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Original Contribution

STUDY OF FROST RESISTANCE OF COMMON WINTER WHEAT VARIETIES

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ABSTACT

Cold resistance of 23 Bulgarian winter common wheat varieties was investigated during the four years period - 2009-2012. Hardening under natural conditions and direct plant freezing test in a controlled frost chamber were applied for the evaluation. As standards were used the following varieties- Mironovska 808, Pobeda, Bezostaya 1, Sadovo 1, N 301 and San Pastore. The genotypes were compared with each other based on the average data received for survival of plants in the frost chamber imposed to temperature levels -20°C. Confidence interval for each standard and variety was calculated . The average data from freezing for a period of four years was evaluated with ANOVA. The genetic distance between the varieties was estimated by determining the euclidean distance between two objects in multidimensional space using program SPSS. Among the all studied genotypes the variety Fermer is distinguished by the highest cold resistance, expressed by the percentage of surviving plants -96.1% - average for four years followed by Sadovo 772, Prelom, Zdravko, Lyusil and Geya-1. The lowest percentage of survived plants - 43.5% and therefore a lowest cold resistance shows variety Sadovo 552. The applied analysis of variance reveals that genotype and environment conditions during the hardening of plants have significant influence on the variation of the studied trait.

Key words: Winter common wheat, cold resistance, direct plant freezing test, standard varieties

INTRODUCTION

The main purpose of any breeding program is the creation of varieties combining high productive potential and good quality under different environmental conditions. Especially, it is important for the new varieties to possess enhanced stability and plasticity (1, 2). Frost tolerance is essential for autumn-planted wheat to survive freezing temperatures during winter in temperate zones (3).

Therefore, the genetic basis of frost tolerance has been investigated for almost a century (4). The improving of frost tolerance is difficult breeding task due to the large number of involved genes and the numerous interactions with the environment (5). It is known that frost resistant cultivars harden faster and deharden more slowly than frost susceptible cultivars. In recent years the genomic selection offers opportunities to predict phenotypes for traits that are controlled by multiple genes with small effects regulated directly or indirectly by the environmental conditions, in particular the low temperature (6, 7, 8).

In the region of Sadovo winters are comparatively milder, but in January and February the radiation temperature decreases abruptly for 2-3 days, being as low as -15 ^{0}C to -18^{0}C with slight or no snow cover, and in some cases it is even lower than -20 ^{0}C. That makes possible the periodic exclusion on the less frost-resistant forms.

The breeding program of common winter wheat in IPGR "K. Malkov" underlines a strategy where for evaluation of breeding materials the method of direct freezing is accepted (9, 10, 11, 12). By imitation of winter conditions and the regulation of low temperatures it is possible more accurate assessment of the breeding materials of common winter wheat when environmental conditions do not allow this. As a results of the joint work of breeders and physiologists are created frost-resistant varieties Fermer,

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Sadovo 772, Prelom, Zdravko, Ljusil, Geya-1 and lines DB 223, KMD 40_{96}, MX 184/37, MX 246/23, BC 7, DB 213, MX 215/10, MX 246/76, MX 247/33, MX 248/41, MO 6, MO 11, MO 17, regardless of the breeding method used to induce genetic diversity. Each of these varieties has been tested for cold resistance during its creation. The lack of simultaneously controlled trial of varieties produced in recent years in IPGR Sadovo and the wide variation in displaying of the trait frost tolerance across years, give us a reason to conduct this investigation.

The objective of this study was to evaluate frost resistance of Bulgarian winter wheat varieties compared with the standard genotypes as adopted by the Executive Agency for Variety Testing and Approbation in Bulgaria (EAVTA) At the same time, we wanted to determine if the low temperature resistance of wheat varieties commercially grown in the country at the present time is sufficient for successful production.

MATERIAL AND METHODS

The investigation was conducted in the Institute of Plant Genetic Resources (IPGR "K. Malkov ") – Sadovo, Bulgaria for a period of four years (2009-2012). Twenty three winter common wheat varieties developed at the IPGR "K. Malkov") by different breeding methods: intervarietal hybridization, interspecific hybridization and experimental mutagenesis **(Table 1)** were studied.

Direct method of freezing plants in freezing chamber at -20 ° C for 24h was applied (13, 10). Plants of each genotype were grown and hardened in two replications under environmental conditions in autumn in special containers with 25 plants. The plants were placed in the chamber during the tillering stage. The freezing was conducted in January, which coincides with the period of maximum cold resistance of plants. After removing plants were reimbursed for a period of 20 days. The percentage of plants survival is counted by the formula:

$$P = \frac{n}{N} \times 100\ (\%),$$

where: P-% survival plants; n-number of survival plants; N-total number of plants (14)

Table 1. *Origin of varieties of common winter wheat*

N	varieties	Origin
1	Sadovo 1	♀ Yubilejna x ♂ Bezostaya-1
2	Pobeda	♀ Triticum sphaerococcum var. rotundatum x ♂ {(Triticum durum x Secale montanum) x Безостая 1 x Meksikan]
3	Katja	♀ [(Fortunato x 301) x ♂ Bezostaya-1
4	Momchil	♀ NS 11-33 x ♂ Avrora
5	Bononija	♀ Krasnodarski kalik x ♂ Ludogorska
6	Sadovo 772	♀ Skitija x ♂ Sadovo 1
7	Zdravko	♀ Charodejka x ♂ Sadovska ranozrejka 3
8	Sadovska belija	♀ Lada x ♂ Sadovo 1
9	Murgavetz	♀ Altimir 67 x ♂ Sadovo 1
10	Mustang	♀ (Sadovo 1 x S1503- 142) x ♂ Sadovo super x Klement)
11	Geya 1	♀ (FD 6405(Fr.) x Zg. 720-1) x ♂ Sadovo 1
12	Diamant	♀ Yubilejna x ♂ Sadovo 1
13	Prelom	♀ Росица x ♂ Tundzha
14	Yunak	♀ Momchil x ♂ Katja
15	Petja	♀ Thessee x ♂ Karmen
16	Sadovo 552	♀ Mironovska 15 x ♂ H 10
17	Boryana	♀ № 4373/9855 x Momchil
18	Guinnees	prepared by physical mutagenesis from Katja
19	Yoana	7/5 П – 8 – М/370 (prepared by physical mutagenesis from Pobeda)
20	Ljusil	♀ Yantar x ♂ Medven
21	Zarevez	♀ Zebrez x ♂ Katja
22	Fermer	1802/15 prepared by physical mutagenesis from Pobeda
23	Nikki	MX 142/6 (Sadovo super x Pobeda)

Thus, it is reported not only the survival rate of plants at low subzero temperatures, but also their ability to restore. For comparison were used standards for cold tolerance accepted of EAVTA as follows Mironovska 808, Pobeda, Bezostaya 1, № 301 and San Pastore, arranged in order of their cold tolerance. Variety Sadovo 1 was included as standard, too. It is characterized with similar cold resistance as variety Rusalka - standard adopted by EAVTA.

The results were processed via one-factor analysis of variance (the years have been used as replications) by means of a program package SPSS 9.

The ranking of varieties based on obtained data was calculated confidence interval for each standard and variety using the Excel program. For estimation of genetic proximity between varieties in regard to the trait cold tolerance and genotypes reaction to the conditions of tempering and thawing a cluster analysis was used (15). The genetic distance of the varieties is calculated by determining the euclidean distance between two objects in multidimensional space using program SPSS.

RESULTS AND DISCUSSION

The average results of the test for resistance to low subzero temperatures for studied genotypes and standards are presented in **Table 3**. Data from the average survival of standards and the range of the confidence interval showed a gradual decrease in cold resistance and classify them into three groups: Highest cold tolerance manifests variety Mironovska 808 (99.3%), in which plants survived adverse conditions and acclimated without any losses during the four years of study. Similar results to those shows Bulgarian variety Pobeda (95.3%). This is probably due to the presence of rye genes in Pobeda's pedigree. According to Chipilski et al. (16) patterns of rye recover after freezing to - 23^{o}C. The varieties Bezostaya 1 and Sadovo 1 showed descending gradation in cold tolerance, respectively 86.5% and 78.6%. Although these both varieties were with lower percent of surviving plants, the differences between them and cv. Mironovska and Pobeda are statistically insignificant and they range to the first group with the best frost tolerance.

The variety № 301 pertain to the second group with satisfactory survival of 65.1% average for the test period. The value of the confidence interval indicates that this variety is with significantly lower frost tolerance in comparison with all genotypes from the first group **(Table 2).** There is an assumption that varieties with similar to № 301 cold tolerance suffer without damage winters in Bulgaria (17, 11).

Most sensitive at low subzero temperature was San Pastore variety which is characterized with low and insufficient survival of plants - 28.1%. The difference is significant at p= 0.05 compared to the all five standards **(Table 3).**

Similar data for gradually decreasing resistance to low temperatures for the six studied varieties were obtained by other authors (13, 17, 11), which allows us to use them as a standard scale.

The results from the investigation of the frost tolerance in Bulgarian common winter wheat varieties are presented in **Table 2** (separately by year) and in Table 3 (average for period of study). Among the all studied genotypes the variety Fermer is distinguished by the highest cold resistance, expressed by the percentage of surviving plants - 96.1% - average for four years. The lowest percentage of survived plants - 43.5% and therefore a lowest cold resistance shows variety Sadovo 552. The variation of plant survival rate in each genotype over the years of study can be explained with the differences in the hardening conditions. As can be seen from the **Figure 1** the average monthly temperatures during the hardening period from October to January in the first year of study – 2009/2010 were higher compared to the same period in other years. The lower survival rate in almost half of the studied varieties - Momchil, Sadovo 772, Giunness, Yoana, Nikki, Borjana, Geya 1, Sadovska belija, Petja, Ljusil, is due to the warm weather at the end of December 2009 and almost the entire month of January 2010, leading to partially dehardening of the plants and reducing of their frost resistance. The lower survival rate in another ten studied genotypes and in almost all standard except St_6-San Pastore in the second year of investigation (2010/2011) can be coupled with a lack of a gradual decline in temperatures during the period of October to December, that had a negative influence on the preparation of plants for the winter. The average temperature in November was higher than in October and in December the temperature dropped sharply and resulted in insufficient tillering. The observed period in the third and fourth years (2011-2012 and 2012-2013) was characterized by temperature conditions suitable to pass the phases of vernalization and hardening. Negative temperatures during most of December and January prevented the plants from dehardening before putting them in the freezing chamber. These favorable hardening conditions resulted in a higher survival rate in the majority of the investigated genotypes.

Table 2. *Survival of common winter wheat plants during four years (2009-2012)*

Varieties	% plant survival rate			
	2009	2010	2011	2012
St_1-Mironovska 808	100.0	97.1	100.0	100.0
St_2-Pobeda	97.1	87.1	100.0	96.8
St_3-Bezostaya 1	87.9	75.0	91.7	91.2
St_4-Sadovo 1	84.4	70.0	74.3	85.5
St_5- N 301	80.0	51.8	52.0	76.6
St_6-San Pastore	64.7	18.8	10.5	18.5
Prelom	91.7	76.5	93.7	96.8
Zdravko	89.7	87.5	91.4	94.7
Sadovo 772	88.9	97.2	100.0	97.1
Ljusil	88.6	93.5	88.9	89.6
Mustang	82.9	63.5	100.0	91.2
Guinness	78.4	85.3	94.1	100.0
Diamant	78.0	66.7	93.9	100.0
Murgavetz	77.8	44.1	100.0	94.6
Momchil	77.5	90.9	100.0	64.1
Yoana	76.5	97.0	94.1	84.0
Sadovo 552	75.7	26.5	34.3	37.5
Geya 1	75.7	76.7	78.4	97.1
Nikki	75.0	97.1	100.0	100.0
Borjana	74.3	90.9	100.0	76.5
Sadovska belija	73.0	88.2	89.5	97.1
Bononija	72.5	62.8	100.0	100.0
Petja	66.7	96.8	100.0	92.3
Katja	65.8	41.4	94.1	67.7
Yunak	57.1	25.9	29.4	74.2
Zarevetz	70.9	52.9	72.2	87.5
Fermer	96.1	96.4	100.0	92.0

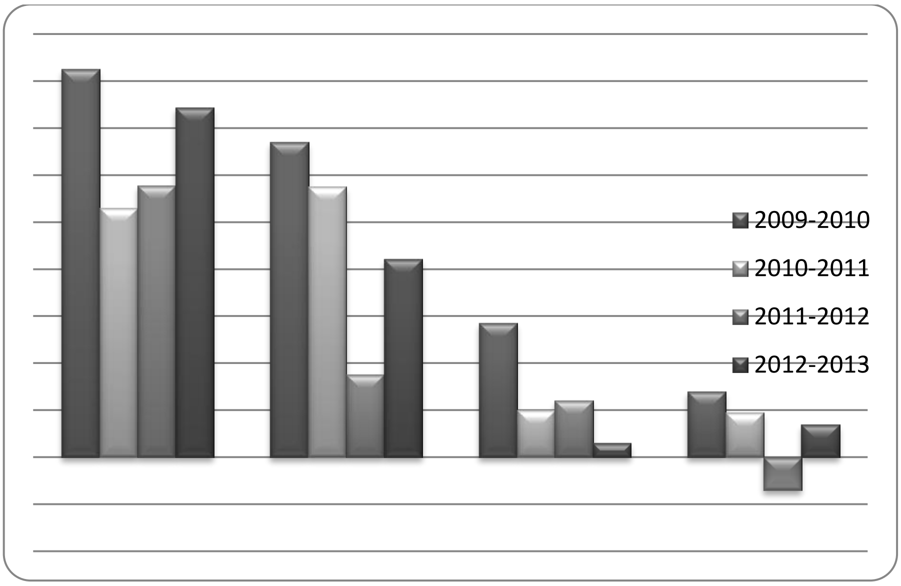

Figure 1. Average monthly temperatures during the hardening period

Table 3. *Average frost resistance of common winter wheat*

varieties	Average survivor of plant at -20^{0}C /24 h (%)	Confidential interval (%)	classifying
St$_1$-Mironovska 808	**99.3**	**100.0-96.6**	**I**
St$_2$-Pobeda	**95.3**	**100.0-88.7**	**II**
St$_3$-Bezostaya 1	**86.5**	**97.1-75.8**	**III**
St$_4$-Sadovo 1	**78.6**	**91.3-65.8**	**IV**
St$_5$- N 301	**65.1**	**79.9-50.3 ***	**V**
St$_6$-San Pastore	**28.1**	**42.1-14.2 ***	**VI**
Bononija	83.8	95.2-72.4	II
Borjana	85.4	96.4-74.5	II
Geya -1	82.0	93.9-70.1	II
Guinness	89.5	99.0-79.9	I
Diamant	84.7	95.8-73.5	II
Zdravko	90.8	99.8-81.9	I
Yoana	87.9	98.0-77.8	I
Katja	67.3	81.8-52.7 *	III
Ljusil	90.2	99.4-80.9	I
Momchil	83.1	94.7-71.5	II
Murgavetz	79.1	91.7-66.5	II
Mustang	84.4	95.6-73.2	II
Nikki	93.0	100.0-85.1	I
Petja	89.0	98.7-79.2	I
Plelom	89.7	99.1-80.2	I
Sadovo 552	43.5	58.9-28.1 *	V
Sadovo772	95.8	100.0-89.6	I
Sadovska belija	87.0	97.4-76.5	I
Fermer	96.1	100.0-90.1	I
Zarevez	70.9	85.0-56.8 *	III
Yunak	46.7	62.1-31.2 *	V

* Significant difference at 5 % compared to standards Mironovska 808 and Pobeda

The single-factor analysis of variance applied for the trait frost tolerance reveals that genotype and environment conditions during the hardening of plants have significant influence on the variation of the studied trait **(Table 4).** The variation of the survival of plants depends in greatest degree on genotype (65.2%) than on hardening conditions during the years of the study (6.0 %). Likewise, the higher value of experimental Fisher's coefficient (F exp.) than table Fisher's coefficient (F critical) demonstrates availability of significant differences between the genotypes. This result confirms the statistical significance of difference between genotypes calculated by confidence interval **(Table 3).**

Table 4. *Analyze of variance of plant survival rate*

Source of variation	df	MS	SS	η^2 %	F exp.	F crit.=5%	F crit.=1%
Total	107		46765.9				
Genotype	26	1172**	30472.5	65.2	6.8	1.64	2.01
Years(replications)	3	936.6**	2809.8	6.0	5.42	2.72	4.04
Errors /E	78	172.9	13483.6	28.8			

** Significant difference at 1 %, S. S.= Sum of Squares, M. S.= Mean Square

The observed in our study variation of plant survival rate in each genotype over the years and the calculated statistical significance of the environment conditions confirms the importance of the preliminary cold hardening of plants for the frost tolerance in common winter wheat, noted by other authors, too (5,18). For a more accurate estimation of the freezing tolerance of a genotypes using the field-laboratory methods several years of testing are needed.

Cluster analysis allows to summarize all data, determine the behavior of the tested varieties in each moment of evaluation. The dendrogram presented in **Figure 2**, shows the distribution of Bulgarian varieties of winter wheat into three major groups according to their cold resistance.

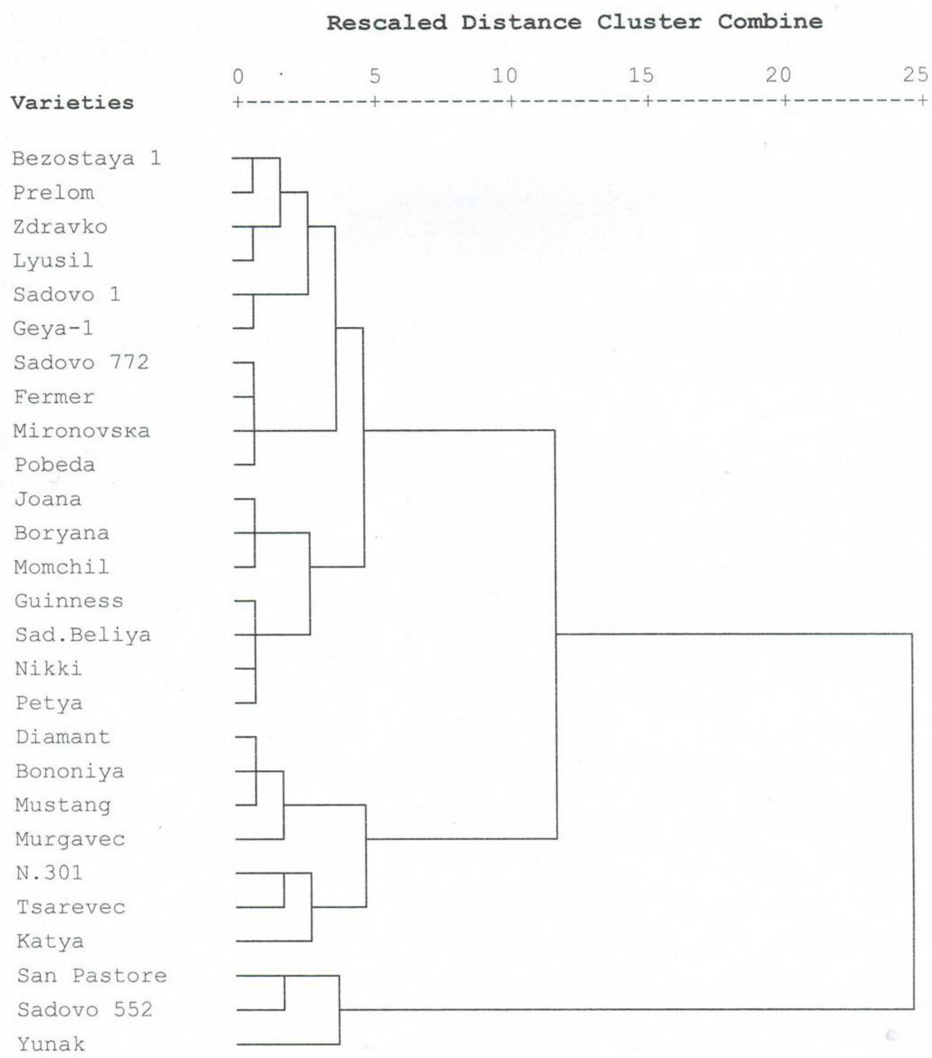

Figure 2. Dendrogram using Ward Method

The first group contains 17 varieties including standards Mironovska 808, Pobeda, Bezostaya 1 and Sadovo 1, which possess excellent and very good cold resistance. When analyzing the graphical distribution of the group is established, that it is divided into six sub-groups. Close to the standards Mironovska and Pobeda are Fermer and Sadovo 772 varieties. Other three subgroups comprises Bezostaya, 1 Prelom, Zdravko, Lusil, Sadovo 1 and Geya-1 are related to the first subgroup with an euclidean distance less than 5 units. All the rest sub clusters from the first major group include together varieties Joanna, Boryana, Momchil, Guinness, Sadovska beliya, Nikky and Petya. Varieties of these sub clusters are related to other subgroups at an euclidean distance of 5 units. It is not surprising that the pedigree of seven of the 13 varieties fall into the first cluster group includes standard varieties Pobeda, Sadovo 1 and Bezostaya, characterized by excellent frost resistance. **(Table 1).**

The second major group includes varieties Diamant, Bononiya, Mustang, Murgavetz, Tzarevetz, Katya and standard № 301, divided into two subgroups and acceding to the first group at an euclidean distance more than 10. Based on the values of the confidence interval mentioned varieties are classified to the standards Pobeda and Bezostaya 1, but because of larger scope of the confidence interval of survival plants is overlaps with the confidence interval of the standard 301 N **(Table 3).** The distribution of the clusters in the dendrogram clearly shows how varieties Tzarevetz and Katya stand closer to N 301. The average score of these varieties was lowest of the group.

Despite of greater variation in plant survival rate over years, these varieties are characterized by very good cold tolerance at the level of standards Bezostaya and Sadovo 1 **(Table 2).**

The third group is the smallest. It consists varieties Sadovo 552 and standard San Pastore, forming hairpin and joining them sort Yunak of an Euclidean distance about 5. These varieties associate to other two major groups in a much larger Euclidian distance of 25 units. Regardless of their proximity to the most sensitive to low temperatures standard San Pastore, varieties Yunak and Sadovo 552 are classified as varieties with good cold tolerance up to standard variety N 301 **(Table 3).** The reason for this is that only the lower limit of their confidence interval overlaps with the range of San Pastore.

CONCLUSIONS

1. The varieties created in the recent years in IPGR-Sadovo are suitable for growing in regions with adverse environmental conditions and there is no risk of yield compromising due to frost.
2. Highest cold resistance varieties are Fermer, Nikki and Sadovo 772.
3. The varieties Yunak and Sadovo 552 have satisfactory resistance to freezing conditions during the winter in the vicinity of Sadovo and Central South Bulgaria.
4. For a more accurate estimation of the freezing tolerance in common winter wheat genotypes using the field-laboratory method several years of testing are needed.

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