Assessment of Frost Tolerance of Wheat Doubled Haploids by Gliadin Electrophoresis

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Abstract: The relationship between gliadin alleles, known to mark frost tolerance, and actual frost tolerance was investigated in 52 doubled haploids (DH) from the cross Florida × Vlada. Frost tolerance was expressed as percentage of plant winter survival in pots placed at different heights above the ground. Gliadin allelic blocks (GLI) were determined by starch electrophoresis. From the six GLI, known as frost tolerance markers, the GLI 1B1, GLI 1D5 or GLI 6D2 was present in 27, 27 or 31 DH, respectively. A weak, but significant correlation of $r = 0.29$ was found in the DH between their survival and frost tolerance prediction values based on the presence of the gliadin frost tolerance markers. The average survival of DH with gliadin frost tolerance marker GLI 1B1 or GLI 6D2, but not with GLI 1D5, was significantly higher than the survival of DH without this block. The DH without any gliadin frost tolerance marker had the lowest survival. Some gliadin genes thus can be used as frost tolerance markers for preliminary selection of frost tolerant lines and for the study of physiological and genetic constitution of frost tolerance in common wheat.

Keywords: gliadins; frost tolerance; marker; doubled haploids; wheat

Frost tolerance, an important trait that ensures plant overwintering, is controlled in common wheat by additive effects of genes carried on at least 10 out of 21 chromosome pairs (SUTKA *et al*. 1997). The first two genes of frost tolerance have been localised recently: *Fr 1* on the long arm of chromosome 5A and *Fr 2* on the long arm of chromosome 5D. Both genes are closely linked to localisation of vernalisation genes (SUTKA *et al*. 1997). Wheat group 5 chromosomes regulate the expression of cold-induced genes localised on group 6 chromosomes (SARHAN *et al*. 1997; LIMIN *et al*. 1997). These are gene families *Wcs 120* and *Wcor 410*.

Some gliadin genes of wheat, localised on group 1 and 6 chromosomes, were found to be in linkage with frost tolerance genes (ČERNÝ *et al*. 1990; SOZINOV 1985; ŠAŠEK *et al*. 1984). Our previous studies on a set of 220 cultivars of winter wheat world collection demonstrated a close linkage between frost tolerance and some alleles of gliadin loci of chromosomes 1B, 1D, 6A and 6D (ŠAŠEK *et al*. 2000). As for gliadin blocks (GLI) determined by starch electrophoresis, a higher markering value of frost tolerance was found in GLI 1D5 and GLI 6A3. GLI 6D2, GLI 1B1 and GLI 6A1 were identified as secondary markers of frost tolerance, less dependent on the existence of frost tolerance. Additive effects of these gliadin markers of frost tolerance were confirmed. Some alleles (GLI 6A2, GLI 1B4 and GLI 1B5) were found to be associated with low frost tolerance.

The objective of the paper was to evaluate a relationship between the presence of some gliadin alleles markering frost tolerance and frost tolerance in doubled haploids from the cross Florida × Vlada. These two parental cultivars have different levels of frost tolerance and different presence of gliadin alleles. Fifty-two doubled haploids produced by crossing of these parental components were available.

MATERIAL AND METHODS

Seed of 52 doubled haploids of wheat (*Triticum aestivum* L.) and two parental cultivars Vlada and Florida came

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from 1997 crop from the Research Institute of Crop Production at Prague-Ruzyně. Survival of the lines and cultivars over the winter season was assessed in 1997/98 and 1998/99 by a provocation pot method used in the long run for determination of cold and frost tolerance in grains (PRÁŠIL & ROGALEWICZ 1989; PRÁŠILOVÁ & PRÁŠIL 2001). Plants were grown in wooden boxes (40 × 30 × 12 cm) placed in winter at two heights above the ground, 5 and 50 cm, and exposed to adverse factors of winter, to frost in particular. 120 seeds from each line were planted, 30 seeds into 4 boxes. The number of living plants was determined in spring and expressed as survival percentage.

Gliadin spectra from at least 6 seeds of each sample were determined by vertical starch gel electrophoresis (SGE) according to ŠAŠEK and SÝKOROVÁ (1989). Allelic gliadin blocks were identified in the electrophoretic gliadin spectra by a previously published method (ŠAŠEK & ČERNÝ 1983). Prediction values of frost tolerance of gliadin allelic blocks (Table 1), i.e. GLI 1D5 = 2.5, GLI 6D2 = 1 and GLI 1B1 = 0.5, were used on the basis of a previous analysis (ŠAŠEK *et al.* 2000).

A relationship between the presence of gliadin allelic blocks and doubled haploid survival was evaluated by correlation coefficient, survival differences by *t*-test and by multiple comparisons (Unistat 5.1) at 5% significance level. Survival data were transformed by arcsin transformation before the analyses.

Table 1. Point values of frost tolerance prediction for gliadin markers according to ŠAŠEK *et al.* (2000)

Gliadin allelic block	Prediction value
GLI 1D5	2.5
GLI 6A3	2
GLI 6D2	1
GLI 1B1	0.5
GLI 6A1	0.25

RESULTS

The survival of 52 doubled haploids and both parental cultivars was considerably different in both winter seasons (Fig. 1). Following a stronger stress in winter 1997/98 the survival of DH ranged from 65 to 10%. Survival average was 43%. After a milder winter 1998/99 the survival of DH ranged from 100 to 62% with average value 89%. There was a large difference in survival between the two parental cultivars in both winter seasons. Survival was always higher in more tolerant variety Vlada than in less tolerant Florida. Survival of the particular samples for both winter seasons indicated a significant correlation ($r = 0.65$). Survival rates of samples in winter 1998/99 were clustered around the value 90% and higher (33 lines

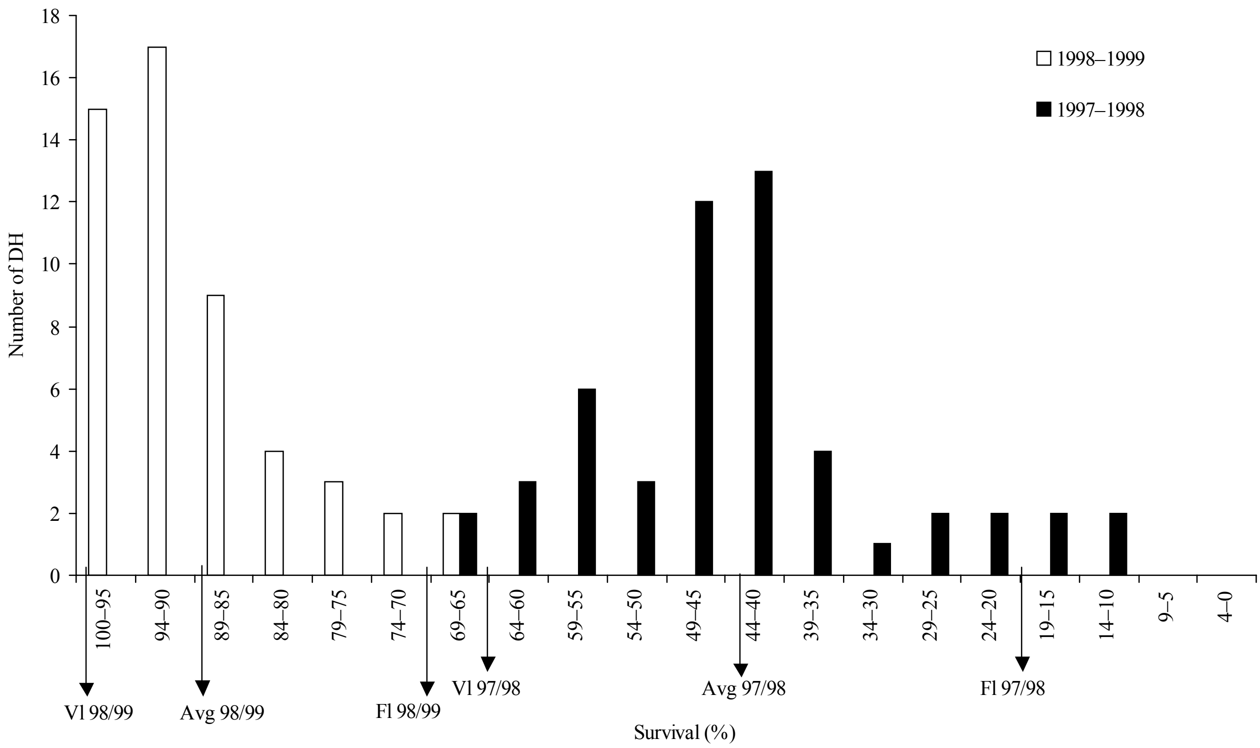

Fig. 1. The number of DH classified according to survival (%) and the survival of parental cultivars Vlada and Florida in winter seasons 1997/1998 and 1998/99 in a provocation pot method

Table 2. Electrophoretic patterns of gliadin blocks (GLI) of parental cultivars

Cultivar	Survival (%)	GLI allele on chromosome						
		1-1A	2-1A	1B	1D	6A	6B	6D
Florida	22	9	0	3	1	4	1	4
Vlada	67	14	3	1	5	2	1	2

in total, i.e. 63%), it means that the survival differences between the DH were lower. This is the reason why the results from winter 1997/98, when differences between the particular lines were larger, were used in further analyses.

The electrophoretic gliadin spectrum of parental cultivars (Table 2) indicated the presence of three GLI markers of frost tolerance (GLI 1D5, GLI 6D2 and GLI 1B1) in more tolerant Vlada, but they were not found in Florida. The presence of these three GLI markers of frost tolerance was even in 52 DH: GLI 1D5 was present in 27 DH, GLI 6D2 in 31 DH and GLI 1B1 in 27 DH. Mean survival difference between DH carrying and not carrying the gliadin frost tolerance marker at the appropriate locus (Table 3) was significantly higher for GLI 1B1 or GLI 6D2,

Table 3. Survival difference between DH carrying and not carrying the gliadin frost tolerance marker at the appropriate locus

Gliadin blocks	Survival difference (%)	Significance*
GLI 1B1–GLI 1B3	9.2	+
GLI 1D5–GLI 1D1	2.8	–
GLI 6D2–GLI 6D4	11.1	+

*, + or – = significant or insignificant at $P < 0.05$

but not for GLI 1D5. The presence of the other GLI blocks carried on chromosome 1A or 6A had no effect on the survival of DH (data not shown).

A frost tolerance prediction value was calculated for each DH line on the basis of the sum of earlier established point values for the presence of GLI frost tolerance markers (Table 1). A weak, but significant correlation of $r = 0.29$ was found in the DH between their survival and frost tolerance prediction values. Further, the DH were divided into five groups according to the frost tolerance prediction values (Table 4). Survival of DH included in a group without GLI frost tolerance markers (i.e. prediction value = 0) was significantly lower in comparison with other groups containing DH with these markers. However, the average survival in these other groups did not explicitly indicate higher survival if the prediction values of frost tolerance were higher.

Another analysis followed when DH were classified according to the presence of GLI alleles, known as frost tolerance markers, and without taking into account their prediction values (Table 5). The average survival of DH with the presence of GLI 1B1 and/or 6D2 was higher than without them. The DH carrying both GLI 1B1 and GLI 6D2 had higher survival than DH carrying only one of them. The presence of GLI 1D5 had no effect on DH line survival. The DH without any gliadin frost tolerance marker had the lowest survival.

Table 4. Classification of DH according to the frost tolerance prediction values of GLI markers and comparisons of their survival

Frost tolerance prediction values	Number of DH lines	Survival (%)*
0	8	27.6 c
0.25–1	7	42.9 b
1.25–2	9	53.2 a
2.25–3	11	42.1 b
3.25–4	17	45.9 b

* survival denoted by the same letter is not significantly different

Table 5. Classification of DH according to the presence of GLI frost tolerance markers (regardless of their prediction values) and comparisons of their survival

GLI markers	Number of DH lines	Survival (%)*
1B1 + 1D5 + 6D2	11	46.6 ab
1B1 + 1D5	6	45.1 b
1B1 + 6D2	9	53.2 a
1D5 + 6D2	6	45.6 b
1B1	1	41.9
1D5	5	38.6 c
6D2	6	42.9 bc
0	8	27.6 d

* survival denoted by the same letter is not significantly different

DISCUSSION

The linkage between some gliadin genes and wheat frost tolerance was shown in the studies published by

Sozinov's team (1978, 1985) and by Šašek and Černý's team (ŠAŠEK *et al*. 1984, 2000; ČERNÝ *et al*. 1990). Genes related to wheat frost tolerance are reported the most frequently on chromosomes 5A, 6A, 7A, 1B, 2B, 4B, 5B, 6B, 1D, 4D, 5D and 6D (SUTKA *et al*. 1997, FOWLER & LIMIN 1997). Gliadin genes localised on group 1 and 6 chromosomes and known as frost tolerance markers are carried on chromosomes 1B, 1D, 6A and 6D (SOZINOV 1985; ŠAŠEK *et al*. 1984, 2000). It seems that though the gliadin genes marker only a limited part of frost tolerance genes, they might be used for preliminary prediction of wheat frost tolerance. It has been confirmed by high correlations between the prediction values of gliadin genes (frost tolerance markers) and classes of frost tolerance determined in a set of wheat cultivars – standards of frost tolerance (ČERNÝ *et al*. 1990) or a collection of 212 cultivars of different geographic origin (ŠAŠEK *et al*. 2000). For example in the latter study on 212 wheat cultivars a highly significant correlation coefficient ($r = 0.86$) was found between the total (additive) prediction value of gliadin frost tolerance markers and the class of frost tolerance of cultivars. Among the gliadin allelic blocks identified by starch gel electrophoresis alleles GLI 1D5 and GLI 6A3 appeared to be major markers of frost tolerance, and GLI 6D2, GLI 1B1 and GLI 6A1 secondary markers.

In this study the performance of gliadin allelic blocks markering frost tolerance in 52 doubled haploids wheat derived from the cross Florida × Vlada was determined. There were three GLI frost tolerance markers in the studied set: GLI 1D5, GLI 6D2 and GLI 1B1. All these alleles were found to be conferring high frost tolerance in Vlada unlike the less tolerant Florida. Although only three out of the six GLI markers of frost tolerance were present, the assessment of 52 DH demonstrated a low, but significant correlation ($r = 0.29$) between survival and prediction values of frost tolerance. The survival of DH carrying the GLI frost tolerance markers was higher than the survival of DH without them. So it was possible to distinguish lines with low frost tolerance from the other lines with medium or high tolerance (Tables 4 and 5). Preliminary selection and monitoring of frost-tolerant lines by GLI markers were confirmed.

It is evident that the presence of GLI 1B1 and GLI 6D2 was more effective than that of block GLI 1D5. This fact can explain the low correlation between survival and frost tolerance prediction values, because the point value of frost tolerance prediction for GLI 1D5 is high according to our previous study (ŠAŠEK *et al*. 2000, and Table1). The low frost tolerance prediction of GLI 1D5 found in this study could also be explained by the genetic background of the cross Florida × Vlada. To make a more detailed assessment it will be necessary to use a larger set of tested DH and/or other hybrid populations.

Cold-induced wheat proteins, which are associated with induction of frost tolerance, are expressed by genes localized on group 6 chromosomes (SARHAN *et al*. 1997; LIMIN *et al*. 1997). The existence of cold-induced genes confirms a possibility of their linkage with gliadin genes localised on these chromosomes. Gliadin genes and/or alleles carried on group 1 chromosomes and displaying a relation with frost tolerance should be another object of physiological and genetic studies of frost tolerance constitution in common wheat.

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Abstrakt

PRÁŠIL I.T., PRÁŠILOVÁ P., ŠAŠEK A., ČERNÝ J. (2002): **Hodnocení mrazuvzdornosti dihaploidních linií pšenice pomocí elektroforézy gliadinů**. Czech J. Genet. Plant Breed., **38**: 104–108.

Byl hodnocen vztah mezi výskytem gliadinových alel – markerů mrazuvzdornosti a mrazuvzdorností 52 dihaploidních (DH) linií sesterského charakteru z kombinace Florida × Vlada. Mrazuvzdornost byla vyjádřena jako procento přežití vzorků po přezimování rostlin v nádobách uložených v různé výšce nad zemí. Gliadinové alelické bloky (GLI) byly stanoveny škrobovou elektroforézou. Ze šesti dříve známých GLI markerů mrazuvzdornosti se ve studovaném souboru vyskytly tři: GLI 1D5 se vyskytoval u 27 DH linií, GLI 6D2 u 31 DH linií a GLI 1B1 u 27 DH linií. Mezi přežitím a bodovou hodnotou predikce mrazuvzdornosti, vypočtenou na základě přítomnosti GLI markerů mrazuvzdornosti, byl u sledovaných 52 DH linií zjištěn nízký, ale významný korelační vztah (r = 0,29). Významně vyšší průměrné přežití bylo zjištěno u DH linií nesoucích GLI 1B1 nebo GLI 6D2, nikoliv však GLI 1D5, proti DH liniím bez těchto gliadinových bloků. Nejnižší přežití bylo zjištěno u DH linií bez výskytu uvedených GLI markerů mrazuvzdornosti. Užití gliadinových genů – markerů mrazuvzdornosti je vhodné nejen pro předběžnou selekci mrazuvzdorných linií, ale i pro studium fyziologicko-genetického založení mrazuvzdornosti pšenice obecné.

Klíčová slova: gliadiny; mrazuvzdornost; marker; dihaploidní linie; pšenice

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