

A Review**Vernalization of Wheat and Genetic Determinism of the Vernalization Reaction****MUREȘAN Diana^{1,2}, Rozalia KADAR², Alexandru GHETE¹,
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Available online 30 June 2019**Abstract**

Winter wheat sown in the spring does not manage to produce grains. Vernalization is necessary for wheat to pass from the vegetative phase to the generative phase. This happens in the same time with wheat “quenching”. Studies show that vernalization can occur during germination, during plant growth or during seed formation and at the full maturity of the grains. Other research emphasizes that vernalization of wheat corresponds to the accumulation of total phytochrome in the vegetative apex and to the accumulation of this pigment in the leaves, and the phenomenon of quenching is achieved by a transformation of the total phytochrome in less sensitive forms to dehydration of cells produced by frost. Vernalization of cultivated wheat is mainly controlled by three loci of the *VRN1* gene, *VRN-A1*, *VRN-B1* and *VRN-D1* located in the middle of the long arms of chromosomes 5A, 5B and 5D respectively. Spring wheat has alleles of the *VRN1* gene that reduce or eliminate the vernalization requirement. As result of research there was identified a fourth gene that controls wheat vernalization requirement (*VRN 4*). They have shown that this gene is a transcribed copy of the *VRN1* gene. The study of Kippes et al. (2015) highlights the importance of the *VRN1* gene as a regulator of vernalization and as a major gene that controls adaptation strategies and the wheat life cycle.

Keywords: *phytochrome, wheat, vernalization.***1. Introduction**

Wheat is one of the main crop plants that provide the basic nutrition of a large part of the world's population.

This culture is characterized by a special ecological plasticity due to its genetic potential to synchronize the moment (the time) of flowering with favorable environmental conditions [17]. This feature allows wheat plants to produce a satisfactory grain yield under very different conditions of temperature, humidity and soil.

The moment of wheat flowering is controlled by three important genetic systems, namely vernalization (Vrn), photoperiod (Ppd) and precocity (Eps) [34, 28, 15]. This work includes a brief incursion into the vast subject called vernalization.

2. Vernalization of wheat

The need for prolonged exposure to low temperatures for plants to become able to flourish is determined by the vernalization requirement [25, 27]. This requirement delays the initiation of the breeding phase and protects floral meristems sensitive to frost damage in winter [5, 33, 37].

Winter wheat sown in the spring, without being vernalized, grows vegetatively but fails to produce grains. In consequence, vernalization is

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necessary for wheat to pass from the vegetative phase to the generative phase.

This is done simultaneously with the "hardening" of the wheat (the slow process of adapting the wheat to the unfavorable winter conditions, whereby the wheat can withstand up to -20 °C at the twining node level). The typical winter wheats need exposure to low temperatures (1-3 °C) for approx. 60 days. There are also winter wheats that vernalize in 30-60 days.

Usually, the duration of winter wheat vernalization is even longer if these have a more pronounced winter character. Typical winter wheat sown in the spring, without being vernalized, grows only vegetative and does not bloom, so it does not form berries. Typical spring wheats manage to complete the vernalization stage in a few days at a temperature of between 5 and 20 °C.

Numerous varieties of spring wheat do not need vernalization to produce seeds. Levy and Peterson (1972) and Hay and Kirby (1991) have proven the insensibility to vernalization of spring wheat [21, 13]. Typical spring wheat sown in autumn gives lower yields, usually being damaged by winter frosts. Facultative wheat (intermediate, semi-winter or facultative) are vernalized for 5 to 30 days, depending on their genotype, towards the spring or winter, at temperatures in between 5 and 10 °C. The facultative (walking) wheat sown in autumn, also suffers like spring wheat, because of low winter temperatures, but less than the typical spring wheat, giving superior outputs to spring wheat.

Sown in spring, "walking" wheat turned out to be more late and sensitive to drought and high temperatures [3]. Under the conditions in our country, the process of vernalization takes occur in the field from the last decade of October and lasts until the end of December.

The temperature and time required for this process vary from one variety to another. The temperature and the low light from autumn - winter period, in temperate regions, act as indicators that allow plants to anticipate season change and hence prepare for frosting temperatures by inducing or amplifying cold tolerance mechanisms.

3. Genetic determinism of the vernalization reaction

The UK's Biotechnology and Biological Sciences Research Council (BBSRC) supports a study based on the genome sequencing of wheat. Professor Keith Edwards of the University of Bristol, the lead author of the study, said that "the wheat genome is five times larger than the human genome," and that is why it is a real challenge for scientists. Consequently, the differences between two wheat

varieties can be identified more quickly and the genes responsible for certain useful characteristics (disease resistance, drought, etc.) can be identified. The genome sequences that have been published give researchers and manufacturers access to 95% of the wheat plant genes.

These genes can be used to amelioration cultivated wheat varieties, through assisted selection, or to obtain genetically modified plants through transgenesis. In this study, the Chinese Spring wheat variety was used (Chinese spring wheat - CS42) was used due to its wide use in genome-related studies. It is estimated that the A genome contains approximately 28,000 genes, the B genome contains 38,000 genes, and the D genome has about 36,000 genes. Also, the number of genes in the hexaploid wheat genome varies between 94,000 and 96,000 [2]. Halloran and Boydell (1967), by using chromosomal substitution lines of the Hope variety in the Chinese Spring variety, have come to the idea that the genes controlling the vernalization reaction are located in 11 chromosomes, of which the genes located in chromosomes 2A, 5A, 3B, 5B, 6B, 7B, 1D and 7D reduce the vernalization stage of the Chinese Spring variety, and the genes located in chromosomes 4A, 2A and 5D prolong this stage [12].

Thus, by using intervartietal chromosomal substitution lines of the hexaploid wheat, it was possible to localize the specific chromosome of the genes influencing the vernalization response [9]. Vernalization is controlled by VRN1-4 genes [3, 29, 38, 18]. Winter wheat varieties possess the recessive alleles of all four genes. Therefore, differences between winter wheats in terms of vernalization requirements seem to be due to multiple recessive alleles [3].

The dominant alleles give the typical spring character and are epistatic for the recessive alleles that give the typical winter character, so spring genotypes carry at least one dominant allele [23]. Thus, depending on the ratio of the dominant alleles to the recessive VRN genes in the three genomes of hexaploid wheat, three types of wheat are distinguished: typical winter wheat (recessive alleles), typical spring (dominant alleles) and the facultative character (various combinations of dominant and recessive alleles) [20].

VRN 1 is a flowering promoter that is activated by low temperatures [31]. These genes directly influence the flowering and maturity times of wheat [6, 37]. VRN 1 transcription levels increase gradually during vernalization; as the cold exposure increases, the level of expression of the VRN1 genes also increases. A high level of expression of VRN1 genes is maintained in apical meristems and leaves after vernalization, and this is responsible for how quickly vernalized plants flourish. Some VRN1

alleles are expressed without vernalization due to deletions or insertions occurring in the promoter or first intron of the VRN1 gene [31]. Using tetraploid wheat mutant lines, Chen and Dubcovsky (2012) have demonstrated that the main role of leaf VRN1 genes is to maintain a low level of transcription of VRN2 genes (which suppress blooming) after vernalization, which allows the production of the FT mobile protein (florigenic) necessary to initiate the flowering [5].

Inside leaves, after the outbreak from the winter season, longer day length and warmer weather conditions favor transcription induction of VRN2. Transcriptional activation of VRN1 genes in leaves during vernalization provides an important regulatory signal that controls the transition from vegetative growth in autumn to reproductive development in the spring. In autumn season, low transcription levels of VRN1 genes result in high expression of VRN2 genes, suppression of FT protein and maintenance of vegetative growth.

During winter season, transcription of VRN1 genes increases, and VRN2 genes decrease. Thus, the presence of VRN1 genes in the leaves prevents the increase of VRN2 genes transcription during longer and warmer days of spring, which facilitates transcriptional activation of FT protein and blooming induction. In conclusion, negative VRN2 regulation by VRN1 in leaves is a key stage in regulating the seasonal responses of winter wheat. The level of transcription of VRN1 genes is significantly increased during vernalization, both on long and short days. Since FT protein transcription levels and VRN2 genes are undetectable on short days, it was concluded that VRN1 is a direct vernalization target [5]. Spring wheat have alleles of the VRN1 gene that reduce or eliminate the vernalization requirement [32]. The vernalization of cultivated wheat is mainly controlled by three loci of the VRN1 gene (VRN-A1, VRN-B1 and VRN-D1) located in the middle of the long arms of chromosomes 5A, 5B and 5D respectively [10, 7, 1, 16, 6].

In hexaploid wheat, the VRN-A1 gene may be present as two or more copies on the haploid genome. There is a hypothesis according to which the number of copies VRN-A1 is correlated positively with the duration of the vernalization requirements and the time of flowering of the wheat. From the three homeologous genes VRN1, polymorphism in the recessive allele coding sequence (which determines the typical winter character), which is associated with the modulation of valuable agronomic features such as frost tolerance, duration of vernalization, length of straw stretching and wheat flour, was revealed only for the VRN-A1 gene [25]. Vrn-A1 is considered to be the main gene that severely reduces the requirements for wheat vernalization [7].

The VRN-A1 alleles is responsible for the typical wheat spring character are dominant and epistatic on the VRN2 genes [5]. A single dominant Vrn-A1 allele is usually sufficient to eliminate the wheat vernalization requirement [18]. Following a study that targeted the genotyping of wheat germoplasm and establishing genetic patterns to represent the typical winter, semi-winter and low winter wheat varieties, it is concluded that the vrn-A1 locus is very sensitive to temperature changes and is closely associated with variations in autumn wheat development that may be caused by the duration of vernalization requirements over the years [37].

This locus is responsible for ear moment and physiological maturity in wheat [37]. By 2009, only two alleles were known at the Vrn-B1 locus: a dominant one (which imprint the typical spring character called Vrn-B1a) and a recessive one (vrn-B1 in the typical winter wheat). Subsequently, two additional dominant alleles were described: Vrn-B1b and vrn-B1c [24].

Recently, in China was discovered the Vrn-B1d allele a dominant allele that confers the typical spring character [40]. At the VRN-D1 locus, three alleles are known: the dominant allele Vrn-D1a, which imparts to the wheat the typical spring character, the vrn-D1 recessive allele associated with the typical autumn character of the wheat and the additional dominant allele Vrn-D1b associated with the character facultative of wheat [11]. This was demonstrated by a study conducted in China on a number of 689 accessions. Following research carried out on a population of 50 homozygous genotypes for Vrn-D1b and 50 homozygous genotypes for vrn-d1, it has been shown that homozygous genotypes for Vrn-D1b have directly influenced production by increasing the number of grains/ear [22]. Following a study in China on 278 wheat genotypes, Zhang et al. (2008) demonstrated that the dominant Vrn-D1 allele is associated with late ear wheat, while Vrn-A1 is associated with early ear and Vrn-B1 with intermediate earing [39].

VRN 2 is a repressor of flowering of wheat in long day conditions which does not have a clear orthologist in Arabidopsis [35]. These genes affect the mod of growth wheat by acting as an indirect repressor on the expression level of Vrn-A1 by Vrn3 repressing [30]. Loss of Vrn2 function through natural mutations or deletions occurs at spring wheat, which does not require the vernalization stage for flourish [35, 6].

The **VRN 3** gene is a homologue of the Arabidopsis FT gene [36, 8] and exhibits increased expression when the dominant allele is present, is manifested by pronounced flourishing and "bypassing" of the vernalization requirement [36]. VRN3 is the main integrator of the vernalization and

photoperiod signals, of herbaceous plants in the temperate zone [5].

VRN 4 is a gene that controls the wheat vernalization requirement. It was only identified in the genome D on the short arm of chromosome 5 [38]. The *Vrn-D4* allele (= *Vrn-4*) has a lesser effect in the acceleration of the flowering than the *Vrn-A1*, *Vrn-D1* and *Vrn-B3* alleles [18]. Kippes et al. (2015) demonstrated that this gene is a translocate copy of the *VRN1* gene [19].

The study of Kippes et al. (2015) highlights the importance of the *VRN1* gene as a regulator of vernalization and as a major gene that controls adaptation strategies and the wheat life cycle [19]. The origin of the wheat *VRN4* gene that reduces the vernalization requirement is shown in Fig. 1. At point A of the figure 1, we can see that at common wheat

ancestral hexaploid genome (*T. aestivum*) had three homologous *VRN1* genes, by one, on the long arm of chromosome 5 for each of the genomes A, B and D. The *VRN1* gene is a promoter of flowering, but the *VRN1* genes are not transcribed until the plants are passing through the vernalization, so the flowering is delayed. In this figure, the haploid equivalent is presented, and there are normally six copies per nucleus (meaning, AABBDD). At point (B), can be observed the *VRN1* gene translocated from the long arm of chromosome 5 (genome A) into the centromeric region of chromosome 5 (genome D, proximal short arm region). The additional gene *VRN1* on chromosome 5D increases transcriptional activity and triggers flowering without vernalization. This additional copy of the *VRN1* gene is the basis of the *VRN4* gene [32].

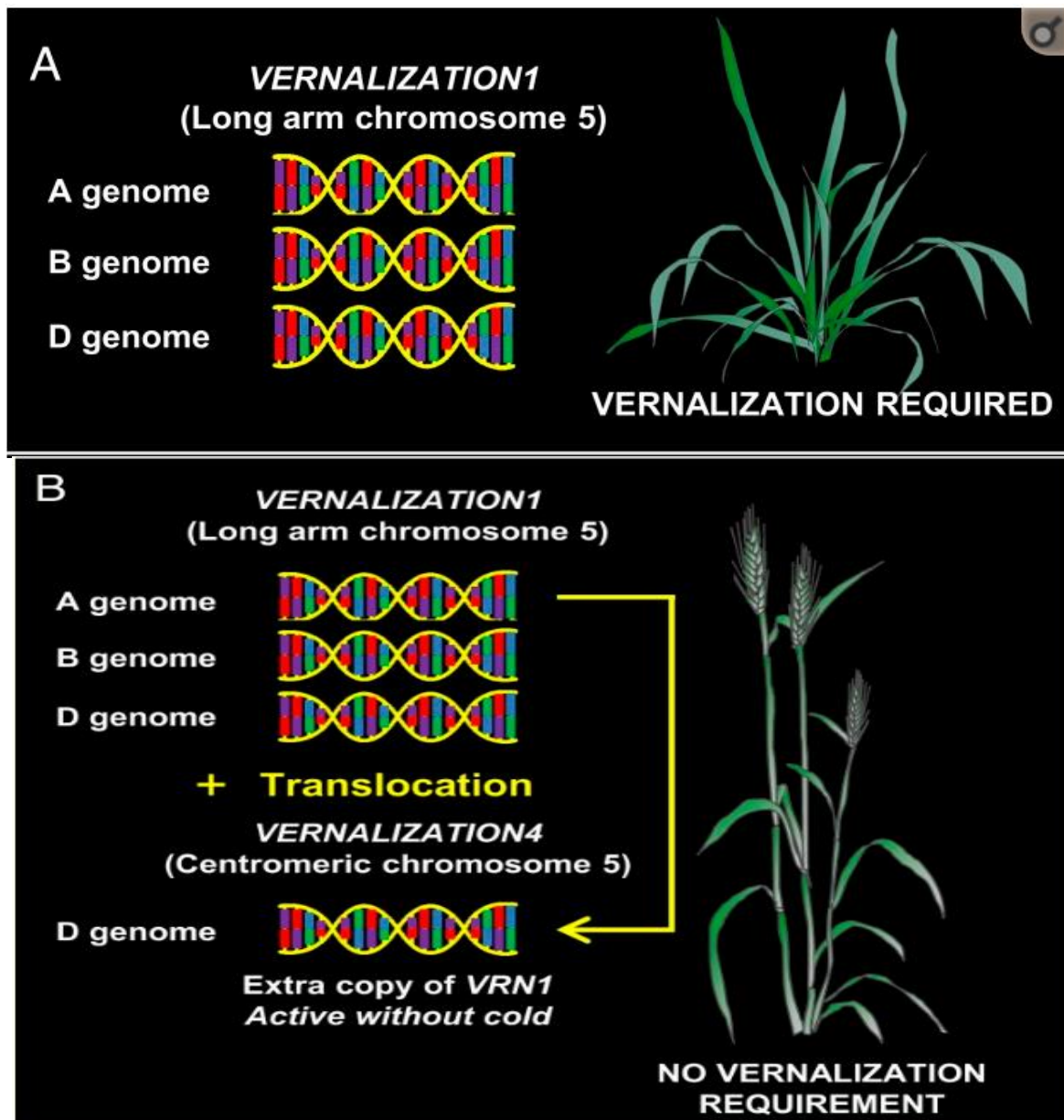


Figure 1. The origin of the wheat *VRN4* gene that reduces the vernalization requirement [32]

The importance of phytochrome (B and C)

The most important phenomenon that occurs during vernalization is the formation and accumulation of phytochrome, considered the essential factor of flowering and fructification.

Phytocroms are dimeric photoreceptors that absorb the maximum light in red and far red, influencing almost every stage of plant growth and development.

They induce large-scale transcriptional changes in response to variation in light quality [26]. Developing knowledge about the phytochrome action has almost exclusively depended on photoreversible change of them [14].

By their action, phytochromes mediate responses such as: floral induction, leaf nictinastical movements, phototropic sensitivity, seed germination, stem extension, leaf and cotyledon expansion, chloroplast development, enzymes activation, protein synthesis, mRNA transcription, chloroplasts movement, transmembrane potential [41]. A high content of chlorophyll causes a decrease in the amount of phytochrome [41].

Therefore, phytochrome accumulation takes place at the same pace as the vernalization process, if the vernalization temperature is correlated with the short day. In long day conditions, phytochrome accumulates in leaves at the same speed as accumulated under short day conditions, but its accumulation at the vegetative tip is slower due to the photoperiodic effects exercised of leaves.

In 1973, Vincent showed that wheat vernalization corresponds to the accumulation of total phytochrome in the vegetative apex and to the accumulation of this pigment in the leaves, and the tempering phenomenon is achieved by a transformation of the total phytochrome in less sensitive forms to the dehydration of the cells produced by frost [33].

Pearce et al. (2016) have shown that during photoperiods of long days, plants that are missing phytochrome B (PHYB) have a severe late flowering, unlike plants to which they are miss phytochrome C (PHYC) [26]. Thus, they concluded that both PHYB and PHYC are necessary for photoperiodic induction of wheat flowering, but only PHYB regulates a large number of genes involved in biosynthesis and hormone signaling, in shadow avoidance response and tolerance to abiotic stresses [26].

Chen et al. (2014) and Pearce (2016) have shown that PHYC is necessary for the early flowering of wheat [4, 26].

In 2014, Chen et al. have stated that in wheat, PHYC is stable and functionally active even in the absence of the others phytochromes, whereas in rice, for good stability and functioning, it requires the presence of the others phytochromes [4].

Conclusion

The reaction to vernalization and photoperiod, which determines the moment of earing and flowering, has a significant influence on the adaptability of wheat to certain environmental conditions and establishing the areas of growing of different varieties.

High winter temperatures lead to insufficient winter wheat vernalization and a delayed reproductive development, so to a low production.

VRN1 genes play an important role in obtaining winter wheat production as it controls the transition from vegetative growth in autumn to generative growth in the spring.

Thus, these genes control adaptation strategies and the wheat life cycle.

Phytochrome is considered the essential factor of flowering and fructification as it plays an important role in wheat tempering, genes transcription, protein synthesis and floral induction.

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