The interphase period “germination–heading” of 8x and 6x triticale with different dominant Vrn genes

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Abstract. The existing spring forms of wheat-rye amphiploids are characterized by late maturity due to the long duration of the interphase period “germination–heading”. The manifestation of this trait is influenced by Vrn-1 genes. Their dominant alleles also determine the spring type of development. The results of studying the interphase period “germination–heading” of spring octoploid and hexaploid forms of triticale created for use in research and breeding programs under the conditions of forest-steppe of Western Siberia are given in this article. The interphase period of the primary forms BxVrnA1, BxVrnB1 and BxVrnD1 obtained by artificial doubling of the chromosome number of the wheat-rye hybrids made by pollination of three lines of the soft wheat ‘Triple Dirk’ – donors of different dominant Vrn-1 genes – by a winter rye variety ‘Korotkostebel’naya 69’ was determined under the field conditions in the nursery of octaploid (8x) triticale. In the nursery of hexaploid triticale, this trait was studied in the populations of hybrids obtained by hybridization of these three primary forms of octaploid triticale with the hexaploid winter triticale variety ‘Sears 57’. In the offspring of crossing BxVrnD1 × ‘Sears 57’, spring genotypes of 6x triticale bearing Vrn-D1 were selected. This fact was determined by PCR. It means that the genetic material from the chromosome of the fifth homologous group of the D genome of the bread wheat is included in the plant genotypes. This genome is absent in the winter 6x triticale ‘Sears 57’. The grain content of spikes of the created hexaploid forms of triticale is superior to that of the maternal octaploid triticale forms. It was shown that plants of the hybrid populations BxVrnA1 × ‘Sears 57’ and BxVrnD1 × ‘Sears 57’ carrying the dominant alleles Vrn-A1a and Vrn-D1a, respectively, have a shorter duration of the “germination–heading” interphase period than the initial parental forms of primary 8x triticale. The short interphase period of “germination–heading” of the 6x triticale is a valuable breeding trait for the creation of early maturing and productive genotypes of triticale.

Key words: octoploid; hexaploid triticale; interphase period “germination–heading”; Vrn-1 genes; hybrids.

Mежфазный период «всходы–колошение» у 8x и 6x тритикале с различными домinantными генами Vrn

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Аннотация. Существующие коллекционные формы яровых пшенично-ржаных амфиплоидов характеризуются позднеспелостью из-за большой продолжительности межфазного периода «всходы–колошение». На проявление этого признака влияют гены Vrn-1, аллели которых в доминантном состоянии обусловливают яровой тип развития. В статье приведены результаты изучения межфазного периода «всходы–колошение» у яровых окта- и гексаплоидных форм тритикале, создаваемых для использования в исследовательских и селекционных программах в условиях лесостепи Западной Сибири. Исследования проводили в питомнике октаплоидных (8x) тритикале в полевых условиях у первичных форм BxVrnA1, BxVrnB1 и BxVrnD1, полученных искусственным удвоением числа хромосом пшенично-ржаных гибридов от опыления пыльцой озимой ржи (сорт Короткостебельная 69) трех линий мягкой пшеницы Triple Dirk – доноров разных домinantных генов Vrn-1. В питомнике гексаплоидных (6x) тритикале изучали этот признак растений в популяциях гибридов от скрещивания трех форм первичных октаплоидных тритикале с гексаплоидным озимым сортом тритикале Сирс 57. С помощью молекулярных маркеров у гибридов определен аллельный состав генов Vrn-1. В потомстве, полученном от скрещивания BxVrnD1 × Сирс 57, выделены и определены методом ПЦР генотипы яровых растений 6x тритикале с домinantным геном Vrn-D1. Данный факт свидетельствует о включении в них генетического материала хромосомы пяты гомеологичной
группы генома D мягкой пшеницы, входящего в геномный состав октаплоидного тритикале. Этот геном отсутствует в озимом 6x тритикале Сирс 57. У созданных гексаплоидных форм тритикале озерненность колоса была лучше, чем у материнских октаплоидных. Показано, что растения из гибридных популяций 8хVrnA1 x Сирс 57 и 8хVrnD1 x Сирс 57, несущие доминантные аллели Vrn-A1a и Vrn-D1a соответственно, обладают более короткой продолжительностью межфазного периода «всходы—колошение», чем исходные родительские формы первичных 8х тритикале. Короткий межфазный период «всходы—колошение» у полученных 6x тритикале является селекционно ценным признаком для создания раннеспелых и продуктивных генотипов тритикале.

Ключевые слова: октаплоидные; гексаплоидные тритикале; межфазный период «всходы—колошение»; гены Vrn-1; гибриды.

**Introduction**

Hundreds of winter and spring varieties and collection forms of triticale (×Triticosecale Wittmack) or wheat-rye amphiploid (WRA) with genomes of wheat (Triticum spp.) and rye (Secale spp.) have been made for more than 130-year history of this artificial crop. According to the latest data of the world organization FAO, in 2017, the total area of this crop reached almost 4.17 million hectares and grain production was 15.6 million tons. In the Russian Federation the area of crops with the maximum value 274.5 thousand hectares in 2014. Grain yield for those years amounted to 500.7 thousand tons tory of this artificial crop. According to the latest data of the

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Hexaploid (6х) forms of triticale (B*B*AARR, 2n = 42) are mainly used for agricultural purpose. They are more cyogenetically stable and fertile compared to octaploid (8х) (B*B*AADDRR, 2n = 56) ones (Lukaszewski, Gustafson, 1987). However, there are some reports of successful cultivation of 8x triticale (Cheng, Murata, 2002).

In Siberia, this crop has not yet been cultivated on a large scale, since selection of spring triticale forms has not been conducted. Spring triticale samples from the world collection of VIR are late-maturing and winter varieties of European selection do not have good winter hardiness and often do not give good grain yield under severe climatic conditions of Siberia. Two winter short-stemmed varieties Sears 57 and Cacak 90 have been created in Siberian Research Institute of Plant Production and Breeding – Branch of the Federal Research Center the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (SibRIPP&B – Branch of ICG SB RAS) for grainforage use. They occupy only several thousands hectares. More than a dozen of spring varieties have been created in Russia (Tyslenko et al., 2016), but there are no Siberian varieties of spring triticale yet, although spring crops yield yearly, unlike winter ones. In order to carry out breeding work with spring triticale successfully, it is necessary to comprehensively study the characteristics associated with the productivity and adaptability of plants, including those related to the type and duration of plant development.

The purpose of this article is to study the duration of the interphase period “germination–heading” of created in SibRIPP&B – Branch of ICG SB RAS spring octaploid and hexaploid triticale forms having different dominant Vrn genes under conditions of the forest-steppe of Western Siberia.

**Materials and methods**

The duration of the interphase period “germination–heading” of octaploid (8х) and hexaploid (6х) triticales with different dominant Vrn genes affecting the duration of the vegetation period of plants was studied in generations: F1 – in 2014, F3 – in 2016, F4 – in 2017, F5 – in 2018 and F6 – in 2019.

Three primary 8x triticale forms were made in SibRIPP&B – Branch of ICG SB RAS by crossing almost isogenic lines of soft wheat Triple Dirk D, Triple Dirk B and Triple Dirk E (Pugsley, 1971, 1972) with winter diploid rye variety Korotkostebel’ naya 69 and by subsequently doubling the chromo-
some number in wheat-rye hybrids (Stepochkin, 2009, 2017). The wheat lines are the sources and donors of dominant genes Vrn-A1, Vrn-B1 and Vrn-D1, respectively. The allelic compositions of Vrn-1 genes of three 8х WRA are Vrn-A1a, vrn-B1a, vrn-D1a, vrn-R1 (8xVrnA1); vrn-A1, vrn-B1a, vrn-D1, vrn-R1 (8xVrnB1); vrn-A1, vrn-B1, vrn-D1a, vrn-R1 (8xVrnD1).

Spring hexaploid forms of triticale were made by selecting early-maturing plants in the offspring of F1-F2 hybrids between primary 8х WRA and winter 6х triticale Sears 57 carrying recessive genes vrn-A1, vrn-B1, vrn-R1 (Fig. 1). The allelic composition of Vrn-1 genes in plants of hybrid populations and parent forms was determined by PCR using allele-specific primers. The structure of primers to Vrn-1 genes and the conditions of PCR are described in articles (Potokina et al., 2012; Likhenko et al., 2015).

Genomic DNA was isolated according to the previously described method (Likhenko et al., 2015). PCR was performed on a BIO-RAD T-100 Thermal Cycler (USA) amplificator in a total reaction mixture of 20 µl, including DNA (50–100 ng/µl) – 1 µl, 10× buffer for Taq polymerase (650 mM Tris-HCl (pH 8.9); 160 mM (NH4)2SO4; 25 mM MgCl2; 0.5 % Tween 20) – 2 µl, dNTPs – 2 µl, direct and reverse primer – 0.5 µl each, Taq polymerase (1 unit/µl) – 1 µl, H2O – up to the final volume of 20 µl. The separation of PCR products was performed by electrophoresis in 1 % agarose gel with the addition of ethidium bromide.

Sowing in the field soil was carried out by hand in the third decade of May (May 21–24 in different years, depending on the weather) in rows of 0.8 m long, 50 seeds in a row on the isolated from other grain crops experimental plot of the SibRIPP&B – Branch of ICG SB RAS, where a three-field rotation was maintained: vegetables – fallow soil – triticale. During the growing season, phenological observations and evaluations were carried out. Statistical processing of the results was performed using the Student’s t-test (Dospekhov, 1985).

**Results**

The evaluation of plants in populations of primary octaploid WRA showed that the duration of the interphase period “germination–heading” in triticale lines 8xVrnA1 and 8xVrnD1 in 2018 and 2019 was shorter than that in 2014, 2016 and 2017 (Table 1). In 2019, triticale 8xVrnA1 had the shortest “germination–heading” period (52.9 days) among all octaploid WRA, while 8xVrnB1 had the longest one (72.5 days). This period of the maternal line 8xVrnD1 lasted 53.8 days. In 2019 hexaploid plants of the hybrid population 8xVrnA1 × 6x Sears 57 was shorter than that of the original octaploid forms, while 8xVrnB1 hybrids did not significantly differ from the spring octaploid parent. When comparing the data of all years of research, one can note that the selection of the most early-maturing plants in each generation led to a significant reduction of the duration of the period from germination to heading of both hexaploid and maternal octaploid triticale forms except for the parental form 8xVrnB1. It did not show significant changes in the duration of this period during all years of research.

Ear morphology of hexaploid triticale plants differs from that of the original octaploid forms (Fig. 2). All octaploid triticale lines are awnless, and the hexaploid forms have, like the paternal winter variety Sears 57, small rudiments of the

**Table 1. Duration of the interphase period “germination–heading” of hybrid hexaploid and maternal octaploid triticale plants with different dominant Vrn-1 genes**

| Hybrids and maternal triticale forms | Duration of the interphase period “germination–heading”, days (m ± sem) |
|-------------------------------------|---------------------------------------------------------------|
|                                     | 2014  | 2016  | 2017  | 2018  | 2019  |
| 8xVrnA1 × Sears 57                  | 69.2 ± 1.3* | 66.1 ± 3.3 | 55.0 ± 1.9** | 45.1 ± 2.5 | 47.3 ± 1.9* |
| 8xVrnD1 × Sears 57                  | 75.9 ± 2.1** | 68.3 ± 1.7 | 63.3 ± 2.4 | 55.2 ± 6.7 | 53.4 ± 1.7 |
| 8xVrnB1 × Sears 57                  | 79.6 ± 4.4 | 71.7 ± 1.5 | 68.6 ± 3.4 | 56.4 ± 2.1** | 57.8 ± 1.5** |
| 8xVrnA1                             | 65.2 ± 1.7 | 65.1 ± 2.0 | 61.7 ± 1.1 | 51.0 ± 1.8 | 52.9 ± 1.4 |
| 8xVrnD1                             | 67.0 ± 1.9 | 66.3 ± 0.7 | 63.9 ± 1.0 | 49.5 ± 2.6 | 53.8 ± 2.1 |
| 8xVrnB1                             | 73.6 ± 1.9 | 71.3 ± 0.9 | 69.0 ± 3.1 | 71.2 ± 4.3 | 72.5 ± 1.6 |

* p < 0.05; ** p < 0.01 – significant differences between a hybrid and its parental form of 8x triticale.
Some ear quantitative characteristics of 8x triticale plants: octaploid 8xVrnA1 × Sears 57 (4), 8xVrnD1 × Sears 57 (3), 8xVrnB1 × Sears 57 (2), 8xVrnA1 × 6xSears 57 (1) and 8xVrnB1 × 6xSears 57 (6).

The interphase period “germination–heading” of 8x and 6x triticale with different dominant Vrn genes

The selected early-maturing hexaploid plants were analyzed by PCR using allele-specific primers for Vrn-1 genes. Their parents – the winter variety triticale Sears 57 and the octaploid maternal forms were taken for comparison (Fig. 3). The analysis showed that the winter variety Sears 57 carries recessive alleles vrn-A1, vrn-B1 and vrn-D1. The maternal forms had the following allelic composition: 8xVrnA1 – Vrn-A1a, vrn-B1, vrn-D1; 8xVrnB1 – vrn-A1, Vrn-B1a, vrn-D1; 8xVrnD1 – vrn-A1, vrn-B1, Vrn-D1a. The offspring from the 8xVrnA1 × Sears 57 cross was heterozygous on the Vrn-A1 gene because they contained two alleles – Vrn-A1a and vrn-A1. In addition, a recessive allele vrn-B1 was revealed, and the alleles of the Vrn-D1 gene were not determined due to the lack of an amplification product. Plants of the hybrid population 8xVrnB1 × Sears 57 have a recessive allele vrn-A1, and the Vrn-D1 gene was not amplified in them. As for the Vrn-B1 gene, the plants are heterozygous and have two alleles Vrn-B1a and vrn-B1. Two recessive alleles – vrn-A1, vrn-B1, and one dominant allele – Vrn-D1a – were identified in plants obtained from crossing 8xVrnD1 × Sears 57.

Discussion

Secondary spring 6x triticale breeding samples possess dominant alleles of Vrn-1 genes and were made by hybridization of primary 8x WRA carrying dominant alleles of Vrn-1 genes with a winter 6x WRA carrying recessive alleles. At the 6x level in the offspring of hybrids in all the studied generations, their Vrn-1 genes retain almost the same ranking (8xVrnA1 × 6x Sears 57 > 8xVrnD1 × 6x Sears 57 > 8xVrnB1 × 6x Sears 57) as at the 8x level in triticale (8xVrnA1 ≥ 8xVrnD1 > 8xVrnB1) in terms of the effect on the reduction of the “germination–heading” period. The original Triple Dirk lines have the same ranking of these genes (Stepochkin, 2009; Stepochkin, Emtseva, 2017). Thus, the effect of the dominant alleles Vrn-A1a and Vrn-D1a leads to a shorter interphase period compared to the effect of the Vrn-B1a allele. It is known that in addition to the Vrn-D1 gene, the Vrn-D4 gene localized in the chromosome 5D can significantly affect the duration of the period from germination to earing (Kipps et al., 2014). Theoretically, it is possible that along with the Vrn-D1 gene, a Vrn-D4 gene can be inserted. However, we exclude this poss-
sibility, because to make primary octaploid triticales, we used the Vrn-isogenic wheat lines Triple Dirk D, Triple Dirk B, and Triple Dirk E, carrying, respectively, only the Vrn-A1, Vrn-B1, and Vrn-D1 genes. A comparison of a set of 8x triticale lines and 6x samples from the VIR world collection showed that the interphase period “germination–heading” of hexaploid triticales is shorter (Stepochkin, Emtseva, 2017). There is an assumption that reducing the level of ploidy can reduce the duration of the period “from germination to heading” in wheat-rye amphiploids. In particular, it was reported that within the crossing combination, octaploid lines formed ears later than hexaploid ones (Kaminskaya et al., 2005).

The hexaploid paternal variety Sears 57 (genomic formula B+B+AARR), has a winter type of development. All its vrn-D genes have recessive alleles. The maternal forms are three spring octaploid triticale lines (genomic formula B+B+AADDRR). Each of them carries one dominant gene: 8xVrnA1 carries a VrnA1a allele on the chromosome 5A, 8xVrnB1 contains a VrnB1a allele on the chromosome 5B, 8xVrnD1 has a Vrn-D1a allele on the 5th chromosome of D genome. It was assumed that in 8xVrnD1 × Sears 57 hybrids in subsequent generations, starting from F2, the chromosomes of the haploid D genome would be lost during the process of meiosis, and the share of winter plants in the hybrid populations would increase. As a result, in the older generations there would be only winter hexaploid forms with the chromosome number 42 without the haploid genome D and without the dominant allele Vrn-D1a. The facts of complete elimination of chromosomes of D genome in such types of crossing are known (Hao et al., 2013). However, by selecting spring plants we were able to create up to the fourth generation populations of 6x forms that could begin transition to the generative development after spring sowing without vernalization. Molecular genetic analysis using the PCR method showed the presence of the dominant Vrn-D1a allele in these forms (see Fig. 3). This means that either as a result of chromosome substitution or translocation, the Vrn-D1 gene remained in the complex genome of hexaploid plants. Some researchers report inclusion of a genetic material of the wheat genome D in the genome of hexaploid triticale forms (Kaminskaya et al., 2005). Unlike plants of 8xVrnD1 × Sears 57 population, hexaploid triticale forms made by crosses 8xVrnA1 × Sears 57 and 8xVrnB1 × Sears 57 do not contain any Vrn-D1 allele, as it was shown by molecular analysis with primers to Vrn-D1 gene, although the maternal lines contain a recessive vrn-D1 allele. The lack of amplification is probably due to the elimination of chromosomes of the D genome.

It is known that octaploid triticales are cytogenetically unstable. As a result of disturbances in meiosis, gametes with an unbalanced number of chromosomes are formed, which leads to appearance of aneuploid plants in 8x WRA populations (Vettel, 1960a, b; Krolow, 1962, 1963). Hexaploid triticale plants with dominant Vrn-1 genes may arise as a result of spontaneous depoliploidization of octaploid WRA carrying these genes. This process is accompanied by the predominant elimination of the chromosomes of D genome of soft wheat in octaploid WRA. At the end of this process, stable 6x triticales appear, which was found in populations of a number of 8x triticales (Stepochkin, 1978; Li et al., 2015).

Conclusion
The presented results showed that the populations of spring octaploid triticales made and maintained at SibRIPP&BR – Branch of ICG SB RAS are donors of different dominant Vrn-1 genes. These populations are used to produce new forms of 8x and 6x WRA and for breeding process. In the hexaploid triticale forms made on their basis, the allelic composition of the Vrn-1 genes was determined using molecular genetic analysis. It was found that plants from the populations of 8xVrnA1 × Sears 57 and 8xVrnB1 × Sears 57 have genes Vrn-A1 and Vrn-B1 in a heterozygous state, so it is necessary to conduct further selection to make homozygous genotypes. In the
created hexaploid forms of triticale, the grain number from ear is higher than that in the original octoploid lines. It is shown that the plants from the hybrid populations $8x$VrnA1 × Sears 57 and $8x$VrnD1 × Sears 57, carrying the dominant alleles Vrn-A1a and Vrn-D1a, respectively, have a shorter duration of the interphase period “germination–heading” than the original parent forms of the primary 8x triticale, which is a breeding-valuable feature for the creation of early-maturing and productive genotypes of triticale.

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