genotypes that quickly lose winter hardiness have higher freezing tolerance without hardening stage. Our results support the hypothesis of two separate genetic systems of freezing tolerance in winter wheat which are determined by *duration* and *rate* of gene expression during cold acclimation.

Key words: freezing tolerance, Fv/Fm, *Triticum aestivum*, winter hardiness.

Introduction

Wheat is one of the major crops in Lithuania and world-wide. Winter wheat is sown in the autumn and harvested the following summer. This allows the autumn-seeded wheat to take advantage of autumn rainfall and thereby yield significantly more than comparable spring-seeded crops. The capability of autumn-seeded varieties to survive winter is often referred to as winter hardiness. Winter hardiness is one of the main limiting factors for winter wheat growing under Lithuanian conditions (Ruzgas, Liutkevičius, 2001). Winter hardiness is a complex trait involving tolerance to freezing, desiccation, anoxia, ice-encasement, resistance to diseases, etc. However, tolerance to low freezing temperatures (frost tolerance) has been considered as the primary limiting factor in most regions (Fowler, Limin, 1997). Freezing tolerance can be defined as the ability of plants to survive freezing temperatures, prevent damage to the vegetative tissues and minimize other negative effects of freezing temperatures on future yield potential (Reinheimer et al., 2004). In winter cereals, frost tolerance is associated with the occurrence of cold-hardening or cold acclimation, which is triggered by induction of battery of *Cor* (cold responsive) genes after exposure of plants to low but non-freezing temperature for certain periods of time (Winfield et al., 2010).

Low-temperature (LT) tolerance is a complex quantitative character that is expressed in anticipation of and during exposure of plants to temperatures that approach freezing. This environmentally induced character is determined by a highly integrated system of structural and developmental genes that are regulated by environmentally responsive, complex pathways (Fowler, Limin, 2007). According to the developmental theory of LT gene regulation (Fowler et al., 1999), *duration* and *rate* of gene expression determine the degree of LT tolerance. The developmental genes (vernalization, photoperiod, etc.) act as the switches controlling the *duration* of expression of LT-induced structural genes while the *rate* of LT acclimation is determined by genotype dependent expression levels of these genes (Fowler, Limin, 2004;

2007). In this system, full expression of cold hardiness genes only occurs in the vegetative stage and plants in the reproductive phase have a limited ability to cold acclimate. Plants that are still in the vegetative stage also have the ability to re-acclimate following periods of exposure to warm temperatures while plants in the reproductive phase have only a limited ability to re-acclimate (Fowler et al., 1999; Fowler, Limin, 2007).

The two major loci that control LT tolerance in *Triticeae* are the frost resistance *Fr-1* and *Fr-2* (Vágújfalvi et al., 2000). The *Fr-1* locus is tightly linked to the vernalization locus *Vrn1*. Allelic variations in the *Vrn1* locus are the main determinants of the winter and spring habit in wheat and barley (Fu et al., 2005). The *Vrn1* and *Fr-1* loci have not been genetically uncoupled, and are hypothesized to be the same locus. The pleiotropic effects of the *Vrn1* locus can explain most of the associated LT tolerance and winter habit (Båga et al., 2007; Dhillon et al., 2010). Low temperatures during winter slow shoot apex development and induce expression of *Vrn1*. Activation of *Vrn1* causes the decrease in frost tolerance that begins when plants are fully vernalized (Fowler, Limin, 2007). *Vrn-A1* region is convergence point for pathway that determines the vegetative/reproductive transition thereby giving it a direct influence on the duration of expression of the rate determining LT tolerance genes (Fowler, Limin, 2007; Laudencia-Chingcuanco et al., 2011). The *Fr-2* locus containing a cluster of C-repeat binding transcription factors is approximately 30 centimorgans proximal to *Vrn-1* (Galiba et al., 1995). The *Fr-2* locus is known to be involved in the regulation of cold induced genes in several plant species including members of the *Triticeae* tribe (Fowler, Limin, 2007; Knox et al., 2008). Transcripts encoding *Cbf-like* proteins have been shown to accumulate rapidly in response to LT in 'Puma' rye and 'Norstar' wheat (Jaglo et al., 2001). Two-tandem clusters of eleven *Cbf* genes map to the *Fr-H2* quantitative trait locus (QTL) in barley (Skinner et al., 2005) and *Rcg1* (regulator for *Cor 14b*) maps to the *Fr-A2* QTL in wheat indicating that *Cbf-like* genes are primary candidates for the *Fr-2* frost tolerance genes (Vágújfalvi et al., 2005). As transcriptional activators for LT tolerance associated genes, the *Cbf-like* genes are also prime candidates for the *rate* genes (Fowler, Limin, 2007).

Frost test can be carried out in the field, growth chamber or combined in the field-laboratory method. Evaluation of frost tolerant cereal genotypes under field conditions is very complicated, as winter hardiness is a complex trait. The field-laboratory method requires considerable space in freezing chambers and depends on many factors including freezing-thawing cycles, snow and ice cover, rainfall, physiological drought that may affect sample under field conditions before being transferred to controlled conditions (Fowler, Gusta, 1979) and gives distinct and even opposite results of freezing tolerant genotypes each year (Rapacz et al., 2008). Frost tests in a controlled environment are more reproducible and many freezing temperatures can be applied in experiments run in growth chamber in order to differentiate the cereal genotypes on the basis of frost resistance (Vágújfalvi et al., 2010). However, the assessment of frost resistance under controlled conditions is not related directly to factors limiting plant survival in the field-laboratory method (Rapacz et al., 2008). There are many methods to evaluate frost test and identify frost tolerant/susceptible plant genotypes. These include the determination of plant survival, the study of photosynthetic system integrity by chlorophyll fluorescence analysis and the estimation of cell membrane damage by electrolyte leakage (conductance) measurements (Reynolds et al., 2001). One of these methods is determination of the maximum quantum yield of the photosystem II (PSII) photochemistry by the ratio of variable to maximal fluorescence in dark-adapted state (Fv/Fm). Fv/Fm analysis is proposed as a rapid method for evaluating frost tolerance in cereals like oat (Rizza et al., 2001), wheat (Rapacz, Wozniczka, 2009) and barley (Rapacz et al., 2008). However, it remains unclear whether this screening method is applicable to local winter wheat breeding programs with highly variable overwintering conditions during the last decade. The objective of this study was to evaluate freezing tolerance of various winter wheat genotypes under field and controlled conditions with cold acclimation and without cold acclimation stage. Fv/Fm parameter was measured under controlled conditions after freezing test in order to assess the ability of chlorophyll fluorescence-based method to screen for frost tolerance in winter wheat genotypes.

Materials and methods

Plant material. Five winter wheat varieties ('Kovas DS', 'Zunda DS', 'Kaskada DS', 'Vikaras DS', 'Ada') and 21 breeding lines (WW-8, WW-13, WW-14, WW-15, WW-18DH, WW-27, WW-29, WW-30, WW-31, WW-32, WW-35, WW-45, WW-62DH, WW-65DH, WW-66DH, WW-67DH, WW-68DH, WW-70, WW-71, WW-75DH, WW-76DH) developed at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, and variety 'Zentos' (Lantmännen SW Seed, Germany) as a standard were chosen for this study.

Field experiment. Field experiments were set up in a nine-course crop rotation in Dotnuva during 2010–2012. The soil of the experimental site is *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*), light loam. It contained 1.5–2.0% humus, available phosphorus (P_2O_5) ranging from 190 to 240 mg kg^{-1}, available potassium (K_2O) – from 180 to 260 mg kg^{-1} and pH 6.5 to 7.0. Black fallow preceded the experiment. Fertilizer NPK rates (in pure elements) before sowing were 30-60-90 kg ha^{-1}. Nitrogen N_{90} kg ha^{-1} was applied after resumption of vegetation. Weeds were controlled by herbicides in the autumn. No other pesticides were applied during the plant growing season. Winter wheat varieties/lines were sown in 3.6 m^2 plots using randomized design with four replications in 2010–2011 and with three replications in 2011–2012 at a seed rate of 4.5 million ha^{-1} with a plot sowing machine "Hege 80" ("Wintersteiger", Austria). The seeds were pesticide-untreated. The evaluation of winter hardiness in natural conditions was conducted in April of 2011 and 2012 by visual scoring on a 1–9 score

basis (Ruzgas, Liutkevičius, 2001). Score 1 means that all plants were killed, while score 9 denotes that all plants survived.

Laboratory freezing test. The seeds of winter wheat varieties/lines were germinated on the moist filter paper in the Petri dishes. After three days, germinated seeds were sown in plastic pots (225 cm^3) filled with universal peat substrate GP0428 ("Durpeta", Lithuania). Each genotype was planted in six replications. Plants were grown at 18°C in a greenhouse with a 16 h photoperiod until the seedlings reached a three-leaf stage. The first experiment included cold acclimation period at 4°C with an 8 h photoperiod for five weeks in a growth chamber. The second experiment was conducted without cold acclimation period. Freezing test was carried out as described by (Skinner, Mackey, 2009) with minor modifications. Just prior to the freezing, plants were counted and the pots were drenched with cold water. Freezing tests were carried out in a programmable freezing chamber PE 2412 UY-LX ("Angelantoni Industrie", Italy). Both acclimated and non-acclimated plants were immediately transferred to freezing chamber. Plants were kept at 4°C for one hour, whereupon the temperature in the freezer was reduced from 4°C to the −12°C over an 8 h period. The temperature was held at the target temperature for 2 h, then raised to 4°C over 8 h and subsequently maintained at 4°C for 24 h. After the freezing test, the plants were moved to the greenhouse. Freezing test survival was scored as the proportion of the plants that had re-grown after two weeks. The Fv/Fm – the yield of the energy trapped in PSII was determined in leaves with a hand-held pulse modulated fluorometer OS-30p (Opti-Science Inc., USA) using the Fv/Fm kinetics option. Leaves were cut off after freezing tests and stored in black bags at 4°C for 24 h. The measurements were taken in 5 replications (leaves) in 5 different spots of leaves holding a leaf by dark-adaptation clips.

Meteorological conditions. The two field experiments performed at 2010–2011 and 2011–2012 autumn-winter seasons had very different climatic conditions (Fig. 1). In 2010–201, the autumn–winter seasons' weather conditions were characterised by a wet second half of autumn and a cold, snowy winter with thaws. November was very wet (precipitation level higher by 26.5 mm compared to annual average). December was cold and wet (temperature was lower by 5.6°C, precipitation rate exceeded annual level by 22.4 mm). Snow depth on the field at the end of December amounted to on average 34 cm. January was warmer (temperature was higher by 2.4°C compared to annual average). Cold weather (temperature was lower by 5.1°C compared to annual average) settled in February. In 2011–2012, the autumn–winter seasons' weather conditions were characterised by dry autumn, unusually warm first half of winter and very cold by the end of winter. October and November were dry (precipitation level lower by 25.9 and 22.9 mm compared to annual average, respectively). In December and the first half of January (average temperature was 5ºC higher compared to the annual average). The weather was unusually cold during the first ten-day period of February

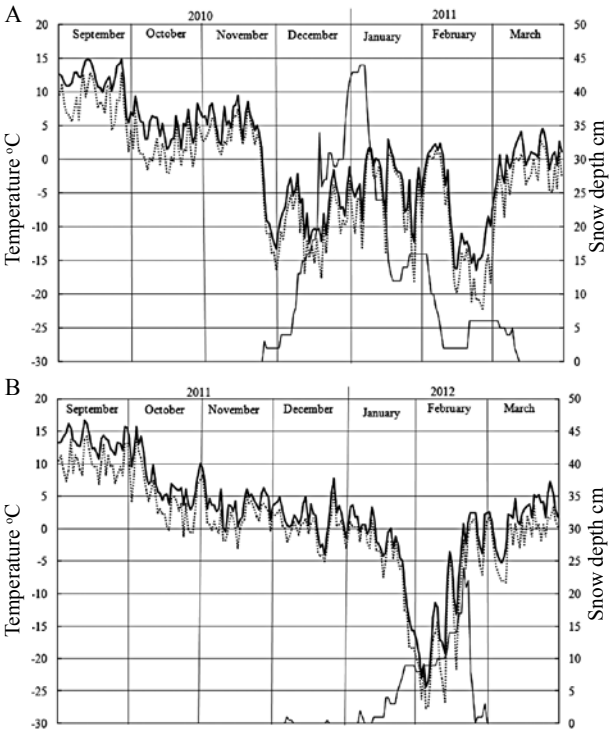


Figure 1. Mean (solid) and minimal daily (dashed) temperatures and snow depth during winters of 2010–2011 (A) and 2011–2012 (B)

(temperature was lower by 14.1°C compared to annual average). The temperature dropped below −27.6°C on February 4 in 2012.

Statistical analysis. Statistical analysis was performed using *Statistica 7* software (StatSoft Inc., Tulsa, USA). Analysis of variance (*ANOVA*) was used to determine significant ($p < 0.01$ and $p < 0.05$) differences between wheat genotypes. The differences between means of wheat varieties/breeding lines were inspected using Fisher's protected significant differences post hoc analysis. Spearman correlation coefficients between mean values of the investigated traits were calculated.

Results and discussion

Wheat winter hardiness in the field. The 2010–2011 winter was favourable for winter hardiness evaluation of wheat genotypes due to the wet autumn, sharp temperature fluctuations with frost and thaw periods and shifting snow cover. Some winter wheat lines were completely killed. Winter hardiness scores ranged from 1.0 (WW-8) to 7.6 ('Zentos') (Fig. 2). Winter wheat variety 'Ada' also showed high score of winter hardiness (6.6), while most plants of 'Zunda DS' were killed. Analysis of variance indicated significant differences of winter hardness ($p < 0.05$) between doubled haploid and conventionally-bred wheat genotypes. The majority of doubled haploid wheat lines were completely killed during the 2010–2011 winter. The 2011–2012 winter was not favourable for evaluation of winter hardiness of wheat genotypes due to beneficial weather conditions during autumn 2011 for cold acclimation and sufficient snow cover during freezing period in February, 2012.

Furthermore, no periods of warming and subsequent deacclimation of plants were observed that year. All wheat genotypes survived the winter of 2011–2012. Winter hardiness scores ranged from 5.8 (WW-31) to 7.8 ('Kovas DS') (Fig. 2). No significant differences of winter hardiness ($p > 0.05$) were found between doubled haploid and conventionally-bred wheat lines.

Wheat genotypes under investigation experienced very different freezing conditions in the field during the two winters (Fig. 1). Consequently, no significant correlation for wheat winter hardiness was observed between years (Table). Variation coefficients for winter hardiness was 13.3% and 3.5% in 2010–2011 and 2011–2012, respectively, suggesting a powerful interaction between the genotypes investigated and weather conditions. Different ranking of genotypes each year in the field-laboratory experiments depends on the interaction between de-acclimation and growth conditions (Rapacz et al., 2008). At the end of the first decade of February 2011 a strong thaw was followed by the cold period (minimal day temperatures dropped to −20ºC) with almost no snow cover. Vegetation of wheat was started during thaw period (wheat released a new roots). Wheat plants could lose their frost tolerance due to the full vernalization saturation and subsequent *Vrn-1* activation and this could lead to the winterkill of some wheat genotypes. No periods of warming were observed during the winter of 2011–2012 and long lasting low above-zero temperatures during autumn and first half of the winter resulted in good acclimation of the plants. Although the minimal temperature of −27.6°C was recorded on 4 February, 2012, snow cover of 9 cm in depth had prevented damage of wheat genotypes under study. Bergjord et al. (2008) noted that quantifying and modelling relationship between temperature, snow cover and fluctuation in frost tolerance could be a suitable first step towards understanding and prediction of winter damage. These observations suggest that depth of snow cover during the second half of the winter is very important for winter survival when freezing tolerance reaches saturation and starts decreasing.

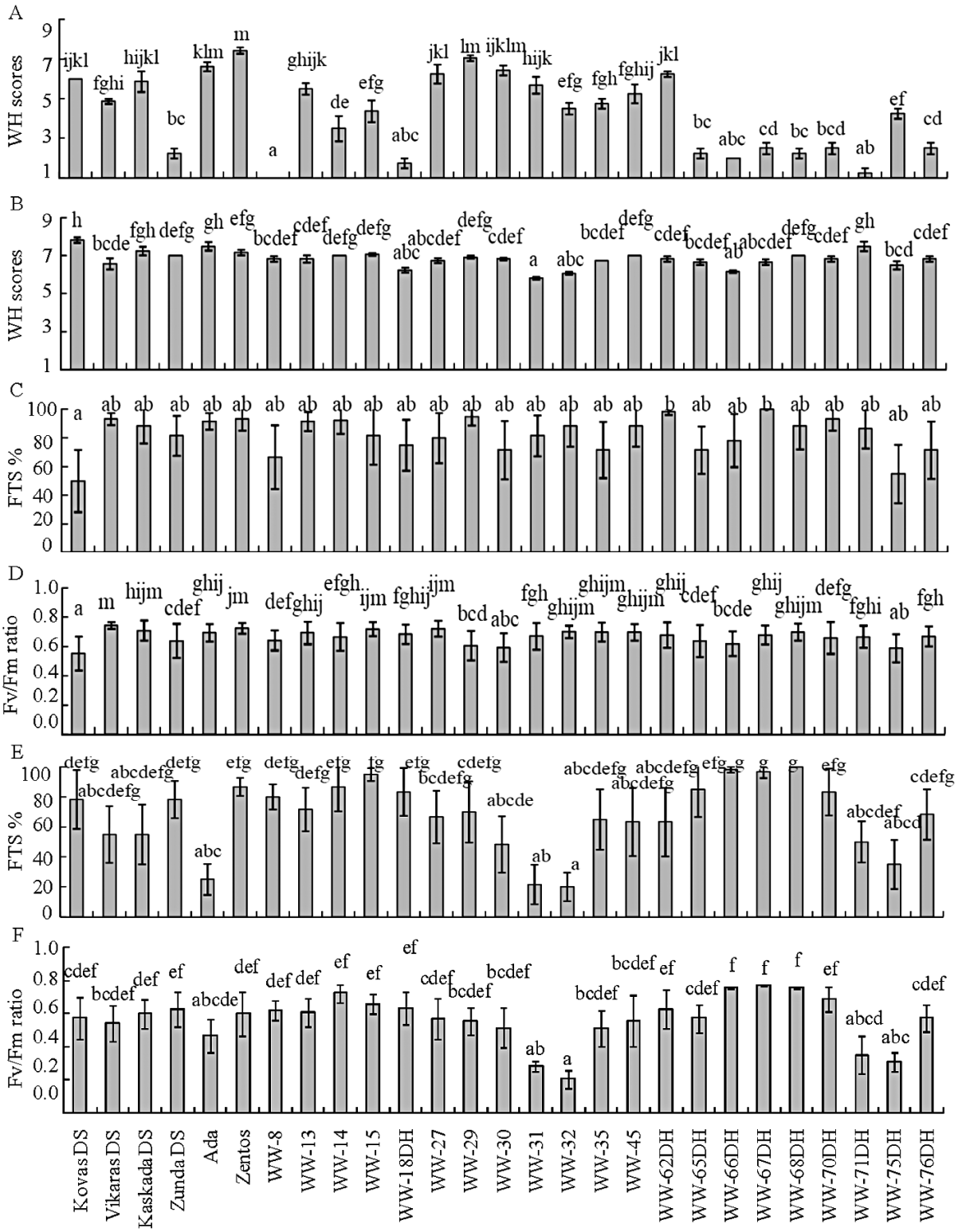
Table. Matrix of Spearman correlation coefficients between winter hardiness of two winters in field experiments, freezing test survival and Fv/Fm ratio in laboratory experiments with hardening stage and without hardening stage

	WH 2010–2011	WH 2011–2012	FTS with hardening	Fv/Fm with hardening	FTS without hardening	Fv/Fm without hardening
WH 2010–2011	1.00	0.24	0.31	0.24	−0.41*	−0.38*
WH 2011–2012		1.00	0.15	0.05	0.12	0.14
FTS with hardening			1.00	0.41*	0.10	0.26
Fv/Fm with hardening				1.00	−0.05	−0.00
FTS without hardening					1.00	0.88**
Fv/Fm without hardening						1.00

* – $p < 0.05$, ** – $p < 0.01$; WH – winter hardiness, FTS – freezing test survival

Wheat freezing tolerance in the laboratory test. Analysis of variance indicated significant differences of Fv/Fm ratio and freezing test survival ($p < 0.01$) between wheat genotypes. Freezing test survival of non-acclimated plants varied from 20% (WW-31) to 100% (WW-68DH). Plants with high survival also had the highest Fv/Fm ratio. The most freezing susceptible wheat plants had an average Fv/Fm ratio value of 0.21 (WW-31), while the most freezing tolerant plants had an average Fv/Fm ratio value of 0.77 (WW-67DH) and 0.76 (WW-68DH). Results of our study also show that the ratio of the maximum variable to maximum total fluorescence in dark-adapted state, Fv/Fm is an efficient method for frost tolerance evaluation in wheat in controlled conditions due to the high correlation between freezing test survival of non-acclimated wheat and Fv/Fm parameter ($r = 0.88$, $p < 0.01$). This relationship has also been demonstrated in oat ($r = 0.87$) (Rizza et al., 2001), barley ($r = 0.91$) (Rapacz et al., 2008) in controlled conditions. Rapacz and Wozniczka (2009) studies show high negative correlation between electrolyte leakage results after freezing test in controlled conditions and Fv/Fm parameter ($r = −0.87$).

Freezing test of acclimated plants shows no significant differences ($p > 0.01$) for freezing tolerance between winter wheat genotypes. Freezing test survival varied from 50% ('Kovas DS') to 100% (WW-67DH). Average Fv/Fm values varied from 0.55 ('Kovas DS') to 0.75 ('Vikaras DS'). The correlation coefficient between the Fv/Fm parameter and freezing test survival of acclimated plants was 0.41, $p < 0.05$. Skinner and Mackey (2009) have shown that the minimum temperature (−12°C) significantly affects survival in all of the winter wheat progeny populations, while the other components such as the total time frozen, the pre-freezing period, the post-freezing warming rate significantly influenced survival in some, but not all of the populations, suggesting genotypic differences in the ability to tolerate variation in specific aspects of the freezing process. Our results show that the minimum temperature of freezing test described by Skinner and Mackey (2009) is inappropriate for freezing tolerance screening of cold-acclimated wheat due to no significant differences between genotypes detected. The much lower correlation between acclimated wheat and Fv/Fm in our study could be explained by the insufficient minimum temperature selected for freezing. We also used larger containers (2650 versus 254 cm^3); therefore minimum temperature of −12°C was too high for evaluation of freezing tolerance differences among wheat genotypes. The LT_{50} value (temperature at which 50% of the plants are killed by cold stress) is selected



Notes. WH – winter hardiness, FTS – freezing test survival. Vertical bars represent ± standard deviation (SD) of the mean. The letters above the boxes indicate statisticaly significant ($p < 0.01$) differences between winter wheat genotypes.

Figure 2. Freezing tolerance of wheat genotypes in the field and laboratory experiments: winter hardiness of wheat lines/varieties in 2010–2011 (A) and 2011–2012 (B) field experiments, freezing test survival (C) and Fv/Fm ratio (D) of acclimated wheat lines/varieties, freezing test survival (E) and Fv/Fm ratio (F) of non-acclimated wheat lines/varieties in laboratory experiments

for freezing tolerance evaluation in many studies. In our freezing test accomplished with cold acclimation period only variety 'Kovas DS' reached LT_{50} at −12°C.

Results of our study show low negative, but significant relationship ($r = -0.41$, $p < 0.05$) between 2010–2011 winter hardiness scores and freezing test survival of non-acclimated plants (Table). Some of cultivars with the highest 2010–2011 winter hardiness, 'Ada' and WW-30, showed one of the lowest freezing test survival in freezing test with no acclimation. However, 'Zunda DS', WW-8DH and some of the other DH wheat lines with the lowest 2010–2011 winter hardiness had one of the highest freezing test survival after freezing with no acclimation (Fig. 2). Fowler and Limin (2007) suggest the existence of two separate genetic systems of freezing tolerance which are determined by the expression of duration and rate genes. The duration of LT tolerance is determined by the rate of phenological development and the time to vegetative/reproductive transition (Fowler, Limin, 2007). The *Vrn-A1* locus is a master switch for pathway that determines the vegetative/reproductive transition and directly influences the duration of LT tolerance (Fowler, Limin, 2007; Laudencia-Chingcuanco et al., 2011). Laudencia-Chingcuanco et al. (2011) have shown that after vegetative/reproductive transition plant continues to lose ability to cold acclimate so that at 70 days after cold treatment the winter-habit genotypes were almost as cold sensitive as the untreated spring-habit genotypes. Bergjord et al. (2008) have noticed a gradual decline of cold tolerance of winter wheat after December. Winfield et al. (2009) have shown that in the winter wheat varieties, transcript levels for *TaVrn1* were initially low but by 9 weeks of vernalisation had increased, and by 12 weeks, when plants were assessed to be fully vernalized, there had been an approximately 10 fold increase in abundance. Activation of *Vrn-1* caused the decrease in frost tolerance that begins when plants are fully vernalized. Dhillon et al. (2010) have shown that *Triticum monococcum* lines with mutations in the *Vrn-1* promoter, showed a significant down-regulation of *Cor14b* under long days.

Vrn-1 gene probably has no effect on the freezing tolerance of wheat in controlled conditions without the period of low temperature exposure. Thus it can be assumed that non-acclimated plants can be affected just by the genetic factors that determine the rate of acclimation. According to Fowler and Limin (2007), the rate component determines the degree that the structural LT tolerance conferring genes are up regulated and *Cbf-like* genes are prime candidates for the rate genes. In *Arabidopsis Cbf* genes are transcriptional factors that are rapidly up-regulated in response to LT treatment and are activators of *Cor* genes. Skinner et al. (2005) demonstrated that in the *Triticeae* – as in *Arabidopsis* – members of the *Cbf* gene family function as fundamental components of the winter hardiness region. Our studies in controlled conditions showed that some non-acclimated wheat genotypes such as 'Kovas DS', 'Zunda DS' and 'Zentos' have high freezing tolerance as well as one of the highest winter hardiness scores. These results can be explained by the fact that wheat grown in the greenhouse started acclimation already at 18°C. It was hypothesized that plants with a warm threshold temperature might better prepare for later freezing stress (Fowler, 2008). It has been suggested that the warmer the induction temperature, the sooner LT tolerance starts to accumulate (Campoli et al., 2009). Rye cultivars with acclimation induced at warmer temperatures were the most coldhardy (Campoli et al., 2009). Rye variety 'Puma' began to acclimate at the temperature as high as 17°C and the *Cor14b* ortholog was expressed in 'Puma' at the temperature higher than 15°C. Previously, Knox et al. (2008) have shown that *TmCbf12*, *TmCbf14* and *TmCbf15* were up-regulated at 12–15°C in the frost-tolerant *T. monococcum* genotype. 'Kovas DS' and 'Zentos' showed good winter hardiness in the field, and these results suggest that wheat genotypes with the higher threshold of cold acclimation and the latest full vernalization saturation can better prepare for the winter.

Conclusions

1. No correlation between winter hardiness scores of 2010–2011 and 2011–2012 winters shows that the winter hardiness is determined by a complex of factors which vary at different climatic conditions. Depth of snow cover during the second half of winter plays an important role in winter survival.

2. High correlation between freezing test survival of non-acclimated wheat and Fv/Fm ratio ($r = 0.88$, $p < 0.01$) shows that this method can speed up the selection for freezing tolerance by evaluating wheat genotypes immediately after the freezing test.

3. Low negative, but significant relationship ($r = -0.41$, $p < 0.05$) between 2010–2011 winter hardiness in the field and freezing test survival of non-acclimated plants supports hypothesis of two separate genetic systems controlling freezing tolerance in winter wheat which are determined by *duration* and *rate* of gene expression during cold acclimation. Wheat genotypes with faster de-acclimation (limited *duration* tolerance) in the field show higher freezing tolerance without acclimation (high *rate* tolerance) in the freezing test.

Acknowledgements

This work was supported by the long-term research programme "Genetics and directional transformation of genotypes in agricultural and forest plants" implemented by the Lithuanian Research Centre for Agriculture and Forestry.

Received 16 01 2013
Accepted 14 05 2013

References

Båga M., Chodaparambil S. V., Limin A. E., Pecar M., Fowler D. B., Chibbar R. N. 2007. Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. Functional and Integrative Genomics, 7 (1): 53–68 http://dx.doi.org/10.1007/s10142-006-0030-7

Bergjord A. K., Bonesmoa H., Skjelvag A. O. 2008. Modelling the course of frost tolerance in winter wheat I. Model development. European Journal of Agronomy, 28: 321–330 http://dx.doi.org/10.1016/j.eja.2007.10.002

Campoli C., Matus-Cadiz M. A., Pozniak C. J., Cattivelli L., Fowler D. B. 2009. Comparative expression of *Cbf* genes in the *Triticeae* under different acclimation induction temperatures. Molecular Genetics and Genomics, 282 (2): 141–152 http://dx.doi.org/10.1007/s00438-009-0451-9

Dhillon T., Pearce S., Stockinger E., Distelfeld A., Li C., Knox A. K., Vashegyi I., Vágújfalvi A., Galiba G., Dubcovsky J. 2010. Regulation of freezing tolerance and flowering in temperate cereals: the *VRN-1* connection. Plant Physiology, 153 (4): 1846–1858 http://dx.doi.org/10.1104/pp.110.159079

Fowler D. B. 2008. Cold acclimation threshold induction temperatures in cereals. Crop Science, 48: 1147–1154 http://dx.doi.org/10.2135/cropsci2007.10.0581

Fowler D. B., Gusta L. V. 1979. Selection for winter hardiness in wheat (*Triticum aestivum* L.). I. Identification of genotypic variability. Crop Science, 19 (6): 769–772 http://dx.doi.org/10.2135/cropsci1979.0011183X001900060005x

Fowler D. B., Limin A. E. 1997. Breeding for winter hardiness in cereals. Acta Agronomica Hungarica, 45 (3): 301–309

Fowler D. B., Limin A. E. 2004. Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. Annals of Botany, 94: 717–724 http://dx.doi.org/10.1093/aob/mch196

Fowler D. B., Limin A. E. 2007. Progress in breeding wheat with tolerance to low temperature in different phenological developmental stages. Buck H. T. et al. (eds). Wheat production in stressed environments. Dordrecht, The Netherlands, p. 301–314

Fowler D. B., Limin A. E., Ritchie J. T. 1999. Low-temperature tolerance in cereals: model and genetic interpretation. Crop Science, 39: 626–633 http://dx.doi.org/10.2135/cropsci1999.0011183X003900020002x

Fu D., Szucs P., Yan L., Helguera M., Skinner J., Hayes P., Dubcovsky J. 2005. Large deletions in the first intron of the *VRN-1* vernalization gene are associated with spring growth habit in barley and polyploid wheat. Molecular Genetics and Genomics, 273 (1): 54–65 http://dx.doi.org/10.1007/s00438-004-1095-4

Galiba G., Quarrie S. A., Sutka J., Morgounov A., Snape J. W. 1995. RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theoretical and Applied Genetics, 90 (7–8): 1174–1179

Jaglo K. R., Kleff S., Amundsen K. L., Zhang X., Haake V., Zhang J. Z., Deits T., Thomashow M. F. 2001. Components of the *Arabidopsis* C-repeat / dehydrationresponsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. Plant Physiology, 127 (3): 910–917 http://dx.doi.org/10.1104/pp.010548

Knox A. K., Li C., Vágújfalvi A., Galiba G., Stockinger E. J., Dubcovsky J. 2008. Identification of candidate *CBF* genes for the frost tolerance locus *Fr-Am2* in *Triticum monococcum*. Plant Molecular Biology, 67 (3): 257–270 http://dx.doi.org/10.1007/s11103-008-9316-6

Laudencia-Chingcuanco D., Ganeshan S., You F., Fowler D. B., Chibbar R., Anderson O. 2011. Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat (*Triticum aestivum* L.). BMC Genomics, 12 (1): 299 http://dx.doi.org/10.1186/1471-2164-12-299

Rapacz M., Wozniczka A. 2009. A selection tool for freezing tolerance in common wheat using the fast chlorophyll a fluorescence transient. Plant Breeding, 128 (3): 227–234 http://dx.doi.org/10.1111/j.1439-0523.2008.01566.x

Rapacz M., Tyrka M., Kaczmarek W., Gut M., Wolanin B., Mikulski W. 2008. Photosynthetic acclimation to cold as a potential physiological marker of winter barley freezing tolerance assessed under variable winter environment. Agronomy and Crop Science, 194 (1): 61–71

Reinheimer J. L., Barr A. R., Eglinton J. K. 2004. QTL mapping of chromosomal regions conferring reproductive frost tolerance in barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics, 109 (6): 1267–1274 http://dx.doi.org/10.1007/s00122-004-1736-3

Reynolds M. P., Nagarajan S., Razzaque M. A., Ageeb O. A. A. 2001. Cold tolerance. Application of physiology in wheat breeding / Reynolds M. P. et al. (eds). CIMMYT, Mexico, p. 111–123

Rizza F., Pagani D, Stanca A. M. Cattivelli L. 2001. Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. Plant Breeding, 120 (5): 389–396 http://dx.doi.org/10.1046/j.1439-0523.2001.00635.x

Ruzgas V., Liutkevičius G. 2001. Investigation of winter wheat cold tolerance in Lithuania for breeding purposes. Icelandic Agricultural Sciences, 14: 29–34

Skinner D. Z., Mackey B. 2009. Freezing tolerance of winter wheat plants frozen in saturated soil. Field Crops Research, 113 (3): 335–341 http://dx.doi.org/10.1016/j.fcr.2009.06.014

Skinner J. S., Zitzewitz J., Szucs P., Marquez-Cedillo L., Filichkin T., Amundsen K., Stockinger E. J., Thomashow M. F., Chen T. H. H., Hayes P. M. 2005. Structural, functional, and phylogenetic characterization of a large *CBF* gene family in barely. Plant Molecular Biology, 59 (4): 533–551 http://dx.doi.org/10.1007/s11103-005-2498-2

Vágújfalvi A., Crosatti C., Galiba G., Dubcovsky J., Cattivelli L. 2000. Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes. Molecular and General Genetics, 263 (2): 194–200 http://dx.doi.org/10.1007/s004380051160

Vágújfalvi A., Aprile A., Miller A., Dubcovsky J., Delugu G., Galiba G., Cattivelli L. 2005. The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. Moleculat Genetics and Genomics, 274 (5): 506–514 http://dx.doi.org/10.1007/s00438-005-0047-y

Vágújfalvi V. Nagy A., Soltész A., Galiba G. 2010. A simplified method to test cereal frost tolerance. Acta Agronomica Hungarica, 58 (2): 143–149 http://dx.doi.org/10.1556/AAgr.58.2010.2.5

Winfield M. O., Lu C. G., Wilson I. D., Coghill, J. A., Edwards K. J. 2009. Cold- and light-induced changes in the transcriptome of wheat leading to phase transition from vegetative to reproductive growth. BMC Plant Biology, 9: 1–14 http://dx.doi.org/10.1186/1471-2229-9-55

Winfield M. O., Lu C., Wilson I. D., Coghill J. A., Edwards K. J. 2010. Plant responses to cold: transcriptome analysis of wheat. Plant Biotechnology Journal, 8: 749–771 http://dx.doi.org/10.1111/j.1467-7652.2010.00536.x

ISSN 1392-3196 / e-ISSN 2335-8947
Zemdirbyste-Agriculture, vol. 100, No. 4 (2013), p. 417–424
DOI 10.13080/z-a.2013.100.053

Paprastojo žieminio kviečio (*Triticum aestivum* L.) atsparumo šalčiui įvertinimas kontroliuojamomis sąlygomis ir lauke

R. Armonienė, Ž. Liatukas, G. Brazauskas

Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas

Santrauka

Paprastojo žieminio kviečio (*Triticum aestivum* L.) atsparumas šalčiui yra vienas pagrindinių veiksnių, lemiančių jo žiemkentiškumą. Siekiant įvertinti žieminio kviečio veislių ir selekcinių linijų toleranciją šalčiui, eksperimentai atlikti lauko ir kontroliuojamomis sąlygomis, naudojant grūdintus ir negrūdintus kviečių augalus. Lauko eksperimentai atlikti 2010–2011 ir 2011–2012 m. Kviečių žiemkentiškumas 2011 ir 2012 m. balandžio mėnesiais buvo įvertintas vizualiai pagal 1–9 balų skalę (1 balas – visi augalai žuvę, 9 balai – visi augalai išgyvenę). 2010–2011 ir 2011–2012 m. žiemos pasižymėjo itin nevienodomis klimatinėmis sąlygomis, todėl esminio koreliacinio ryšio tarp kviečių žiemkentiškumo skirtingais metais nebuvo nustatyta. Kviečių žiemkentiškumo balai 2010–2011 m. svyravo nuo 1,0 (WW-8) iki 7,6 (‘Zentos’), o 2011–2012 m. – nuo 5,8 (WW-31) iki 7,8 (‘Kovas DS’). Žieminio kviečio veislių ir linijų atsparumas šalčiui dirbtinėmis sąlygomis įvertintas remiantis lapų chlorofilo fluoresencijos rodiklio Fv/Fm ir išgyvenimo po šaldymo testo rezultatais. Nustatyta stipri (r = 0,88, p < 0,01) koreliacija tarp negrūdintų kviečių išgyvenimo po šaldymo testo ir Fv/Fm santykio parodė, kad šis metodas gali pagreitinti šalčiui atsparių genotipų atranką, juos vertinant iš karto po šaldymo testo. Tyrimų rezultatai parodė silpną, tačiau esminį (r = −0,41, p < 0,05) ryšį tarp 2010–2011 m. žiemkentiškumo lauko eksperimente ir negrūdintų kviečių išgyvenimo po šaldymo testo. Žieminio kviečio genotipai, kurie greitai praranda žiemkentiškumą, yra atsparesni šalčiui be grūdinimosi stadijos. Tai rodo, kad egzistuoja dvi atskiros žieminio kviečio atsparumo šalčiui genetinės sistemos, kurias lemia *užsigrūdinimo trukmės* ir *spartos* genų raiška.

Reikšminiai žodžiai: atsparumas šalčiui, Fv/Fm, *Triticum aestivum*, žiemkentiškumas.