UDC 580.085: 633.11
Original scientific paper

IN VITRO SCREENING FOR LOW TEMPERATURE TOLERANCE OF WHEAT GENOTYPES

Ankica KONDIĆ ŠIPKA, Nikola HRISTOV and Borislav KOBILJSKI

Institute of Field and Vegetable Crops, Novi Sad, Serbia

Kondić-Šipka Ankica, Nikola Hristov and Borislav Kobiljski (2006): *In vitro screening for low temperature tolerance of wheat genotypes*. - Genetika, Vol. 38, No. 2, 137-144.

Low temperature (LT) tolerance of 12 wheat (*T. aestivum* L.) genotypes was investigated in an *in vitro* zygotic embryo culture. Isolated embryos were grown on a modified MS medium for two months. Calluses were prepared by gradual decrease of temperatures (17°C-7 days, 5°C-7 days, -5°C-1 day, -10°C-1 day) and then exposed to LT treatments (-15°C, 24 h and 48 h). After LT treatments, calluses were acclimated by gradual increase of temperatures and grown for another month. Based on the differences in callus fresh weight between the control group and calluses exposed to the LT treatments, levels of LT tolerance of wheat genotypes were determined. The results have shown that out of the 12 wheat cultivars, 6 had a high level of LT tolerance, 5 had a medium and only one spring cultivar (Venera) had a low level of LT tolerance. These results were compared with the results of the standard *in situ* test in a cold chamber. Only partial disagreement of results was determined in two cultivars with the medium level of LT tolerance (NSR-2 and Balkan). It can be concluded that the *in vitro* test can be successfully used for the separation of genotypes with high and

Corresponding author: Ankica Kondić-Špika, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia, Phone: +381 21 4898 224, E-mail: ankica@ifvcns.ns.ac.yu

low levels of LT tolerance, but for more precise separation within the medium level of LT tolerance, the test should be improved.

Key words: low temperatures, *in vitro* test, tolerance, wheat

INTRODUCTION

Frost stress during the developmental stages of reproductive organs reduces wheat yield in some years. Breeding for long duration cultivars and delaying sowing are the main strategies to avoid spring frost. These strategies have a limit, as the yield is reduced by 15% per week delay in anthesis past the optimum date (GUNAWARDENA *et al.*, 2004).

Cold acclimation/hardening is prior exposure of plants to cold, nonfreezing temperatures that increases their tolerance to subsequent freezing (KALENGAMALIRO *et al.*, 2000). It plays an important role in frost tolerance in wheat cultivars. Frost resistant cultivars harden faster and deharden more slowly than frost susceptible cultivars (SAULESCU and BRAUN, 2001).

FOWLER *et al.* (1981) identified 34 biochemical, physiological and morphological characters of winter wheat (*Triticum aestivum* L.) that were associated with cold acclimation and winter hardiness. Screening new selections for winter hardiness in the field is an expensive and time consuming, since test winters are rare and unpredictable. Evaluation of cold hardiness *in vitro* would allow testing in controlled conditions, accelerate breeding process and reduce long term program investment (PALONEN and BUSYARD, 1998).

In vitro cultures can be hardened by exposure to low temperatures (CASWELL *et al.*, 1986) or by exogenous application of ABA (JOHNSON-FLANAGAN *et al.*, 1991) and sugars (PALONEN and JUNTILA, 1999). Acclimated *in vitro* cultures can be used to compare cultivars for their frost hardiness (BARTOLOZZI *et al.*, 2001).

The aim of this study was to evaluate the effectiveness of the *in vitro* test for the assessment of low temperature tolerance in wheat cultivars by comparing results with those obtained in the standard *in situ* test.

MATERIAL AND METHODS

Twelve randomly selected wheat (*Triticum aestivum* L.) cultivars were used to initiate callus culture. Seeds were surface sterilized as described in an earlier paper (KONDIĆ *et al.*, 1998). Isolated zygotic embryos were inoculated onto a modified MS (Murashige and SKOOG, 1968) nutrient medium containing 1.5 mgl^{-1} 2,4-D, 0.5 mgl^{-1} NAA and 0.5 mgl^{-1} thiamine HCl (vitamin B_1). During the two months of growing at 25°C and 16 h photoperiod, calluses were subcultured every 15 days onto the fresh MS medium.

Cold acclimation of calluses was carried out at 5°C for 7 days. Freezing was done in a cold chamber by gradual decrease of temperatures (-5°C-1 day, -10°C-1 day). All of these freezing temperatures were considered as a preparation

for subsequent low temperature (LT) treatments, which were performed at -15°C for 24 h (treatment I) and 48 h (treatment II). After the LT treatments, calluses were exposed to gradual decrease of temperatures (-10°C-1 day, -5°C-1 day, 5°C-7 days, 17°C-7 days) and grown at 25°C for another month. The control group of calluses was exposed to the same temperature regime except for the LT treatments. At the end of the experiment, callus fresh weight was measured. Based on the differences in callus fresh weight between the control group and calluses exposed to the LT treatments, levels of LT tolerance of wheat genotypes were determined.

The *in situ* test for LT tolerance of the same wheat cultivars was performed as described by HRISTOV *et al.* (1997). This standard test was used as a check for the validity of results obtained in the *in vitro* experiment. The *in vitro* results for fresh weight of treated calluses in relation to the fresh weight of the control group of calluses (%) were compared with the *in situ* results for survived leaf mass (%).

The experimental design was a randomized complete block with three replicates. To provide sufficient callus mass for analysis, three tubes were used for each replication. The results were statistically processed by the analysis of variance and the significance of differences between the treatments was determined using the LSD test. All statistical analysis were done using the computer program MSTAT-C.

RESULTS AND DISCUSSION

Low temperature treatments had an inhibitory effect on callus growth in wheat cultivars. While treatment I caused significant suppression of callus growth in 6 cultivars, treatment II had an inhibitory effect in 9 cultivars (Table 1.). The most tolerant cultivars were Partizanka, Centurk and GK Cipo. In these cultivars LT treatments had no effect on callus growth. The spring cultivar Venera was the most sensitive genotype, in which both treatments had lethal effect on callus growth.

The rate of callus fresh weight decrease relative to the control ranged from 2.2% to 86.0% in treatment I (Figure 1.) and from 12.5% to 84.9% in treatment II (Figure 2). Most of the genotypes had similar reaction to both LT treatments. Only two cultivars (NSR-2 and Zlatka) had more then 15% difference in callus fresh weight between the treatments. In both treatments 6 cultivars (Bezostaja 1, Mironovska 808, Partizanka, Centurk, Stamena and GK Cipo) had callus fresh weight more then 75% relative to the control, which can be concerned as a high level of LT tolerance. Five genotypes (NSR-2, Pobeda, Nevesinjka, Balkan and Zlatka) had callus fresh weight between 50 and 75% relative to the control in both treatments and a medium level of LT tolerance. In the spring cultivar Venera, which was the most sensitive genotype, callus fresh weight decreased relative to the control by more then 85% in both treatments.

Comparisons of the *in vitro* (% of callus fresh weight in relation to the control) and the *in situ* (% of survived leaf mass) results (Figures 1. and 2.) have shown high level of agreement between them.

Table 1. - Effect of LT treatments on fresh callus weight of wheat genotypes

Genotype	Treatment	Fresh callus weight (mg)
Bezostaja 1	Control	103.0
	I	100.3
	II	90.1**
Mironovska 808	Control	71.4
	I	69.8
	II	61.6*
NSR-2	Control	67.8
	I	49.8**
	II	37.1**
Partizanka	Control	38.0
	I	32.6
	II	30.7
Pobeda	Control	106.3
	I	76.2**
	II	64.2**
Nevesinjka	Control	65.2
	I	46.3**
	II	38.3**
Centurk	Control	40.7
	I	37.5
	II	33.5
Balkan	Control	40.0
	I	26.1**
	II	24.5**
Venera	Control	46.4
	I	6.5**
	II	6.1**
Stamena	Control	45.6
	I	40.1
	II	35.1**
Zlatka	Control	64.0
	I	45.1**
	II	32.7**
GK Cipo	Control	41.5
	I	40.1
	II	35.4
LSD	0.05	7.537
	0.01	9.952

*significant at 5%; **significant at 1%

In both treatments two cultivars (NSR-2 and Balkan) from the group of genotypes with the medium level of LT tolerance have shown partial disagreement between the results obtained in the different tests. Even though both cultivars had better results in the *in vitro* then in the *in situ* one, they were assigned to the same tolerance level group by both tests.

Wheat cultivars used in this study differed in regard to the level of LT tolerance. Our *in vitro* test included cold acclimation because findings of other authors indicated that varietal differences in frost tolerance could not be detected in some species without acclimation (PALONEN and BUSZARD, 1998; BARTOLOZZI *et al.*, 2001). Varietal differences in cold hardiness of sugar beet (*Beta vulgaris* L.) could be detected without acclimation when benzyladenine was omitted from the medium and the sucrose level was lowered to 1% (DIX *et al.*, 1994). Use of acclimated *in vitro* cultures is better, since the degree of frost tolerance in different cultivars may depend upon their different capability to acclimate to cold (BARTOLOZZI *et al.*, 2001).

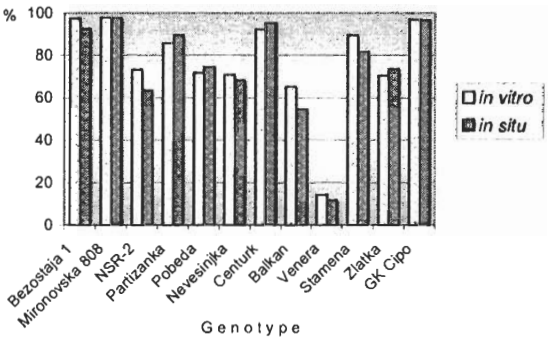

Figure 1. - A comparison of the *in vitro* (% of callus fresh weight in relation to the control) and the *in situ* (% of survived leaf mass) results for wheat at LT treatment I

Low temperature tolerance of wheat genotypes estimated by the *in vitro* test corresponded well to their LT tolerance in the *in situ* test. This clearly highlighted the effectiveness of *in vitro* cultures for evaluating frost tolerance. These results are in agreement with ZATYLNI *et al.* (1993), PALONEN and BUSZARD (1997) and KALENGAMALIRO *et al.* (2000). The effects of freezing temperatures on wheat calluses obtained by *in vitro* culture are similar to those on *in vivo* plants. *In vitro* cultures will offer many advantages for studying the mechanisms of frost tolerance in wheat. For instance, *in vitro* cultures require a limited space, provide a high amount of plant material in a relatively short period of time, but mostly allow to work under strictly controlled conditions.

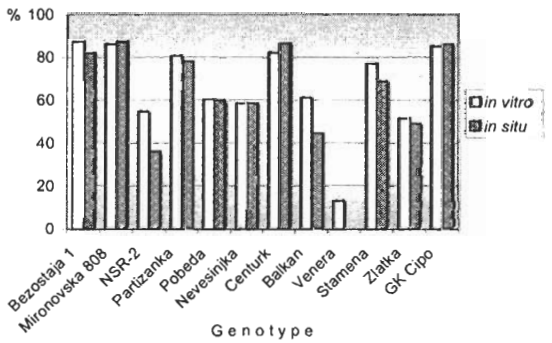

Figure 2. - A comparison of the *in vitro* (% of callus fresh weight in relation to the control) and the *in situ* (% of survived leaf mass) results for wheat at LT treatment II

In order to make this *in vitro* test more precious and effective, the process of acclimation should be improved in future experiments by using different temperatures and durations of the procedure. Also, LT treatments should be improved by using a wider range of temperatures and selecting the best one for the separation of wheat genotypes with different levels of tolerance.

New possible investigations will attempt to screen wheat cultivars in terms of frost tolerance by using *in vitro* cultures and the study of biochemical modifications induced by acclimation, including the possibility to use this methodology for an early selection within breeding programs.

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Received May 17, 2006
Accepted July 3, 2006

IN VITRO SKRINING TOLERANTNOSTI GENOTIPOVA PŠENICE PREMA NISKIM TEMPERATURAMA

Ankica KONDIĆ ŠIPKA, Nikola HRISTOV i Borislav KOBILJSKI

Naučni institut za ratarstvo i povrtarstvo, Novi Sad, Srbija

I z v o d

Ispitivana je tolerantnost 12 genotipova pšenice (*T. aestivum* L.) prema niskim temepraturama (NT) u *in vitro* kulturi zigotnog embriona. Izolovani embrioni gajeni su na modifikovanoj MS podlozi dva meseca. Proizvedeni kalusi izloženi su prvo dejstvu pripremnih temperatura (17°C-7 dana, 5°C-7 dana, -5°C-1 dan, -10°C-1 dan), a zatim i dejstvu NT (-15°C-24h i 48h). Nakon tretmana, kalusi su aklimatizovani postepenim povećavanjem temperature (obrnutim redosledom pripremnih temperatura) i gajeni još mesec dana. Na osnovu razlike u porastu sveže mase kalusa, između kontrolne grupe i kalusa izloženih dejstvu NT, utvrđen je stepen tolerantnosti pojedinih genotipova pšenice.

Rezultati su pokazali da je od 12 ispitivanih sorti, 6 imalo visok stepen tolerantnosti prema NT, 5 je imalo srednji nivo tolerantnosti, dok je samo jedna jara sorta (Venera) imala veoma nizak nivo tolerantnosti. Ovi rezultati su poređeni sa rezultatima standardnog *in situ* testa u hladnim komorama. Utvrđeno je delimično nepoklapanje rezultata samo kod dve sorte srednjeg nivoa tolerantnosti (NSR-2 i Balkan). To nam ukazuje na činjenicu da se *in vitro* testom mogu pouzdano razdvojiti genotipovi visoke i niske tolerantnosti prema NT, dok je za razdvajanja u okviru srednjeg nivoa tolerantnosti potrebno doraditi ovaj test.

Primljeno 17. maja 2006.
Odobreno 3. jula 2006.