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Identification and effects of alleles of gene Vrd2 according to economically valuable attributes of wheat in conditions of South Steppe of Ukraine

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The purpose. To identify recombinant-and-inbred lines of combination of crossing Orenburgskaya 48//Cappelle Desprez/2B Chinese Spring and to assess effects of alleles of gene Vrd2 on some economically valuable attributes. **Methods.** Temporal vernalization, genetic research, biological statistics. **Results.** Genotypes are identified of parent ingredients and recombinant-and-inbred lines of wheat Orenburgskaya 48//Cappelle Desprez/2B Chinese Spring for allelic state of gene *Vrd2.* Cappelle Desprez/2B Chinese Spring and 43 lines are the carrying agents of dominant allele of gene *Vrd2*, while grade Orenburgskaya 48 and 18 lines — of recessive *vrd2*. Effect of alleles of gene *Vrd2* on separate economically valuable attributes is evaluated. **Conclusions.** In comparison with lines-carriers of recessive allele *vrd2*, presence of dominant allele Vrd2 results into decrease of winter- and frost resistance, cutting time to heading increase of weight of grains for an ear and grain yield.

Key words: *wheat, alleles of gene Vrd2, vernalization, genotype, heading, yield, recombinant-and-inbred lines.*

The requirement for a low temperature (vernalization) is one of the most important adaptive mechanisms that enables winter crops of mild climate to overwinter due to the delayed development at the vegetative stage in autumn and to form a yield under favorable environment of the spring and summer period of vegetation. A more durable need for vernalization causes slower development at the initial stages and the transition to the formation of a differentiated growing point and the rudiments of reproductive organs in these genotypes is observed much later [1]. The durable vernalization requirement before the transition to reproductive development determines both the level of plant resistance to negative temperatures [2] and the duration of the period before the decrease in this hardiness [3].

The winter bread wheat cultivars revealed significant differences in the duration of vernalization requirement from 15 to 60 or more days [4, 5, 6]. The cultivars of a particular region are characterized by a certain duration of vernalization requirement, which indicates the adaptive value of similar genotypes for certain growing environment. The modern cultivars of winter wheat in the Southern steppe of Ukraine (V-VII periodsof cultivar changing SGI-NCSCI, Odessa) are characterized by 30-40 days vernalization requirements [7], in contrast the old cultivars II-IV ones of a given region of cultivar Odes’ka 16 and Myronivska 808, which required vernalization near 50-60 days for the transition to generative development. This has become a result of widespread use during the 50s-60s years of the last century in the winter wheat breeding in the South of Ukraine of the semi-dwarf cultivars from Mexico, the USA, India, which are the sources of a number of useful traits that have a positive effect on the rate and intensity of biological and actual yield accumulation [8].

The tendency to reduce the vernalization requirement is also observed even in cultivars of the central part of the Ukrainian forest-steppe [9]. Among the cultivars of MIW named after V.M. Remeslo for already 76% stocks 30-40 days vernalization is enough for transition to generative development. And only 24% ones head

after 50-60 days vernalization. However, in the Czech Republic after 2000, a significant shift was observed in the direction of cultivars breeding with a more durable vernalization. [10]

Differences in the duration of vernalization requirement affect the heading date, drought tolerance, winter hardiness, frost resistance, the weigth of grains per head and yield [11, 12, 13, 14]. In the south of Ukraine, the reduced vernalization requirement contributes to a reliable increase in grain yield, but leads to a decrease in winter hardiness and frost resistance [15].

In winter bread wheat three genes were found: *Vrd1*, *Vrd2* and *Vrd3* with varying expressiveness controlling the differences in the duration of vernalization requirement [16]. The *Vrd1* gene is located on chromosome 4A, the gene *Vrd2* — on 5D, and *Vrd3* — on one of chromosomes 1A or 6A or 4B. [17]. The triple recessive *Vrd* genotype is characterized by the duration of vernalization requirement from 55 to more than 60 days, the presence of the dominant *Vrd1* gene reduces it to 25-35 days, and *Vrd2* or *Vrd3* to 35-45 days [18]. The use of the dominant *Vrd2* allele was recommended for the creation of new frost resistant cultivars for the Southern steppe of Ukraine [19], since *vrd1Vrd2vrd3* the genotype has higher winter hardiness and frost resistant and don't differ significantly by yield from the most common at that time in production *Vrd1vrd2vrd3* and *Vrd1Vrd2vrd3* genotypes [20].

Aim of the research is to identify the recombinant inbred lines of the Orenburgskaya 48 / Cappelle Desprez / 2B Chinese Spring cross and evaluate the effects of the *Vrd2* gene alleles on some economically valuable traits.

Research methods. The research objects are 64 recombinant-inbred lines (RIL) F_{∞} from the crossing of the cultivar Orenburgskaya 48 to substituted in 2B chromosome Capelle Desprez / 2B Chinese Spring line. The cultivar Orenburgskaya 48 and the Capelle Desprez / 2B Chinese Spring line differed in the field environment in terms of plant height, heading date, the number of spikel ets in the main head, glume hairing and others [20]. Of the more than 190 RILs created by the SSD (single seed descent), 64 lines were selected for which all possible combinations of these differences between parental stoks were presented.

The genetic analysis by the genes *Vrd* of cultivar Orenburgskaya 48 and the Capelle Desprez / 2B Chinese Spring line was carried out according to the methodology developed in the SGI-NCSCI [16]. The testers were near isogenic lines of Erythrospermum 604-*Vrd1*, Erythrospermum 604-*Vrd2*, and Erythrospermum 604 and Mironivs'ka 808 cultivars (both *vrd1 vrd2*). To identify the *Vrd* genotypes of 64 RILs, the 5-days seedlings of each line were vernalized during 40 and 30 days in a CST-1 chamber at 2°C and illuminated 12 hours at 3000 lux, but the cultivar Orenburgskaya 48 and the Capelle-Desprez / 2B Chinese Spring line did during 50 days additionally. After vernalization the seed lings were planted on April 26 in 5-liter vessels ten plants per each and were grown in a vegetation plots area under the environmet of a natural day length (from 14 hours to 20 minutes to 15 hours 46 minutes) day. To determine duration of the period to heading (DPH) heading dates of individual plants were recorded.

To evaluate the effects of *Vrd2* gene alleles seeds of RILs and parental stocks were sown in the fall of 2011-2013 (on October 22, 12 and 4, respectively) in plot areas of $3m^2$ 500 grains per $1m^2$ in the experimental field of the department of general and molecular genetics of the Institute. The experiment was repeated for three times. The seedlings frost resistance was estimated at -12 ºC [22], and the vegetative phase did at -13 ... -14ºC [23]. For the latter case, in the second decade of January or in the first decade of March (depending on the presence of snow cover), 75-90 plants of each genotype were taken from the field (25-30 plants per each repetition). Winter hardiness was determined in the field by counting the number of plants at the end of October and those overwintered in the spring. During vegetation, heading date was recorded in the presence of 75% headed plants on the plot, which was transformed (from May 1) to duration of the period to heading (DPH). After harvesting in 30 plants of each line (10 per repetitions) the height of plants (HP), productive tillering (PT), namber of grains per head (NGH) and weight of grains per head (WGP), weight of 1000 grains (WTG), weight of grain and straw of the plant were registered to calculate harvest index (HI), as well as the number of productive tillers per unit area (NPT) and grain yield (GY).

Meteorological parameters during the research period included the whole range of possible adverse environmental factors that are common in the Ukrainian Steppe. This allowed to evaluate objectively the source material for the average adaptivity under those environment and also made it possible to differentiate on al-

ternative alleles of the *Vrd2* gene in parental stocks and RILs of winter bread wheat for a set of economically valuable traits.

Statistical data processing was carried out using generally accepted methods for calculating mean, t and χ^2 criteria and variance analysis [24].

Results of research and discussion. The parental cultivar Orenburgskaya 48 and the substituted on 2B chromosome Capelle Desprez / 2B Chinese Spring line differ in the duration of vernalization requirement (Fig. 1). For plants of the Capelle Desprez / 2B Chinese Spring line there is inherent a 30-days vernalization requirement. The heading of this line plants after given duration of vernalization was observed at 49.0 and 48.2 days in 2010 and 2011, respectively. A further increase in the duration of the previous vernalization to 40 or 50 days did not lead to a significant reduction of the period to heading in the Capelle Desprez / 2B Chinese Spring line. On the contrary plants of the cultivar Orenburgskaya 48, required a vernalization not less than 50 days for a generative development. Plants of this cultivar have headed after 50-days vernalization on 59,6 and 69,2 days in 2010 and 2011, respectively. With the vernalization duration of 40 and 30 days plants of the cultivar Orenburgskaya 48 were in the tillering phase up to the end of the experiment (97-98 days). Only in 2010 after 40-days vernalization we observed heading in six from ten plants at 62-88 days.

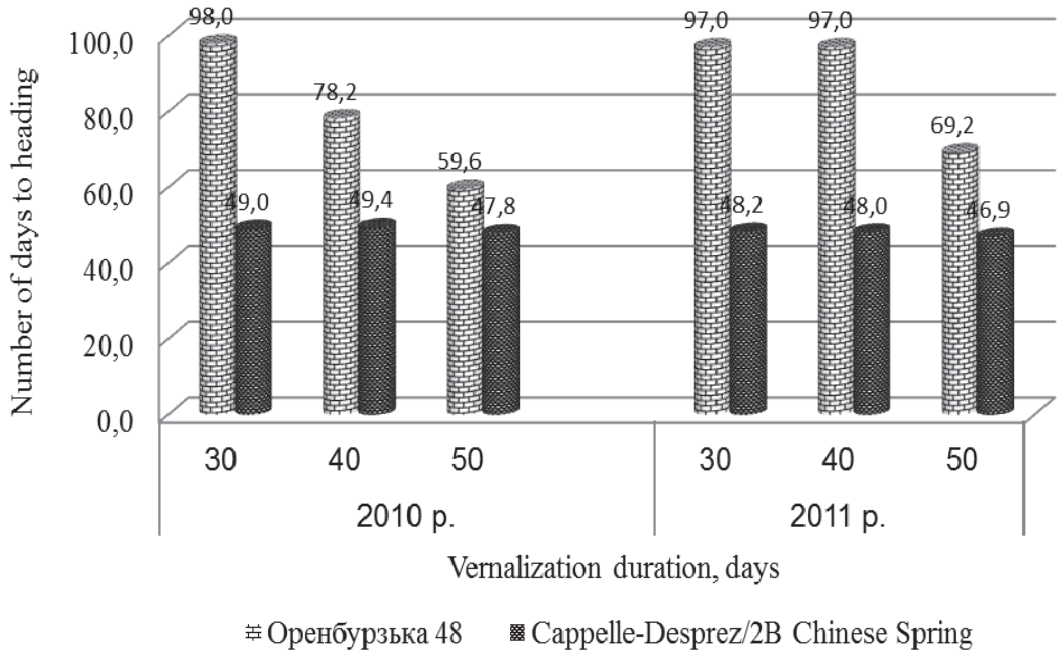

Fig.1. Days to heading in plants of Orenburgskaya 48 and Cappelle Desprez / 2B Chinese Spring after vernalization of various duration in 2010 i 2011 years

In 2012 among F_1 plants of the population got from cross of cultivar Orenburgskaya 48 to the Cappelle Desprez / 2B Chinese Spring line, 53 headed plants and 20 non-headed plants were detected after 40 days of vernalization. This relationship corresponded to the theoretically with the theoretically expected 54,75 : 18,25, showing the differences in the alleles of one gene (3 : 1). The criterion for compliance $\chi^2_{3:1}$ is 0,77. In 2016, a segregation in F_1 for this combination also consisted of 56 headed plants to 13 non-headed plants and corresponded to monogenic one ($\chi^2_{3:1}$ = 1,40) suggesting a monogenic difference between parental stocks.

According to the results of the genetic analysis, it can be concluded that the Capelle Desprez / 2B Chinese Spring line is the carrier of the dominant *Vrd2* gene (Table 1). This conclusion was based on the presence of monogenic differences in segregation on headed and non-headed plants after 40-days vernalization in the population F_1 from cross the indicated line to the recessive by *vrd* genes tester. At the same time there was an absence of segregation (all plants were headed) in cross the Capelle Desprez / 2B Chinese Spring

line to the dominant by *Vrd2* gene tester and the presence of digenic differences in crosses to dominant by *Vrd1* or *Vrd3* genes tester.

1. Segregations to the headed and non-headed plants in F_2 populations from crosses of the cultivar Orenburgskaya 48 and the Capelle Desprez / 2B Chinese Spring line to the *Vrd* gene testers after 40-day vernalization

Stocks	Year	Testers			
		Recessive	*Vrd1*	*Vrd2*	*Vrd3*
Capelle Desprez / 2B Chinese Spring	2012	63:12*	59:7**	77:0	66:4**
	2013	56:12*	69:8**	73:0	71:4**
	2015	67:15*	72:8**	77:0	72:3**
	2016	65:13*	88:3**	-	-
Orenburgskaya 48	2012	0:36	24:38	52:13*	39:30
	2013	0:54	43:11*	65:20*	49:17*
	2016	-	60:19*	49:18*	52:28

Note. *$\chi^2_{3:1} \leq 3{,}84$; **$\chi^2_{15:1} < 3{,}84$

Unlike the Capelle Desprez / 2B Chinese Spring line in the cross of the cultivar Orenburgskaya 48 to the recessive by *Vrd* genes tester, the segregation was not revealed after 40-day vernalization. All plants were in the tillering phase. However, in F_2 populations when crossed the specified cultivar to monogenically dominant *Vrd1* or *Vrd2* or *Vrd3* genes testers we revealed segregations into headed or non-headed plants, though which was not always coincided to the theoretical expectation for the digenic differences of parents. Nevertheless, the very fact of the segregation on plants that headed or did not head after 40-days vernalization in our opinion, can testify to the presence of genetic differences of the cultivar Orenburgskaya 48 and testers of the gene *Vrd1* in 2012 or *Vrd3* in 2012 and 2016. Thus, the Orenburgskaya 48 has a recessive genotype by the *Vrd* genes.

Since the differences in vernalization requirement between the cultivar Orenburgskaya 48 (50 days) and the Capelle Desprez / 2B Chinese Spring line (30 days) are only due to allelic differences in the *Vrd2* gene (*vrd2* or *Vrd2*, respectively), and the use of 40- and 30-days vernalization can identify the genotypes of recombinant-inbred lines for the *Vrd2* gene. The presence of the dominant allele of the *Vrd2* gene in the genotype of the lines will contribute to early heading (reduction of period to heading), and the allele *vrd2*, on the contrary, will hold back the development that phenotypically is manifested in increasing the duration of the period to heading in lines with this allele [20]. RILs significantly differed in the duration of the period to heading (Table 2). Thus, the scope of the variation of the period to the RILs heading was from 47,2 (line 58) up to 88,7 days (line 139) at the 40-days vernalization and 57,6-91,5 days after vernalization of 30 days, respectively, lines 58 and 177. In both variants of the vernalization duration of, non-headed lines were observed. As the criterion for the attribution of the RIL to carriers of the dominant or recessive allele of the *Vrd2* gene was the use of the heading date (62 days) at the 40-day vernalization of the first plant of the cultivar Orenburgskaya 48 carrier of the recessive allele *vrd2*. Therefore, lines 58, 50, 10, 96, 44, 60, 17, 33, 23, 45, 131, 105, 24, 92, 28, 148, 71, 97, 59, 74, 57, 164, 117, 98, 165, 121, 80, 112, 166, 151, 89, 146, 90, 185, 177, as well as the Cappelle Desprez / 2B Chinese Spring line after 30- and 40-day vernalization, may be characterized as carriers of the *Vrd2* gene. At the same time, in the lines that did not head at 30-day but they headed at 40-day vernalization for 51.8-57.2 days (lines 94, 13, 39, 40, 29, 56, 5, 14) were also attributed to the carriers of the dominant *Vrd2* gene.

2. Genotypes by the alleles of the *Vrd 2* gene and the duration of period to heading (DPH) of the recombinant inbred lines Orenburgskaya 48 // Capalle Desprez / 2B Chinese Spring after 30- and 40-days vernalizations, days

Line	Genotype	40 days	30 days	Line	Genotype	40 days	30 days	Line	Genotype	40 days	30 days
58	*Vrd 2*	47,2±0,80	57,6±2,16	164	*Vrd 2*	60,8±1,24	65,5±2,25	36	*vrd2*	59,8±2,43	n
50	*Vrd 2*	48,2±0,20	54,3±1,11	117	*Vrd 2*	61,2±0,80	84,5±7,80	111	*vrd2*	61,0±1,58	n
10	*Vrd 2*	49,0±1,26	52,0±0,89	98	*Vrd 2*	62,0±0,95	81,4±6,96	77	*vrd2*	62,0±5,10	n
96	*Vrd 2*	49,2±1,20	55,8±2,13	165	*Vrd 2*	62,0±1,14	84,2±5,64	109	*vrd2*	62,8±3,20	n
CD*	*Vrd 2*	49,4±0,51	49,0±1,76	121	*Vrd 2*	64,0±1,61	75,2±1,96	65	*vrd2*	63,4±2,62	n
44	*Vrd 2*	50,2±1,39	58,6±1,72	80	*Vrd 2*	64,6±2,62	71,6±0,98	124	*vrd2*	63,5±2,40	n
60	*Vrd 2*	51,6±1,54	58,6±2,16	112	*Vrd 2*	64,8±3,66	91,8±6,20	133	*vrd2*	65,7±1,90	n
17	*Vrd 2*	53,6±1,17	82,3±10,27	166	*Vrd 2*	65,6±1,25	74,5±1,50	35	*vrd2*	66,0±1,05	n
33	*Vrd 2*	53,8±0,20	72,6±6,76	151	*Vrd 2*	65,7±0,67	73,3±0,85	118	*vrd2*	66,0±0,58	n
23	*Vrd 2*	54,0±0,63	63,0±2,08	89	*Vrd 2*	66,0±1,58	84,8±8,14	143	*vrd2*	66,0±1,00	n
45	*Vrd 2*	54,8±2,84	65,8±8,07	146	*Vrd 2*	67,0±1,00	82,8±8,83	38	*vrd2*	68,0±0,70	n
131	*Vrd 2*	54,8±0,86	67,2±8,12	90	*Vrd 2*	67,7±1,33	93,6±4,40	140	*vrd2*	69,3±1,31	n
105	*Vrd 2*	55,0±0,89	58,0±0,91	185	*Vrd 2*	68,0±0,45	86,5±6,64	108	*vrd2*	70,0±3,03	n
24	*Vrd 2*	55,4±0,98	60,2±0,97	177	*Vrd 2*	72,5±5,50	91,5±6,50	122	*vrd2*	75,5±7,50	n
92	*Vrd 2*	55,8±1,80	75,8±7,07	94	*Vrd 2*	51,8±0,58	n**	Or.*	*vrd2*	78,2±8,14	n
28	*Vrd 2*	56,6±1,29	61,0±0,89	13	*Vrd 2*	52,0±0,77	n	139	*vrd2*	88,7±9,33	n
148	*Vrd 2*	57,0±1,10	66,2±2,22	39	*Vrd 2*	54,5±1,19	n	152	*vrd2*	n	n
71	*Vrd 2*	57,0±0,58	92,2±5,80	40	*Vrd 2*	56,0±0,95	n	153	*vrd2*	n	n
97	*Vrd 2*	57,2±0,49	67,0±2,10	29	*Vrd 2*	56,2±1,32	n	161	*vrd2*	n	n
59	*Vrd 2*	58,0±1,22	82,2±6,46	56	*Vrd 2*	56,8±1,55	n	136	*vrd2/Vrd2*	73,4±10,2	n
74	*Vrd 2*	58,4±1,94	76,5±3,50	5	*Vrd 2*	57,0±2,26	n	31	*vrd2/Vrd2*	78,6±8,4	n
57	*Vrd 2*	59,0±0,41	73,0±8,38	14	*Vrd 2*	57,2±1,16	n	181	*vrd2/Vrd2*	90,5±7,5	n

Note. * CD - Cappelle Desprez / 2B Chinese Spring; Or - Orenburgskaya 48; ** n - plants in this variants did not headed

Other lines of this group are 36, 111, 77, 109, 65, 124, 133, 35, 118, 143, 38, 140, 108, 122, 139, 152, 153, 161, which headed on 59.8-88 ,7 or which did not head at all, can be described as carriers of the recessive allele *vrd2.*

Most plants of the studied lines headed within three days interval. At the same time, the variation of duration the period to heading of individual plants of the lines 181, 31, and 136 amounted from 30 to 39 days. On that basis, these 3 lines were characterized as possible populations for the both alleles of the *Vrd2* gene (*Vrd 2* / *vrd2*). Thus, the identified *Vrd* genotypes 64 RILs F_9 of cross Orenburgskaya 48 / Cappelle Desprez / 2B Chinese Spring: 43 lines are carriers of dominant *Vrd*2, 18 lines — *vrd2*, and 3 lines — the possible populations by alleles of the given gene.

The variance analysis results according to various economically valuable traits in the studied set (wich we evaluated in 2011-2013) indicated with high probability a significant role (Table 3) of genetic differences between the sub-sets of the carriesr of the alleles *Vrd2* or *vrd2* for the four traits: winter hardiness ($P < 0,01$), duration the period to heading, the weight of grain per head, grain yield (all at $P < 0,05$).

3. Results of two-factor (year and genotype) variance analysis of the traits differences in studied RILs Orenburgskaya 48 // Cappelle Desprez / 2B Chinese Spring for the alleles of the gene *Vrd2*, 2011-2013.

Trait	Year	Genotype	Interaction	Random factors
df	2	1	2	180
Wint.	4,5***	1,4**	0,4	0,2
DPH	1664,8***	26,6*	0,7	5,1
HP	18326***	7	59	74
PT	0,644***	0,001	0,127	0,091
NGH	2370,4***	33,4	3,9	10,4
WGH	5,415***	0,090*	0,037	0,019
WTG	381,9***	11,5	7,3	8,8
NPT	664887***	293	3485	3288
HI	1,33***	0,02	0,01	0,01
GY	0,026***	0,015*	0,001	0,003

Notes: * - Reliable at $P \leq 0,05$; ** - at $P \leq 0,01$; *** - at $P \leq 0,001$.

The presence in the genotype of RIL of the dominant allele *Vrd2* significantly reduced winter hardiness by 8%, shortened the duration the period to heading by 0,9 days, increased the weight of head grain by 0,049 g comparing to the lines carrying the recessive allele *vrd2* (Table 4). The grain yield of RIL-carriers of the *Vrd2* allele was 0,336 kg / m^2 and exceeded on 0,020 kg / m^2 of the carriers of *vrd2* (0,316 kg / m^2). By other traits differences of the RILs subsets were not significant, although there was some tendency to increase the number of grains per head, the weight of thousand grains, and the harvest index in lines with the dominant allele *Vrd2*.

4. Average values of economically valuable traits in groups of Orenburgskaya 48 // Cappelle Desprez / 2B Chinese Spring RILs differed by alleles of the *Vrd2* gene

Trait		*Vrd2*	*vrd2*	$F_{ф}$	$HIP_{0,05}$
Winter hardiness %		76,0	84,0	6,20	1,2
Frost resistance %	January 2011, -14°C	28,3	39,0	1,11	
	January 2013, -14°C	54,6	70,3	1,51	
	March 2011, -13	43,2	56,7	3,16	
	March 2012, -13°C	80,8	90,5	5,97	1,8
	Sidlings 2012, -12°C	13,4	21,2	1,74	
	Sidlings 2013, -12°C	9,1	14,1	2,47	
DPH, days		15,6	16,5	5,20	0,7
HP, cm		109	109	0,10	
PT, t.		1,7	1,7	0,02	
NGH, g.		26,7	25,8	3,20	
WGH, g		0,940	0,891	4,72	0,043
WTG, g		32,7	32,2	1,30	
NPT, p./m^2		432	429	0,09	
HI		0,50	0,47	2,66	
GY, kg/m^2		0,336	0,316	4,94	0,015

Note: $F_{ф}$= 3.84 at $P \leq 0.05$

Artificial freezing in the phase of sprouting and tillering did not reveal the benefits of one or another allele of the *Vrd2* gene in cold resistance, with the exception of freezing at -13° C in early March 2012, when lines of *vrd2* genotype were higher frost resistant than such of *Vrd2* genotype.

Conclusions

Genotypes of 64 RILs F9 Orenburgskaya 48 // Cappelle Desprez / 2B Chinese Spring were identified, as carriers of dominant or recessive alleles on *Vrd2* gene. The presence of the dominant *Vrd2* allele in the RILs genotypes led to a decrease in winter hardiness, frost resistance, to shortening of the period to heading and to an increase in the weight of grain per head and grain yield compared with the lines-carryings of the recessive allele *vrd2*.

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