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EFFECT OF VERNALIZATION REQUIREMENTS ON HEADING DATE AND GRAIN YIELD OF NEAR-ISOGENIC LINES OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Choosing the most appropriate vernalization requirements can contribute to optimizing wheat life cycle for maximum use of climatic resources in the target environment. Spring and winter forms, undistinguishable under full vernalization, were selected from two released cultivars (Lovrin 34 and Fundulea 4), both originated from crosses involving a Mexican spring cultivar (Nadadores 63). These near-isogenic lines were tested in yield trials at Fundulea, in a traditionally winter wheat growing environment, for 6 years at two planting dates. The difference between heading dates of winter (*vrn* carriers) and spring (*Vrn* carriers) near-isogenic lines varied from 0 to 3 days depending on weather conditions of different years and on planting date. Grain yield was strongly influenced by environment, with the effect of planting dates being less important than the effect of years and of interaction between years and planting dates. The significant effect of genotypes was mainly due to the *Vrn* alleles, while the effect of genetic background and of interaction between vernalization requirements and background was, on average, not significant. The significant interaction between genotypes and environments was due to the interaction of both *Vrn* alleles and genetic background with years, and not with planting dates. On average, spring isolines over-yielded the winter ones by over 400 kg ha^{-1} at the normal planting date and by about 160 kg ha^{-1} at late planting. A significant correlation (r = 0.80*) was found between the effect of *Vrn* alleles on heading date and the effect on grain yield. The effect of the Vrn allele on heading date or yield was not correlated with the degree-days cumulated in autumn, but was significantly correlated with visual scores for winter damage and an index of winter severity. The potential use of *Vrn* allele for increasing yield in South Romania depends on risks of winter damage, which might be influenced by future climate changes, or by possible improvement of freezing resistance in *Vrn* carriers.

Key words: *Vrn* alleles, yield, heading date, winterhardiness, wheat.

INTRODUCTION

Maximizing yield in any environment requires the maximum use of available resources, such as water and radiant energy, by the wheat plant and avoidance of stress conditions during growth and grain filling. For that, wheat must have an appropriate flowering time and life cycle duration, which "fine tunes" the life cycle to the target environment (Snape et al., 2001). In wheat, like in many other crops, the rate at which wheat (*Triticum aestivum* L.) reaches anthesis and other developmental stages depends largely on the variation of three major factors: vernalization (*Vrn*), photoperiod (*Ppd*), and earliness per se (*Eps*) (van Beem et al., 2005).

Vrn and *Ppd* gene systems have been investigated in various studies related to adaptation, and results have shed some light on the benefits that could be obtained through their conscious manipulation within spring or winter types (Worland, 1996; Snape et al., 2001).

Stelmakh (1990) suggested that the geographic distribution of *Vrn*-genes is partially related to their effects on adaptation to specific regions. Mustăţea et al. (2000) found that, in South Romania, spring isolines, with no vernalization requirements, yielded significantly more than the winter lines when emergence was very late, but significantly less when winter was severe.

Vernalization insensitivity or low vernalization response is known to be under control of an orthologous series of dominant alleles at the *Vrn-A1* (former *Vrn1*), *Vrn-B1* (former *Vrn2*), and *Vrn-D1* (former *Vrn3*) loci

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that are located in the long arms of group 5 chromosomes (Worland, 1996).

On the other hand, it is known that vernalization and photoperiod responses regulate the expression of Low Temperature (*LT*) tolerance genes, through their influence on the rate of plant development (Mahfoozi et al., 2001).

This paper reports data on the effect of vernalization requirements on heading date, grain yield and winterhardiness, during six years, in a traditionally winter wheat growing environment.

MATERIAL AND METHODS

During a study on vernalization requirements of Romanian wheat cultivars, two cultivars (Lovrin 34 and Fundulea 4) were found to be mixtures between a winter form (requiring vernalization temperatures for about 40 days) and a spring form, which could develop to heading without vernalization. These forms were undistinguishable morphologically, and headed concomitantly in environments which provided conditions for full vernalization. They could be differentiated by heading date only when vernalization was not complete.

Lovrin 34 was selected in F4 from the cross Ranniaia 12/Nadadores 63//Lovrin 12, where Nadadores 63 is a CIMMYT spring cultivar, selected from the cross Penjamo 62/ 2*Yaqui 54, while Ranniaia 12 and Lovrin 12 are winter cultivars (Paraschivoiu et al., 1987). According to Syukov (2003) (cited at http://genbank.vurv.cz/wheat/), Nadadores 63 was determined to be a carrier of the *Vrn2* (synonym *Vrn-B1a*) allele for spring habit, but we could not find any independent report confirming this.

Fundulea 4 was selected in F5 from the cross Ranniaia 12/Nadadores 63//Lovrin 12/3/ Fundulea 29, involving the same spring parent and three winter parents. In contrast with Lovrin 34, it is a photoperiod sensitive cultivar (Saulescu and Ittu, 1989).

We can assume that the plants, representing the final selections from which these two cultivars originated, were heterozygous for the alleles of the *Vrn 2* locus; therefore the spring and winter forms isolated 6 to 8 years after the final selection could be considered near-isogenic lines, differing mainly by the *Vrn* or *vrn* alleles.

The *Vrn* and *vrn* near-isogenic lines of both Lovrin 34 and Fundulea 4 were tested in yield trials at Fundulea (located at 44°33' Northern latitude, 24°10' Eastern longitude and 68 m altitude) for 6 years (1996-1998 and 2008-2010), at two planting dates: normal (providing 220 to 460ºC degree days till winter), and late (providing 38 to 235ºC degree days till winter) (Table 1).

Table 1. Planting dates and corresponding degree days cumulated till winter in the six years of testing

Season	Normal planting		Late planting	
	Date	Degree days till winter	Date	Degree days till winter
1995-1996	October 16, 1995	220ºC	November 14, 1995	38ºC
1996-1997	October 14, 1996	460ºC	November 7, 1996	235ºC
1997-1998	-	-	October 26, 1997	210ºC
2007-2008	October 18, 2007	242ºC	November 9, 2007	71ºC
2008-2009	October 15, 2008	363ºC	October 30, 2008	180ºC
2009-2010	October 23, 2009	310ºC	November 6, 2009	180ºC

The six years of this study were quite different in temperatures resources during autumn, in winter conditions (minimum temperatures from -15ºC in 1996 to -24ºC in 2010) and in rainfall (from 298.2 mm in 2009 to 405.8 mm in 2010). Heading date and grain yield were recorded. ANOVA was used to analyze yield data. Winter severity was estimated by visual scores for winter damage and by the sum of temperatures below -9ºC estimated at crown level, with the insulation effect of snow layer, calculated according to Aase & Siddoway (1979) (cited by Lazăr et al., 2005).

RESULTS AND DISCUSSION

Heading date of Lovrin 34 and Fundulea 4 *vrn/Vrn* near-isogenic lines varied from May 7 in Lovrin 34*Vrn* in 2008 normal planting date to June 3 in Fundulea 4*vrn* in 1996 late planting (Table 2). Years produced the largest variation, both at normal planting date (13 days in Lovrin 34*vrn*, 14 days in Lovrin 34*Vrn*, 8 days in Fundulea 4*vrn* and 19 days in Fundulea 4*Vrn*) and at late planting date (15 days in Lovrin 34*vrn*, 16 days in Lovrin 34*Vrn*, 19 days in Fundulea 4*vrn* and 17 days in Fundulea 4*Vrn*).

Table 2. Heading date of Lovrin 34 and Fundulea 4 *vrn*/*Vrn* near-isogenic lines

Year	Planting date	Lovrin 34		Fundulea 4	
		vrn	*Vrn*	*vrn*	*Vrn*
1996	Normal	May 18	May 18	May 21	May 21
	Late	May 25	May 24	June 3	June 1
1997	Normal	May 20	May 17	May 22	May 21
	Late	May 22	May 21	May 25	May 24
1998	Late	May 13	May 13	May 19	May 19
2008	Normal	May 9	May 7	May 14	May 12
	Late	May 17	May 15	May 20	May 18
2009	Normal	May 11	May 8	May 14	May 12
	Late	May 13	May 11	May 15	May 14
2010	Normal	May 10	May 8	May 15	May 14
	Late	May 10	May 8	May 17	May 16

Late planting produced a delay of 0 to 8 days in Lovrin 34 and from 1 to 13 days in Fundulea 4, depending mostly on the year. Fundulea 4 lines always headed later than Lovrin 34 lines, by at least 2 days and by maximum 9 days.

The difference between heading dates of winter (*vrn* carriers) and spring (*Vrn* carriers) near-isogenic lines varied from 0 to 3 days depending on weather conditions of different years and on planting date (Table 3).

Table 3. Difference between heading dates of lines carrying *vrn* and *Vrn* alleles

Year	In Lovrin 34 background			In Fundulea 4 background			On average over both backgrounds		
	NPD	LPD	Average	NPD	LPD	Average	NPD	LPD	Average
1996	0	-1	-0.5	0	-2	-1	0	-1.5	-0.75
1997	-3	-1	-2	-1	-1	-1	-2	-1	-1.5
1998		0	0		0	0		0	0
2008	-2	-2	-2	-2	-2	-2	-2	-2	-2
2009	-3	-2	-2.5	-2	-1	-1.5	-2.5	-1.5	-2
2010	-2	-2	-2	-1	-1	-1	-1.5	-15	-1.5

Grain yield was strongly influenced by environment, with the effect of planting dates being less important than the effect of years and interaction between years and planting dates (Table 4). The significant effect of genotypes was mainly due to the *Vrn* alleles, while the effect of genetic background and of interaction between vernalization requirements and background was, on average, not significant.

The significant interaction between genotypes and environments was due to the interaction of both *Vrn* alleles and genetic background with years, and not with planting dates.

Table 4. ANOVA for grain yield of near-isogenic lines

Sources of variation	SS	df	MS	F
Environment	91724959	10	9172496.0	148.91***
- Planting dates	(1362928)	(1)	1362928.0	22,12**
- Years + Years*Dates	(90362031)	(9)	10040226.0	162,99**
Genotypes	978148.7	3	326049.6	5.29**
- *Vrn* alleles	(845543.2)	(1)	845543.2	13.73**
- Background	(84946.6)	(1)	84946.6	1.38
- IA *Vrn**Bckg	(47658.9)	(1)	47658.9	0.77
*IA Genotypes*Environment*	4702074	30	156735.8	2.54**
- IA *Vrn**Env.	(2505094)	(10)	250509.4	4.07**
-IA *Vrn**Planting dates	(65802.5))	(1)	65802.5	1.07
- IA *Vrn**Years+Y*Dates	2439292	(9)	271032.4	4.40**
- IA Bckg*Env.	(1561651)	(10)	156165.1	2.53*
- IA Bckg*Planting dates	(941.3)	(1)	941.3	0,02
- IA Bckg*Years+Y*Dates	(1560710)	(9)	173412.2	2.81**
- IA *Vrn**Bckg*Env	(635328.4)	(10)	63532.8	1.03
Error	3449540	56	61598.9	

The effect of the *Vrn* alleles on yield was significant in all years, except one (1997). The effect of the genetic background was significant in most years, with the exception of 1997 and 2008 (Table 5). However, the background effect was different in different years, with lines in Lovrin 34 background yielding more than those in Fundulea 4 background in 1996 and 2010 and less in 1998 and 2009, so that on average the background effect was not significant. The effect of the interaction between *Vrn* alleles and background was significant in only 2 of the 11 environments.

Table 5. F values for genotype effects in the six testing years

Year	Normal planting date			Late planting date		
	Vrn alleles	Background	IA	*Vrn* alleles	Background	IA
1996	**46.39****	**8.68***	0.0004	**81.75*****	**41.11****	1.32
1997	1.53	2.21	0.06	1.81	0.17	0.28
1998	-	-	-	**29.31*****	**9.31***	1.81
2008	**17.34***	0.96	3.69	**20.16***	5.38	0.04
2009	**410.39*****	**158.61*****	**47.41****	**22.76****	**10.79***	0.36
2010	**45.11****	**258.89*****	0.85	**91.62*****	**165.61*****	**7.95***

Winter type isolines yielded significantly more than their spring equivalents in 2 out of 11 comparisons, while the spring isolines were significantly higher yielding in 6 out of 11 comparisons, both in Lovrin 34 and Fundulea 4 backgrounds (Table 6). On average, spring isolines over-yielded the winter ones by over 400 kg ha^{-1} at the normal planting date and by about 160 kg ha^{-1} at late planting. This can be explained by the results of Snape et al. (2001), who found that the presence of the spring allele resulted in faster rate of development with both vegetative and reproductive primordia being produced at a quicker rate. As a result, spring isolines could use better the sometimes limited water resources, mainly water accumulated in the soil during winter.

The yield variation of winter lines tended to be smaller at normal planting date, but was larger at the late planting date (Table 7).

Table 6. Grain yield difference between spring (*Vrn* carriers) and winter (*vrn* carriers) near-isogenic lines

Year	In Lovrin 34 background			In Fundulea 4 background			On average over both backgrounds		
	NPD	LPD	Average	NPD	LPD	Average	NPD	LPD	Average
1996	-437*	300*	-68	-435*	387*	-24	-436*	343*	-46
1997	267	-100	83	200	-17	91	233	-58	87
1998	-	-753**	-753**	-	-413*	-413*	-	-583*	-583*
2008	510*	-25	242*	1385**	1090*	1237**	947**	533*	740**
2009	1402***	243	822*	691*	313	502*	1046**	278	662*
2010	335*	584**	459*	254*	318*	286*	294*	451*	373*
	415*	41	131*	419*	280	280*	417*	161	206*

Table 7. Average yield and coefficient of variation in Lovrin 34 and Fundulea 4 *vrn/Vrn* near-isogenic lines

Genotype	Normal planting date		Late planting date	
	Average yield	s%	Average yield	s%
Lovrin 34*Vrn*	4809	32.59	3818	36.68
Lovrin 34*vrn*	4394	31.64	3777	42.37
Fundulea 4*Vrn*	4746	38.21	3830	41.11
Fundulea 4*vrn*	4327	31.58	3550	49. 72

A significant correlation (r = 0.80*) was found between the effect of *Vrn* alleles on heading date and the effect on grain yield (Figure 1). Environments which allowed a faster development of the spring isolines, as compared with the winter ones, were conducive to a larger yield advantage due to the *Vrn* allele.

These differences were not correlated with the degree-days cumulated in autumn, the correlation coefficients being r = -0.19 n.s. and r = -0.03 n.s. for the differences in heading dates and differences in yield respectively.

Mustățea et al. (2000) associated the relative performance of spring isolines with no vernalization requirements, with the severity of overwintering conditions. As expected, as a result of the effect of vernalization response on the expression of *LT* tolerance genes (Mahfoozi et al., 2001) the Vrn isolines, in both genetic backgrounds, were less resistant to freezing in artificial tests, and their susceptibility increased to the end of winter, as compared to January (Table 8). This is supported by the results of Petcu et al. (1997), who reported that the winter isoline of Lovrin 34 had an increased activity of peroxidase isoenzymes, as compared to the spring isoline.

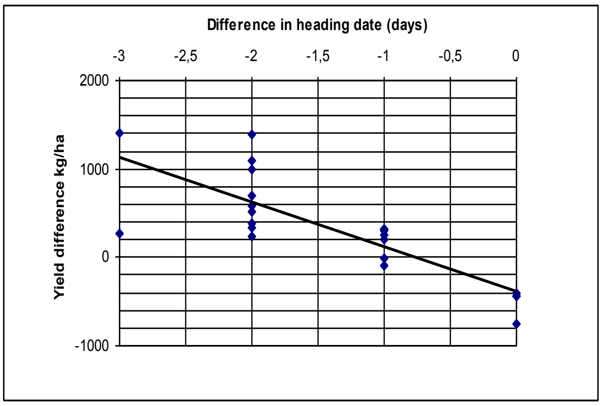

Figure 1. Relationship between the difference in heading date and the difference in grain yield between the spring (*Vrn*) and the winter (*vrn*) isolines

Table 8. Average artificial freezing scores in Lovrin 34 and Fundulea 4 *vrn/Vrn* near-isogenic lines

Freezing date	Lovrin 34		Fundulea 4	
	vrn	*Vrn*	*vrn*	*Vrn*
January	3	5	4	6
February	4	7	5	8

1 = no damage, 9 = no survival.

In field natural conditions, *Vrn* isolines always showed more winter damage than *vrn* lines, but large differences between winter damage scores of *Vrn* and *vrn* lines were recorded only in some years, particularly in

1996, 1998 and 2010 (Table 9). *Vrn* lines in Fundulea 4 background were less affected than those in Lovrin 34 background, as they developed slower due to their day-length requirements.

The visual scores for winter damage were significantly correlated with the yield differences between *Vrn* and *vrn* lines (r = -0.58*). Yield differences were also correlated with the sum of temperatures below -9ºC estimated at crown level (under the snow cover if present) (r = -0.46*).

Table 9. Winter damage scores in Lovrin 34 and Fundulea 4 *vrn/Vrn* near-isogenic lines

Year	Planting date	Lovrin 34		Fundulea 4	
		vrn	*Vrn*	*vrn*	*Vrn*
1996	Normal	3	7	3	6
	Late	Spring emergence	Spring emergence	Spring emergence	Spring emergence
1997	Normal	2	3	2	3
	Late	2	4	2	3
1998	Late	2	7	1	5
2008	Normal	1	3	1	3
	Late	Spring emergence	Spring emergence	Spring emergence	Spring emergence
2009	Normal	2	3	2	2
	Late	2	3	2	2
2010	Normal	3	5	2	4
	Late	5	7	3	6

1 = no damage, 9 = no winter survival.

This suggests that the yield advantage of spring isolines can be annulated and even reversed in more severe winters. Therefore, the potential use of *Vrn* allele for increasing yield in South Romania will depend on the risk of winter damage, which might be influenced by future climate changes, or by possible improvement of freezing resistance in *Vrn* carriers. Lazăr and Lazăr (2010), based on simulation studies, suggested that cultivars with very low requirements for vernalization are not suitable for South-Eastern Romania, due to lower yield level and very poor yield stability. The disagreement between their conclusions and the results of the present studies is most probably due to the relatively small number of testing years, which might not be representative for the longer series of years included in the simulation study. However, we cannot exclude the possibility that the simulation model used did not describe completely the complex effects of *Vrn* alleles, and this deserves further studies.

The effects of the spring habit allele will also depend on the vernalization locus involved. Zhmurko et al. (2004) showed that lines with different *Vrn* gene differ in carbohydrate accumulation. Stelmakh et al. (1997) reported that the frost resistance of *Vrn* carriers depended on the respective gene, with carriers of *Vrn2* and *Vrn3* having a considerable higher frost resistance than *Vrn1* carriers. Further research should address the effect of spring alleles at different *Vrn* loci, in an attempt to find the best compromise for South Romania between sufficient winterhardiness and the rapid growth in spring.

CONCLUSIONS

Even in a traditionally winter wheat growing area, such as South Romania, carriers of *Vrn* allele for spring habit significantly over yielded their respective winter isolines in half of the six testing years. The yield advantage of the *Vrn* carriers depended on overwintering conditions, being cancelled in severe winters. If future climate changes will reduce the risk of severe winters, or if freezing resistance of *Vrn* carriers will be significantly improved, *Vrn* alleles, can offer a possibility for increasing yield in environments similar to South Romania.

REFERENCES

Lazăr, C., Micale, F., Genovese, G., 2005. *Simulation of frost resistance of winter wheat in Europe.* Italian Journal of Agrometeorology, 9 (1): 62-63

Lazăr, C. and Lazăr, D.A., 2010. *Simulation of temperature increase influence on winter wheat yields and development in South-Eastern Romania.* Romanian Agricultural Research, 27: 7-15.

Mahfoozi, S., Limin, A.E., Fowler, D.B., 2001. *Influence of vernalization and photoperiod responses on cold hardiness in winter cereals.* Crop Science, 41 (4): 1006-1011.

Mustăţea, P., Săulescu, N.N., Ittu G., 2000. *Efectul genelor de reducere a taliei şi al genelor care controlează cerinţele de vernalizare asupra reacţiei grâului la semănatul întârziat (Vernalization and day-lenght requirements in present Romanian wheat cultivars).* An. ICCPT Fundulea, 67: 7-18.

Paraschivoiu, R., Săulescu, N.N., Băjireanu, I., Şiclovan, V., 1987. *Preocupări şi realizări privind ameliorarea grâului în partea de vest a ţării.* In Lucrări ştiinţifice – Volum omagial 1946-1986. S.C.A. Lovrin. Redacţia de Propagandă Tehnică Agricolă: 35-50.

Petcu, E., Hagima, I., Zglimbea, R.G., 1997. *Effect of vernalisation genes on frost tolerance in some wheat cultivars.* Proceedings of the Int. Symp. on „Cereal adaptation to low temperature stress in controlled environments”, 2-4 June, Martonvasar, Hungary: 121-127.

Saulescu, N.N. and Ittu, G., 1989. *Reacţia unor soiuri româneşti de grâu la vernalizare şi fotoperioadă (The effect of semidwarfing genes and vernalization requirement genes on the response to late emergence in winter wheat).* Probl. Genet. Teor. Aplic., 21(3): 111-121.

Snape, J., Butterworth, K., Whitechurch, E., Worland, A.J., 2001. *Waiting for fine times: Genetics of flowering time.* In: Wheat in a Global Environment. Proceedings of the 6th International Wheat Conference, 5-9 June 2000, Budapest, Hungary (Editors Bedö Z. and Láng L.): 67-74.

Stelmakh, A.F., 1990. *Geographic distribution of Vrn-genes in landraces and improved varieties of spring bread wheat.* Euphytica, 45:113-118.

Stelmakh, A.F., Musicz, V.N., Avsenin, V.I., 1997. *Effect of Vrn and Ppd genes on frost resistance in bread wheat.* Proceedings of the Int. Symp. on „Cereal adaptation to low temperature stress in controlled environments”, 2-4 June, Martonvasar, Hungary: 32.

Syukov, V.V., 2003. *Geneticheskie aspekty selektsii yarovoi myagkoi pshenitsy v Srednem Povolzhe.* Avtoref. diss. d-ra biol. nauk. Saratov. 52 pp. (cited at http://genbank.vurv.cz/wheat/)

van Beem, J., Mohler, V., Lukman, R, van Ginkel, M., William, M., Crossa, J., Worland, A.J., 2005. *Analysis of genetic factors influencing the developmental rate of globally important CIMMYT wheat cultivars.* Crop Science, 45 (5): 2113-2119.

Worland, A., 1996. *The influence of flowering time genes on environmental adaptability in European wheats.* Euphytica, 89: 49-57.

Zhmurko, V.V., Avksentyeva, O.A., Gerashchenko, O.U., and Stelmakh, A.F., 2004. *Manifestation of Vrn gene effects in isogenic winter wheat lines.* Annual Wheat Newsletter, 50: 176-178.