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RESEARCH ARTICLE

USING SPRING TYPE OF WHEAT FOR SHORTENING TIME TO EAR EMERGENCE OF WINTER WHEAT BY BREEDING

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ABSTRACT

The study was conducted to examine whether there are real opportunities for the creation of ultra early-winter wheat, using wheat with spring type of development. To achieve the target 24 hybrid combinations between 6 and 8 early spring winter wheat varieties in two consecutive years are made. With different vernalization requirement of plant populations in an artificial climate in the greenhouse winter and spring type plants habit are separate through consistent double selections. Subsequently all the developed lines are checked in respect of field winter cold tolerance for two winter seasons. Combination of spring and winter wheat provides a real opportunity to obtain ultra early forms with winter wheat type of development. About 16% of the plants from crosses of spring parents have similar time to ear emergence (TEE) as the respective spring parent. Time to ear emergence of the spring form does not directly affect the probability of obtaining the desired early plants. The later time to ear emergence of the winter parent, the smaller is the share of the earliest genotypes in the hybrid combinations. The selection of a very early date of time to ear emergence using spring wheat is completely possible and unexpectedly easy to implement. However, preparation of early breeding lines is not sufficient, because they have different grain yield. To have a high productivity must for native climate is to have a high cold resistance. It achieves much more difficult against the earlier date of ear heading date. The results show that it is implicitly associated with the winter cold tolerance of winter parents in every cross combination.

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INTRODUCTION

In the practical wheat breeding in Bulgaria the using of spring wheat has been very limited, in terms of published studies (Popov *et al.*, 1985; Panayotov *et al.*, 2005). Basic and important reason for this is the drastic reduction of drought and winter resistance of hybrid materials that it causes (Braun, 1997; Braun *et al.*, 2001). Back in the 80s some attempts to use various hybrid combinations of winter cross spring wheat (Boydjieva, 1995; Morgunov *et al.*, 1998) were published. In terms of Dobrudzha region (North East Bulgaria) every winter "spring" type of plants or frost or so adversely affected by the cold, even in "soft" winter that the selection did not reach them, or if they do it is more the exception (Tsenov *et al.*, 2011). Selected plants in these combinations hardly have the desired transfer properties or traits of "spring" type. This is largely renders the breeding work in this direction.

Compromise behavior by the selection in connection with the lowering of cold tolerance is generally negative for the new lines. Even if are highly productive, they have quite low cold for tolerance our conditions, reaching the proper level of № 301 (check for suitable cold tolerance) is extremely rare. Over the past few winters since 2003, season conditions have shown us that cold tolerance close to that № 301 is completely insufficient for successful survival of plants in the harsh winter environments in northern Bulgaria especially (Tsenov *et al.*, 2012). Hundreds of varieties and spring wheat samples that have been tested by the 80s of last century in Bulgaria possess many valuable to native breeding traits (Gotsov, 1984; Boydjieva, 1995; Panayotov *et al.*, 2005, Rajaram and Braun, 2008). Moreover, they have the earlier date of heading they have a low and stable to lodging stem, which is formed by the presence of (Rht-B1b, Rht-D1b or both genes together), (Mathews *et al.*, 2006; Rebetzke *et al.*, 2012) strongly fertile spikes, which formed over 5-7 grains in short spikelet (Rattey and Shorter, 2010), very high harvest index (0.40 ÷ 0.45) (Fletcher and Jamieson, 2009), high tolerance to brown black

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and yellow rusts (Huerta-Espino *et al.*, 2011). High and complex disease resistance is due, unless a specific genetics (Lr, Sr, Yr-genes) and presence of wheat-rye translocation 1B/1R, transferred to a large number of varieties (Table 2) (Lagudah *et al.*, 2009). Much spring wheat cultivars has serious genetic resistance to *Fusarium* (Del Ponte *et al.* 2012), *Septoria* (Ali *et al.* 2008) and other diseases. In recent years the efficiency of utilization and use of nitrogen and phosphorus has increased (Sandras and Lawson, 2013). Tolerance to high temperatures and drought in spring wheat was significantly improved (Ortiz *et al.*, 2008; McIntyre *et al.*, 2010; Hall and Richards, 2013) because it is grown in countries (North Africa, Australia, Argentina, Middle East), where this is an annual problem. Without shine with high quality grain from many samples are rare in our breeding of high bands (Glu B1: 13 + 16 and 17 + 18) and low molecular glutenins (Glu -A3: f and d; Glu-B3: h), which are associated with high grain quality in our environments (Todorov, 2006, Tsenov *et al.*, 2010). Spring kind of wheat except the lack of cold tolerance, however, has other negative effects for us: more of the lines have a white grain and vitreous is extremely low. The majority of samples are selected for irrigation (Sharma *et al.*, 2010) and for intensive growing technology, which involves genetically low productive tillering in the absence of irrigation. The aim of the study was to explore the possibility of establishing an early-winter wheat, using a spring type of wheat and to establish interconnections with other traits associated with the productivity of wheat.

MATERIALS AND METHODS

Initial plant material

Spring varieties are selected on the basis of pre-test conditions of DAI in 2004. Seeds have received for experience named 4 IAT (International Adaptation Trail) from CIMMYT, Mexico is grown according to pre-specified methodology for this. A total of 80 varieties were evaluated which the implementation of the idea six of them are selected, only. These are varieties: Super Seri # 1, Westonia, Attila, Galvez Dwarf, Babax and Tui, which when growing as wintering, have shown the highest possible productivity and stability compared to the other in terms of northeastern Bulgaria, and high potential grain quality (Table 3). The high grain yield is obtained thanks to their high resistance to diseases, low and resistant to lodging stem, availability of rye translocation resistance to rusts and *Septoria*, etc. (Pinto *et al.* 2010; Lopes *et al.*, 2013). Selected for mating winter wheat varieties Milena, Pobeda, Laska, Slaveya, Sadovo 1 Kristal, Enola and Galateya, which differ significantly by date of ear heading and for which sufficient information on their biological and economic characteristics has been published (Popov *et al.*, 1975, Mangova and Stankov, 1987; Tsenov *et al.* 1999). They differ genetically in level of cold tolerance (Tsenov *et al.*, 2012) and the height of the stem (Zheleva *et al.*, 2006), which is also registered in their choice for parents. In both cycles spring wheat variety was used as female component in the crossing. Hybrid combinations are made in field conditions in two consecutive cycles: 1) 2005: The late in DH, high in stem and very cold tolerant varieties of winter wheat Milena, Pobeda, Laska and Slaveya were combined with earlier in DH and "low stem" varieties of spring wheat Super Seri # 1, Westonia, Attila; 2) 2006: selected varieties are: Sadovo 1 Enola, Kristal and Galateya (early with different cold tolerance typical winter wheat) are crossed with an average early and lower stem varieties typical spring wheat varieties (Tui, Babax, Galvez Dwarf). In 2005, they made two "control" crosses between winter wheat and early Enola and Kristal (such as female parent) and late variety Milena, as male component. The reason for this is to correlate the dates of ear heading of the earliest lines derived from three groups of crosses. Plants from crosses between winter wheat are grown only in the greenhouse and field conditions depending on their specific habits and subject to the same scheme of cultivation of F2 to F5.

First hybrid generation (plants) is grown in field conditions, as part (30%) of the resulting F1-seeds are left in reserve for possible frost in winter. The reason is that all the plants in this generation are fully spring type. In both years (2006 and 2007) in winter conditions were relatively soft with snow cover, which allow normal wintering of all hybrid F1-plants.The main problem that exists when using such a design is to find an effective way of select of winter type plants, without relying on random and variable impact of conditions in the winter for this. In our case, except that we need winter plants, they must be at least as early as its spring parents, which is our ultimate purpose.When the study was in progress there is still lacked information on the specific genetic control of vernalization, both spring and winter wheat varieties. Based on theory and distribution of genes in regions (Flood and Halloran, 1986; Stelmakh, 1998; Allard *et al.* 2012), we have assumed that the maximum possible number of vernalization genes in both groups of wheat parents has to be three. According to Mendel's laws at three gene-control and the classic segregation of the recessive allele and the dominant share of the plants of the "winter", but recessive alleles is approximately 1/5 of all hybrid plants (Ayala and Kiger, 1984).

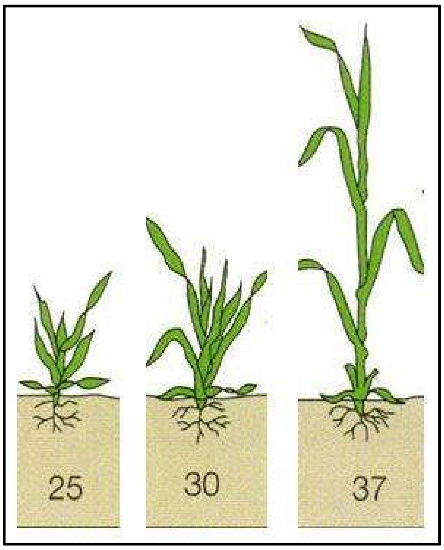

Figure1. Schematic visual difference in plants from different phases of development without vernalization, according to scale of Zadoks *et al.* (1974)

This theoretical assumption was the reason to be pledged for greenhouse test not less than 250 plants. From the resulting F2 seed 240-260 single plants of each combination are sown in the

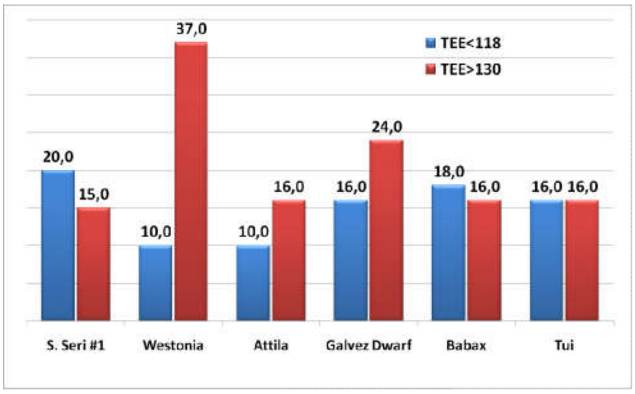

Figure 2. Percentage of plants with different ear emergence time according to any one of the early spring female parents

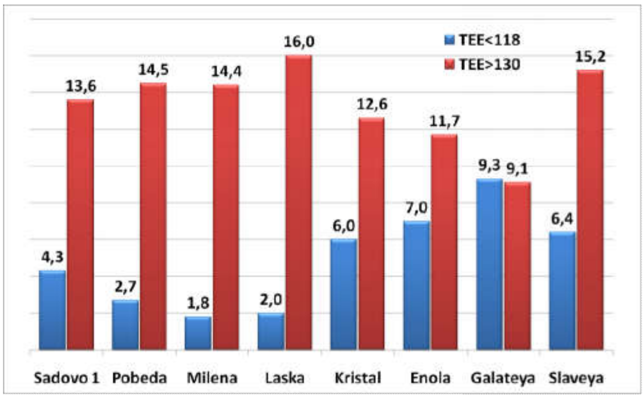

Figure 3. Percentage of plants with different of ear emergence time according to any one of the late male winter parent

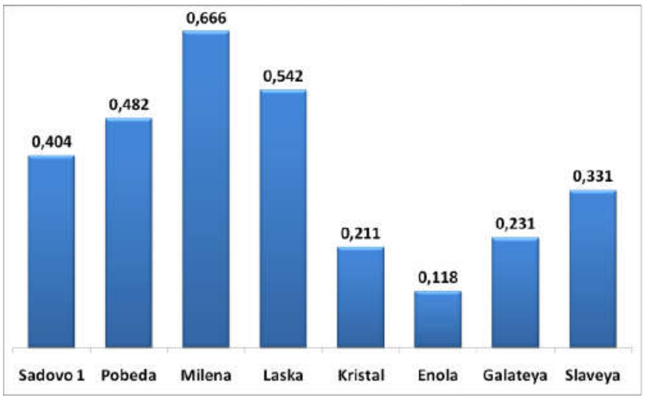

Figure4. Correlation between cold tolerance and productivity in groups of crosses according to winter component in them

greenhouse in the laboratory complex, (DAI) without prior vernalization, they are grown at the optimal temperature (25 ^{0}C + / 18^{0}C day/night). Hybrid plants are grown in pots until the time the first stem node (the beginning of spindling) at some of the plants. This procedure in is going on both years by mid of February. Because the length of the natural day of the season, in the conditions in which they grow is between 9-10 hours, the fastest and steadily grow and developing plants possessing dominant vernalization genes that are fully spring (VRN) (Figure 1). These plants, which are in phase stem elongation reached 37 Zadoks stage - first-second stem node is discarded immediately as completely "spring." The remaining plants, visibly divided into two groups: "fast" growing and "slow" growing. Heterozygous (winter-spring) plants differ in that they have a faster growth rate and fewer tillers. The second group, which is the smallest fully "winter" plants in which the alleles are recessive and they are in stage of tillering (25-27 by Zadoks). In some of such crosses visual separation plant was impossible. Therefore all left plants subjected to vernalization for about 40 days, more than enough to allow the plants to reach the ear heading and maturation. After this period, the plants were moved to field conditions (about 18 to 25 March) and grown to full maturity in real field conditions. The same approach applies to the next F3-generation hybrids. Received seeds from the relatively small number of "winter" plants after the double selection cycle in the early hybrid generations were a prerequisite to be used bulk method in F4. In this generation in field conditions about 4500-5000 plants from each cross have been grown. There are marked and subsequently selected plants across the available range of the date of ear emergence time. Thus keep the whole range of differences in the occurrence of this moment; it is paying particular attention to early plants, the objective of the study. Thus marked and selected 80-120 F4-plants were grown as F5 offspring in single rows of the breeding nursery. In this generation the individual descendants are estimated for date of ear emergence, up to physiological maturity, according to the methods of (Tsenov, 2009) and all these traits. The resulting breeding lines presented in the tables of results are determined based on the date of ear heading of the corresponding spring or winter parent.

Studied traits

Selected F5 advanced lines are cultivated in a separate unit of the nursery yield trail, in three replications each, with experimental plot size of 6 m^{2}. On each replication all possible traits related to productivity: date of ear formation (Z-59), number of days from January 1 to the date of ear formation and grain yield were tested. The differences in the date of ear emergence between parents and created advanced lines are analyzed. Particular attention is paid to the earlier than mean for a cross date of ear heading. By criterion x^{2} is checked theoretically expected share of "winter" plants of any combination, depending on its specific set of genes. Thus, the difference between the genetic controls of vernalization practically is determined by the results and was discussed according to the information about the parental varieties, collected during the study. Correlations between grain yield, the date of the ear emergence and field cold tolerance on the basis of group of advance lines are calculated.

RESULTS

When combining spring with winter wheat segregation of various seasonal types according to theoretical expectations in our case should be 63 spring: 1 winter. This results from the three genes for vernalization (2) and photoperiod (1) that are used by each spring varieties. In this three-gene control expected segregation ratio is: 27: 9: 9: 9: 3: 3: 3: 1. According to Mendel's laws on every 64, 27 hybrid plants is fully spring type and should be eliminated completely. 36 of rest are

Table 1. Summary of participating in cross-breeding spring and winter wheat varieties (* number of days from January 1, ** see the abbreviations)

Variety	Origin, Year of realize	Date of heading *	Genes for vernalization	Genes for photoperiod	Seasonal type [1,2,3]
Super Seri #1	Mexico, 1982	117	*VRN-B1, VRN-D1*	*PPD-B1*	Spring [1,2]
Westonia	Mexico, 1985	116	*VRN-A1, VRN-B1*	*PPD-B1*	Spring
Attila	Mexico, 1987	119	*VRN-D1*	*PPD-B1*	Spring
Galvez Dwarf	Mexico, 1990	120	*VRN-D1*	*PPD-B1*	Spring
Babax	Mexico, 1992	121	*VRN-B1, VRN-D1*	*PPD-B1*	Spring
Tui	Australia, 1997	122	*VRN-D1*	*PPD-B1*	Spring
Sadovo 1	IPGR**, 1973	137	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter [3]
Pobeda	IPGR, 1982	136	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Milena	DAI**, 1996	138	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Laska	DAI, 1996	135	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Kristal	DAI, 1996	130	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Enola	DAI, 1997	129	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Galateya	DAI, 1999	128	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
Slaveya	DAI, 2000	127	*vrn-A1, vrn-D1*	*Ppd_D1a*	Winter
SE		1,23			

1 - Lukman (2003); 2- van Beem *et al.*, 2005;; 3- Kolev *et al.* (2010)

Table 2. List of the studied hybrid combinations between spring and winter varieties

№	♂ component	♀ component	№	♂ component	♀ component
	in 2005			*in 2006*	
1	S. Seri #1,	Sadovo 1	13	Galvez Dwarf	Kristal
2	S. Seri #1,	Pobeda	14	Galvez Dwarf	Enola
3	S. Seri #1,	Milena	15	Galvez Dwarf	Galateya
4	S. Seri #1,	Laska	16	Galvez Dwarf	Slaveya
5	Westonia	Sadovo 1	17	Babax	Kristal
6	Westonia	Pobeda	18	Babax	Enola
7	Westonia	Milena	19	Babax	Galateya
8	Westonia	Laska	20	Babax	Slaveya
9	Attila	Sadovo 1	21	Tui	Kristal
10	Attila	Pobeda	22	Tui	Enola
11	Attila	Milena	23	Tui	Galateya
12	Attila	Laska	24	Tui	Slaveya

Table 3. Summary information on the tested varieties of international experience of CIMMYT Mexico by specialized program for varieties GRIS (G*enetic* R*esources* I*nformation* S*ystem for Wheat and Triticale*), (*http://wheatpedigree.net/*)

Name	Pedigree:	Valuable major genes of resistance	High-molecular gluten genes			Low-molecular gluten genes		
			Glu *A1*	Glu *B1*	Glu *D1*	Glu *A3*	Glu *B3*	Glu D3
Super Seri #1	Cid139513/Seri M 82	*Lr19, Lr23, Lr26; Sr31; Yr2, Yr7*	1	17+18	5+10	c	h	c
Tui	Hermosillo-77/ Sapsucker//Veery	*Lr13, Lr26, Sr31,Yr9*	1	7+8	5+10	b	h	c
Galvez Dwarf	Bluebird/Gallo// Carpintero/3/Pavon-76	*Lr3a, Lr10, Lr13 Rht 1+2*	1	17+18	5+10	c	h	c
Attila	Nord-Desprez / Vg-9144//Kalyansona/Bluebird/3/Yaco/4/Veery	*Lr26, Lr46, Sr31, Yr9, Yr27, Yr27, Yr29*	2*	7	5+10	c	h	c
Babax	Bobwhite/Nacozari-76//Veery/3/Bluejay/Cocoraque-75	*Yr31 Pm4b, Tolerant to Septoria sp.*	2*	7+9	5+10	c	h	c
Westonia	CO-1190-203/84-W-127-501	*Lr1, Lr3a, Sr6, Sr9g,Yr6, Yr7*	2*	17+18	2+12	c	h	c

Method of cultivation and selection

Table 4. Number of plants of different combinations and ratios between winter and spring type in the first group of F2-cross populations

№	Cross	ST#	SWT	WT	Theoretical	χ^2	*p-value*
1	S. Seri #1/Sadovo 1	138	88	45	9:6:3	0.066	0.0012
2	S. Seri #1/Pobeda	132	86	43	9:6:3	0.066	0.0012
3	S. Seri #1/Milena	133	94	45	9:6:3	0.029	0.0001
4	S. Seri #1/Laska	59	121	55	*1:2:1*	0.016	0.0000
5	Westonia/ Sadovo 1	130	95	44	9:6:3	0.185	0.0123
6	Westonia/ Pobeda	136	90	47	9:6:3	0.007	0.0000
7	Westonia/ Milena	137	85	46	9:6:3	0.029	0.0001
8	Westonia/ Laska	62	121	59	*1:2:1*	0.066	0.0012
9	Attila/ Sadovo 1	133	94	43	9:6:3	0.029	0.0001
10	Attila/ Pobeda	136	92	48	9:6:3	0.007	0.0000
11	Attila/ Milena	137	83	44	9:6:3	0.029	0.0001
12	Attila/ Laska	61	123	66	*1:2:1*	0.016	0.0000
	All without 4, 8, и 12	1212	807	375	9:6:3	0.001	0.0000
	Crosses: 4, 8, и 12	182	365	180	1:2:1	0.007	0.0000

ST Spring type; **SWT**- Spring-Winter type; **WT**- Winter Type

Table 5. Number of plants of different combinations and ratios between winter and spring type in the second group F2-cross populations

№	Cross	ST#	SWT	WT	Theoretical	χ^2	*p-value*
13.	Galvez Dwarf/Kristal	65	130	65	1:2:1	0.007	0.0012
14.	Galvez Dwarf/ Enola	63	129	68	1:2:1	0.029	0.0001
15.	Galvez Dwarf/Galateya	68	128	64	1:2:1	0.118	0.0012
16.	Galvez Dwarf/ Slaveya	61	134	65	1:2:1	0.029	0.0000
17.	Babax/ Kristal	59	128	73	1:2:1	0.266	0.0123
18.	Babax/ Enola	60	135	65	1:2:1	0.118	0.0001
19.	Babax/ Galateya	64	134	62	1:2:1	0.000	0.0000
20.	Babax/ Slaveya	58	134	68	1:2:1	0.007	0.0012
21.	Tui/ Kristal	58	129	73	1:2:1	0.185	0.0001
22.	Tui/Enola	64	131	65	1:2:1	0.066	0.0001
23.	Tui/ Galateya	63	125	72	1:2:1	0.007	0.0000
24.	Tui/Slaveya	63	134	63	1:2:1	0.007	0.0000
	Total	564	1169	607	1:2:1	0.011	0.0000

STSpring type; **SWT**- Spring-Winter type; **WT**- Winter Type

heterozygous, having recessive genes for winter type of habit. These plants should be selected, because in the next generation they produce winter type, again. In crosses in which there are two genes, a phenotypic segregation is 15: 1 and 9: 3: 3: 1 by genotype.

DISCUSSION

Purpose of the study is to analyze the possibility of obtaining early winter with the type of development, the cultivation of which do not pose a risk in the harsh winters of the Balkan Peninsula. About 15% of the plants in hybrid populations have an earlier date of ear emergence of the corresponding spring parent (Figure 2). The highest share of the early plants in combination with the participation of a variety Super Seri # 1 (20%), followed by those of the variety Babax (18%) and the lowest share of early in crosses where varieties Westonia and Attila, were used (only 10%). Other crosses occupy an intermediate position between already said. It is interesting to be noted the low proportion of plants with early heading of early varieties Westonia (EED=116) and their high share in combinations involving much later the group of spring wheat - Babax (EED=121). The largest is proportion of early plants with the participation of most late spring variety Tui (EED = 122). These data show no link between the likelihood of obtaining early-stage and early date of ear emergence. Rather it is directly connected only with the specific genetic nature of participating in a particular cross-type spring and winter varieties

In the case considerably more important is the fact that each of the seven plants of selected "winter" type one is at least as early spring as its parent, which is a breeder achievement. Similar outcomes were also reported in the studies of Morgunov *et al*. 1998 and Braun, 1997. The group cross of Westonia is significantly higher proportion of late plants, with about 1.8 times the average for all combinations, although it is among the earliest spring varieties, as already noted. Receiving of early plants is possible, regardless of date of ear emergence of the spring parent. Then, is it necessary to analyze whether the date of ear heading of the winter parent matter? The data in Figure 3 provide the answer to this question. If we compare the earliest lines presented against the background of a winter variety used, the picture changes dramatically. An average of only 3.8% of all the "winter" plants are obtained earlier offspring at a level close to that of spring type of wheat. Differences between crosses are immaterial with the exception of those involving varieties Milena and Laska, who are later in the whole experiment. Early plants with their participation are only about 2%. For comparison of a cross between early winter wheat 7% offspring for combinations of Enola and about 9.5% for those with Galatea are early ones. Therefore, the early winter is a component of the crosses, the greater the proportion of plants in early winter habit in them. On the other hand, the share of the later heading forms is smaller again at the earlier parents: 9-11% (at Galateya and Enola) against 14.5-16% for later Milena and Laska. Large differences in the cold tolerance of winter wheat used are prerequisites to assume that much of the choicest early lines, though winter type will have low genetic tolerance to cold. The largest share of the early heading plants with participation of low cold tolerant varieties Enola and Galatea and vice versa, but the lowest in combination with the cold-tolerant varieties, Laska and Milena. The production of plants with the earliest time of ear heading is not sufficient in terms of winter tolerance. They must have the genetics for cold tolerance at least the level of Enola or Galatea (Tsenov *et al*. 2012). Exactly this combination is the most difficult to achieve, especially if we turn our selection to ultra early in heading plants. Answer to this essential question for our climate survey gives the additional study of 30 F5-breeding lines from each group crosses in respect to winter parent. Figure 4 shows the correlation between assessment scores for wintering in real field conditions and the resulting grain yield. This is a sufficient guarantee for the level of cold tolerance during two consecutive winter period (2012-2013). Data show definite difference between the crosses of winter wheat varieties. The more high level of cold tolerance of the variety (Milena), the higher the grain yield of early lines with his participation (r=0.666**). Conversely, featuring varieties like Enola and Kristal and reduces considerably the level of cold tolerance of super early lines, which have a negative impact on their grain yield (r = 0.118 ns). The correlation value copies the genetic level entirely from the cold of winter parent, without exception. This finding is very important in getting early-winter wheat. They must be not only in winter habit, but also in cold tolerance. In conclusion we can say that the selection of lines with a very early date of ear heading using spring wheat is completely possible and unexpectedly easy to realize. The development of early breeding lines is not sufficient, because they have a relatively low yield of grain. To have higher productivity prerequisite is to have a high cold tolerance. It achieves much more difficult against the earlier date of ear emergence. It is also implicitly associated with the winter cold tolerance of the winter type component when crossing. This, in turn, significantly reduces the likelihood of obtaining early plants with sufficiently high productive potential. At the end of 24 combinations only 15 lines that have a sufficiently high level of tolerance to cold and date of ear heading, similar to spring component, were obtained. This is a negligible part of the total volume of the created material by selection and constitutes only 0.05% of it. Such low efficiency is the main reason to avoid using spring wheat varieties, as a general rule. According to Allard *et al*. (2012), the requirements for the duration of vernalization is not a measure of cold tolerance and is probably the reason for relatively easily obtain early lines. In our case the presence of vernalization requirement was used as an instrument for separate type of seasonal development and it proved quite effective. Similar to our approach, using winter x spring lines, reports Larsen (2012) and Sharma *et al*. (2012). In the latest study, however, winter forms are used for the improvement of spring wheat, which is radically different from our goals. Continental climate of the country known threshold winter- and cold tolerance of the breeding material is mandatory (Tsenov *et al*. 2012). Similar is the opinion of the review published by Gusta and Wisnewski, (2013), in which cold tolerance in winter wheat is required to achieve high grain productivity.

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