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THE USE OF *Vrn* GENES FOR CREATION OF TRITICALE FORMS WITH DIFFERENT LENGTH OF VEGETATION PERIOD

(review)

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Abstract

The advantages and disadvantages of triticale culture are briefly reviewed. The control of the length of vegetative period of spring triticale forms and spontaneous spring triticale mutants is viewed more particularly. Triticale (× *Triticosecale* Wittmack) is a new agricultural culture that combines valuable traits of wheat and rye. The advantages of triticale are its ability to grow on poor, acid, waterlogged soils; higher, than in wheat, content of protein in grains; its resistance to many fungus diseases. The disadvantages are undersized grains, its tendency to sprouting, lodging, a partial toxicity of grains due to the presence of alkylresorcinols, and a longer, compared to parental forms, vegetation period. The biggest influence on the length of the vegetation period of cereals have vernalization response genes — *Vrn*. Spring plants have one or more dominant *Vrn* genes, in winter plants all *vrn* genes are recessive. Common wheat carries genes *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, located on the chromosomes 5AL, 5BL, 5DL respectively (A.J. Worland, 1996), *Vrn-D4* gene, located on the centromeric region of chromosome 5D (N. Kippes et al., 2015) and *Vrn-B3* gene on the chromosome 7BS (L. Yan et al., 2006). Rye has *Vrn-R1* gene on the chromosome 5RL (J. Plaschke et al., 1993). In triticale there were detected *Vrn-A1a*, *Vrn-B1a*, *Vrn-B1b* and *Vrn-B1c* alleles (M. Nowak et al., 2014; O.I. Zaitseva et al., 2015). The same alleles were detected previously in common wheat (D.K. Santra et al., 2009; A.B. Shcherban et al., 2012, 2015; J. Milec et al., 2013; I.E. Likhenko et al., 2014). Heading time of plants can be influenced not only by an alteration of nucleotide sequence of *Vrn* genes, but also by a change of the copy number of these genes (A. Diaz et al., 2012). *Vrn* genes can influence heading time in the combination with each other. For example, cultivars with three dominant *Vrn* genes are ripening earlier, than cultivars with one or two dominant *Vrn* genes, but they have the least productivity (A.F. Stelmakh, 1993; M. Iqbal et al., 2007). It was also reported, that introgression of chromosome 2D shortened the period of triticale vegetation (A.A. Shishkina, 2008; L.V. Koren et al., 2010). Triticales have more prolonged vegetation period compared to parental wheat lines, which can be due to the inhibition of the *Vrn* genes by rye genome (L.N. Kaminskaya et al., 2005; I.N. Leonova et al., 2005). The genetic control of growth habit of spontaneous spring mutants is currently unknown. It was determined, that the majority of spring mutants are late ripening, and, after autumn sowing, they survive in different extent, what can mean, that they are facultative (P.I. Stepochkin, 2008; P.I. Stepochkin et al., 2008). In Siberia winter triticales occupy considerable areas, but the breeding of spring triticales hasn't been carried on yet. Spring triticales could increase biodiversity of spring cultures. Thereby creation of spring triticales with different length of vegetative period is of great breeding interest.

Keywords: hexaploid and octaploid triticale, spontaneous spring mutant, wheat, length of vegetative period, *Vrn* genes

Triticale (× *Triticosecale* Wittmack), or a wheat-rye amphiploid (WRA),

is an artificially created culture derived from wheat (*Triticum* spp.) and rye (*Secale* spp.) crossing. Octaploid, hexaploid and tetraploid triticale are distinguished. Octaploid triticale ($2n$ = 56, $A_1A_1B_1B_1$DDRR genome with A_1, B_1 of soft wheat genome, and A, B of durum wheat genome) are obtained by crossing 42-chromosome wheat (mainly a soft wheat *T. aestivum* L.) with rye (mainly *S. cereale* L.) followed by the chromosome set doubling. These WRAs are cytologically unstable and generate aneuploids at a high frequency. Over a number of generations, they lose chromosomes until reaching a stable hexaploid level [1, 2]. Because of a reduced grain number per spike, octaploid (×8) triticale are not used in commercial plant growing, These WRAs should be cytologically monitored, and the typical plants should be selected to maintain the ×8 number of chromosomes [3].

Hexaploid (×6) triticale ($2n$ = 42, AABBRR genome) derive from crossing 28-chromosome wheat (mainly *T. durum* Desf.) with rye and subsequent doubling of the chromosome number. These forms are more cytologically stable than ×8 triticale.

Hexaploid and octaploid triticale are primary forms created by doubling the chromosome number of F_1 hybrids between hexaploid or tetraploid wheat and rye. However, most varieties are secondary hexaploid triticale developed by crossing ×8 triticale with ×6 triticale or ×6 triticale with wheat. In the second generation, due to the peculiarities of the A and B genomes of soft and hard wheat, some genotypes appear with a higher productivity and a significantly lower frequency of meiotic disturbances [4].

Tetraploid (×4) triticale ($2n$ = 28) was first obtained by pollination of wheat-rye F_1 hybrids with rye pollen. Among F_2 hybrids, a plant was found with 28 chromosomes, 14 from wheat and 14 from rye [5]. The yield of ×4 triticale is very low. However, the ×4 triticale plants are more cytologically stable than ×8 and ×6 ones; therefore, the fertility of ×4 triticale may be increased by selection methods [5].

This review focuses on the diversity of genes and alleles encoding development processes in triticale, wheat and rye to involve these gene pool in breeding varieties for regions with different lengths of the growing season.

Triticale possesses a number of advantages. Although wheat was crossed with rye mainly in order to give it winter hardiness of rye, triticale on this basis, as a rule, does not differ from winter wheat. As assumed, this is due to the suppression of the rye chromosome activity by wheat cytoplasm [6]. According to some reports, ×8 triticale is more winter-hardy than ×6 triticale [7], however, a decrease in ploidy from ×8 to ×6 led to an increase in frost resistance [8]. Comparing triticale with the parental wheat lines showed more winter-hardiness due to the presence of rye chromosomes [9]. Molecular markers revealed three loci responsible for winter hardiness on chromosomes 5A, 1B, and 5R of hexaploid triticale [10].

The triticale, like rye, is superior to wheat in the ability to grow on acidic, infertile, flooded soils. Triticale is more resistant to powdery mildew, yellow rust and smut. However, triticale, like rye, is affected by brown rust to which ×8 plants are more susceptible than ×6 plants, and by stem rust, ergot, root rot and snow mold [7].

Another valuable trait of triticale compared to wheat is a higher protein content in grain. However, triticale flour is inferior to wheat flour for bread baking because of low quality of gluten, and, therefore, it is used as a 30-50% mixture with wheat flour. This improves elasticity and increases the bread volume [11]. Bread with triticale flour surpasses wheat bread and rye bread in nutritive

value, and also has a characteristic sweetish taste. Pasta, cookies, biscuits, crackers, diet bread made from triticale flour are gluten-free dietary foods for people suffering from metabolic disorders. A way to improve baking qualities of triticale is the replacement of the rye chromosome 1R with the homeologous chromosome 1D of wheat [12]. Substitution of rye chromosome 1R with 1U *Aegilops umbellulata* chromosome 1U is also of potential interest for improving baking qualities [13].

Grain of triticale is used in brewing and alcohol manufacturing. The yield of alcohol in this case is 3-5 % higher than for other cereals [14]. Triticale is a successful forage and feed crop. Triticale grain is a high-protein feed with better digestibility than that of wheat and barley. Cattle prefer green mass of triticale as compared to wheat and rye due to higher content of sugars and carotenoids. Feeding triticale green mass increases milk yield, milk fat content, and animal weight gain [7].

However, triticale also has some disadvantages. Primary triticale exhibits reduced grain number per spike resulting from disturbances in meiosis, which lead to the emergence of aneuploid plants and, consequently, a decreased productivity [15]. The presence of only one rye chromosome in the wheat genome causes structural changes in the karyotype [16]. The problem of low grain number is solved by selection.

Triticale has a shrunk, poorly filled grain due to violated accumulation of fine-grained starch, as well as premature release of α-amylase which decomposes starch [17]. Grain protein content inversely correlates with grain filling, thence selection for grain filling leads to a decrease in its protein content, and vice versa [7]. The activity of α-amylase determines one more drawback of triticale, i.e. the tendency of grain to germinate prior to harvesting, which reduces grain quality and yield. Presence of chromosome substitution 2R/2D increases the resistance to pre-harvest sprouting [18].

Most triticale varieties are long-stemmed and prone to lodging. Plants more than 91 cm in height have a predisposition to lodging [19]. Plants with 2R/2D substitution are shorter than other plants [20, 21]. The replacement of the 2R rye chromosome with the 2U *Aegilops umbellulata* chromosome led to a decrease in height of triticale hybrids [13].

Another disadvantage of triticale is grain toxicity caused by antimetabolites alkylresorcinols. As per the content of these substances, triticale occupies an intermediate position between wheat and rye. Feed containing more than 50% of triticale grain inhibit animal weight gain, and may cause diseases of liver and gastric mucosa. The amount of alkylresorcinols decreases during grain processing and the preparation of feed mixtures [5].

Triticale has a longer vegetation period than the parent forms. It is known that the growing season of polyploids increases with an increase in ploidy [5]. Moreover, due to the hybrid origin, many biological processes in triticale proceed more slowly than in wheat [7]. The period from heading to flowering in this crop is several days longer than in wheat [5]. The phase of dough-like ripeness in triticale is long and can last up to 3 weeks [5]. Triticale grain ripens 3-20 days later than wheat grain [7, 8]. The ×6 triticale occupies an intermediate position between soft wheat and ×8 triticale as to the period before heading [3, 22], and F_1 hybrids of wheat and rye, on the contrary, develop more rapidly than wheat plants [5]. Winter rye has a higher rate of growth and apex development than wheat, and triticale is closer to rye than to wheat on this trait [23].

Wheat, rye, and triticale forms can be of spring, winter, and alternate type of development [24]. Winter forms need a long (1-3 months) exposure to low

positive temperatures for the transition to generative development (vernalization); spring crops are able to go to earing without it. Alternates can develop both in spring and in winter mode. The time of the onset of generative development and the time of heading are important adaptive traits. The type of development, as well as the duration of the growing season, is controlled by the *Vrn* genes (response to vernalization).

In common wheat, *Vrn-A1*, *Vrn-B1*, *Vrn-D1* genes are located on 5AL, 5BL and 5DL chromosomes, respectively [25, 26)], *Vrn-D4* is in the near-centromere region of the 5D chromosome [27, 28], *Vrn-B3* is on chromosome 7BS (29). The rye gene *Vrn-R1* is located on 5RL chromosome [30]. The spring type of development is controlled by one or several dominant *Vrn* genes, the winter type is controlled by recessive *vrn* genes in all these loci [31]. Alternates can carry dominant genes *Vrn-B1* [32], *Vrn-D1*, *Vrn-D4* [33-36] or a "weak" allele of the dominant *Vrn-A1* gene [37].

On the example of substituted and isogenic wheat lines, it was shown that the dominant *Vrn-A1* gene is epistatic with respect to other *Vrn* genes and determines the absence of a response to vernalization, while plants with the *Vrn-B1*, *Vrn-D1*, *Vrn-D4*, and *Vrn-B3* genes respond to varying degrees to vernalization by acceleration of heading [31, 38, 39]. According to the influence on the heading time, *Vrn* genes can be arranged as following: *Vrn-A1* > *Vrn-D1* > *Vrn-D4* > *Vrn-B1*, where the plants with the dominant *Vrn-A1* gene are the earliest, and with the dominant *Vrn-B1* gene are the latest [40, 41]. Among Chinese wheat varieties, plants with the dominant *Vrn-D1* gene, on the contrary, mature later than plants with *Vrn-B1*, which can be explained by the presence of different *Vrn-D1* alleles [34]. The dominant *Vrn-B3* gene in combination with other *Vrn* genes determines a very early heading [34].

Vrn loci are characterized by multiple allelism resulting from the differences in the structure of regulatory regions (the promoter or the first intron). To detect alleles of *Vrn* genes, primers for these regions have been designed. The *Vrn-A1* gene of common wheat has the alleles *Vrn-A1a* (insertion and duplication in the promoter region), *Vrn-A1b* (deletion of 20 bp in the promoter region) [42] and *Vrn-A1c* (deletion of 5504 bp in the first introne) [43]. Alleles *Vrn-A1d*, *Vrn-A1e* and *Vrn-A1f* were found in tetraploid wheat (deletions of 32, 54 and 50 bp in the promoter region, respectively) [42, 44]. In diploid wheat, there are *Vrn-Am1f*, *Vrn-Am1g*, and *Vrn-Am1h* alleles with deletions and/or insertions in the promoter region and/or in the first intron [45]. Hexaploid wheat *T. compactum* has a new variant of the dominant allele *Vrn-A1a*, characterized by the presence of a 16 bp deletion and four single-nucleotide polymorphisms (SNPs) in a mobile genetic element in the promoter region, as well as a new *Vrn-A1j* allele containing a 54 bp deletion in the promoter region [46]. In tetraploid wheats *T. turgidum* and *T. durum*, the *Vrn-A1i* allele with SNP was found in the sequence of the adenine (A) tract of the VRN-box of the *Vrn-A1* gene, presumably determining a reduced sensitivity to vernalization and a facultative type of development [46]. In wheats ×4 and ×6, five variants of the *Vrn-A1b* allele were identified, which differ in the polymorphism of the A-tract and C-enriched segment in the VRN-box sequence [46]. In *T. dicoccum*, the dominant allele *Vrn-A1k* with a 42 bp deletion was found in the promoter region; this allele is responsible for the decrease in the need for vernalization and the spring type of development [47].

The identified wheat *Vrn-B1* gene alleles are *Vrn-B1a* with 6850 bp deletion in the first intron [43], *Vrn-B1b* which differs from *Vrn-B1a* by an additional 36 bp deletion in the first intron [48], and *Vrn-B1c* which, in addition to the

deletion as in *Vrn-B1a*, carries in the first intron an 820 bp deletion and a 431 bp duplication shifted to the beginning of this deletion [49, 50]. In tetraploid wheats *T. turgidum* and *T. turanicum* Jakubz. the promoter regions of the dominant *Vrn-B1* gene have a 5463 bp and 127 bp deletions, respectively [44, 51]. In wheat ×4 *T. carthlicum*, the *Vrn-B1(ins)* allele was detected, which is characterized by the insertion of a retrotransposon in the promoter region [46]. In *Vrn-B1*, two variants were found, differing from the recessive allele *vrn-B1* in 7 bp, 3 bp, 2 bp deletions and 8 SNPs [46].

The *Vrn-D1a* allele of spring soft wheat has a 4235 bp deletion in the first intron [43]. The *Vrn-D1b* allele of alternates differs from *Vrn-D1a* in a single-nucleotide substitution in the promoter region [36]. In three common wheat varieties from China, the *Vrn-D1c* allele with a 174 bp insertion was detected in the promoter region [52]. *Aegilops tauschii* has *Vrn-D^{t}1* allele with a 5437 bp deletion in the first intron [53], *T. spelta* and *T. compactum* have *Vrn-D1s* with an 844 bp insertion in the first intron [54]. Five haplotypes of the *Vrn-D1* gene, Hap-7Tu and Hap-8T, differing in the length of T-tract at the –428 bp were found in five ×6 wheat samples [55]. The *Vrn-D4* gene appeared due to the insertion of a 290 kbp region of 5AL chromosome carrying the *Vrn-A1* gene into the short arm of the 5D chromosome [56].

The *Vrn-B3a* allele in the substituted Chinese Spring/Hope 7B line has a 5295 bp insertion in the promoter region [29], *Vrn-B3b* allele has an 890 bp insertion in the promoter of recessive gene *vrn-B3*, and *Vrn-B3c* has 20 bp and 4 bp deletions in the promoter of the dominant *Vrn-B3a* gene [57].

Allele-specific markers revealed in triticale the dominant alleles *Vrn-A1a*, *Vrn-B1a*, *Vrn-B1b*, and *Vrn-B1c* [58, 59], previously detected in common wheat [48, 60-63].

Not only a change in the nucleotide sequence of the *Vrn* genes, but also an increase in the number of copies was recently reported to affect earing of wheat. Thus, an increase in the number of copies of the *Vrn-A1* dominant allele to two and three caused a delay in flowering initiation compared to wild-type plants bearing one copy of *Vrn-A1* [64].

Dominant *Vrn* genes can affect the length of growing period in combination with each other. Varieties with two *Vrn* genes enter the earing phase earlier than varieties with one dominant gene, and varieties with three *Vrn* genes are the most early-season but the lowest in productivity [65, 66]. Triticale varieties with earlier earing show higher spike fertility and 1000-grain weight [67].

The dominant *Vrn* genes differ in their effect on plant growing period mainly due to the fact that they determine different durations of the second stage of organogenesis (tillering) [41, 68]. So, it is the smallest in early-ripening genotypes with one dominant *Vrn-A1* gene (*Vrn-A1 vrn-B1 vrn-D1*) and two dominant genes (*Vrn-A1 Vrn-B1 vrn-D1* и *Vrn-A1 vrn-B1 Vrn-D1*) and the largest in the late-ripening line with one dominant *Vrn-B1* gene [68]. Moreover, the shorter the period of vegetative development of plants, the longer the period from heading to ripening, and vice versa [41, 69]. Loci affecting the rate of plant development were found in triticale in different chromosomes [70].

Vrn genes are associated with wheat productivity traits indirectly through regulation of carbohydrate and nitrogen metabolism [71, 72].

L.V. Koren and L.V. Khotyleva note [73] that the ×6 triticale with introgression of 2D soft wheat chromosome ripens most early as compared to all lines they studied. Another study also reports that triticale lines with 2R/2D chromosome substitution have a significantly shorter vegetation period compared to lines with a complete set of chromosomes, and triticale lines with 2B/2D sub-

stitution and T:2RS.2RL-2BL translocation ripen later than forms with substitution 2R/2D [21].

Many researchers observed spontaneous appearance of spring plants among winter wheat, rye and triticale upon spring sowing [74, 75]. Genetic control of the type of development of spontaneous spring mutants is not currently known, but the cause of their occurrence is assumed to be mutations either in the promoter region or the first intron of the *Vrn* genes [42, 43], or an epigenetic change in chromatin state in these regions that does not affect the DNA sequence [76, 77]. In both cases, recessive *vrn* genes become dominant, resulting in induction of generative development [78]. The type of development of spontaneous spring mutants is determined by the heterozygous dominant gene, since the progeny from self-pollination of the mutants segregates into spring and winter forms [8]. In the F_2 from crossing between mutant spring rye plants segregation of spring and winter forms was close to 3: 1, which indicates a monogenic dominant control of springiness [8]. Two spring mutants of winter wheat variety Lutescens 105 differed in vegetation duration by almost a month. A genetic analysis of the F_2 generation of these mutants revealed spring to winter type segregation close to 15: 1. Therefore, the genes that determine the type of development of the studied mutants are in different loci [8].

The earliest spontaneous spring mutants of winter triticale eared in late July to early August, but most of the mutants were late ripening and eared in September [75]. In addition, all spontaneous spring mutants of wheat, rye, and triticale sown in autumn overwinter to varying degrees. Therefore, we can assume that they belong to the alternates [8].

The spring mutants occur more frequent with an increase in the shelf life of seeds, as well as with an increase in temperature in June and the amount of precipitation in July [75]. It is assumed that these stresses activate mobile elements leading to mutations in the *Vrn* genes [75]. The findings [79-81] also confirm the effect of physical and chemical mutagens on the duration of the growing season of spring plants.

The researchers from the Institute of Genetics and Cytology of Belarus reported on production of octaploid and hexaploid triticale lines with dominant *Vrn* genes [22]. The maternal forms were the isogenic lines of common wheat with dominant alleles *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, obtained on the basis of the varieties Triple Dirk, Mironovskaya 808 and Bezostaya 1, and a pollinator was the winter diploid rye variety Voskhod and spring alloplasmic rye [22]. In these lines, the inhibitory effect of the triticale genetic background on the expression of the dominant *Vrn* genes was revealed. The triticale plants eared later than the corresponding wheat lines. Moreover, the dominant *Vrn-A1* gene which determines early earing was suppressed to a greater extent than *Vrn-B1* and *Vrn-D1* [22, 82]. Later, octaploid triticale lines with dominant *Vrn* genes were created at the Siberian Institute for Plant Industry and Breeding, Siberian Branch RAS, by crossing Triple Dirk common wheat isogenic lines with *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-D4* genes with winter diploid rye Korotkostebelnaya 69. A sequential arrangement of dominant *Vrn* genes in these triticale lines, as per their influence on heading time, were the same as in wheat lines with these genes [3].

Thus, triticale is a promising crop with the ability to grow on poor soils and resistance to a number of fungal diseases, with higher protein content in grain than wheat, high alcohol yield and frost resistance. The *Vrn* genes have the greatest influence on the duration of the growing season of wheat, rye, and triticale. These genes to varying degrees affect the earing time and the vernalization effect. Numerous alleles of *Vrn* genes differ in mutations located in the promoter

region and/or the first intron, which increase the diversity of plants by the length of the growing period and response to vernalization. Allele-specific primers revealed in triticale the alleles *Vrn-A1a*, *Vrn-B1a*, *Vrn-B1b* and *Vrn-B1c* previously detected in common wheat. In addition, by varying the number of copies of *Vrn* genes or by combining different dominant *Vrn* genes with each other, the length of the plant growing season can be manipulated. Some winter triticale, wheat, and rye seeds sown in spring produced spontaneous spring plants. The type of development of such spontaneous spring mutants is determined by the heterozygous dominant gene, since the offspring from their self-pollination segregates into spring and winter forms. In Siberia, winter cultivars of triticale are successfully grown. Given an unpredictable climate change and increasing demand for feed grain in animal husbandry, it is of interest to involve different dominant *Vrn* alleles and their combinations in breeding spring forms of triticale, differing in the length of growing season.

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