Allelic variability by vernalization and photoperiodic sensitivity loci of a number of wheat varieties (*Triticum aestivum* L.) grown in Estonia

Master thesis

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Vernaliseerimise ja fotoperioodi tundlikuse lookuste alleelne varieeruvus Eestis kasvatatavates nisu (*Triticum aestivum* L.) sortides

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Declaration

Hereby I declare that I have compiled the paper independently and all works, important standpoints and data by other authors have been properly referenced and the same paper has not been previously been presented for grading.

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Annotatsioon

Nisu on oluline toidu- ja söödateravili, 2021 aastal oli nisu ülemaailmne kogusaak 776 miljonit tonni. Nisu kasvatatakse kõigil kontinentidel peale Antarktika – see on võimalik tänu nisu erakordsele võimele adapteeruda erinevate keskkondadega. Nisu kohandumine on vastus *VRN* (vernaliseerimist kontrollivate) geenide ja *PPD* (fotoperioodi kontrollivate) geenide alleelsete variantide muutumisele. Vernaliseerimine ehk mõjutamine madalate temperatuuridega vastutab vegetatiivse/generatiivse kasvfaasi ülemineku ajastamise (FT) eest. *VRN* ja *PPD* geenide alleelide kompositsioon on aluseks nisu adapteerumisele mltmete keskkonna parameetriga. Eesti jaoks on eriti oluline kohanemine madalate temperatuuridega.

Käesolevas töös oli esmaülesandeks tuvastada *VRN* ja *PPD* geenide alleelsed kommbinatsioonid, mis esinevad Eesti sordilehe (seisuga juuni 2021) nisusortides. 87-l heksaploidse nisu sordil määrati alleelspetsiifiliste markerite abil alleelid üheksas *VRN* geeni lookuses: *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Vrn-A2*, *Vrn-B2*, *Vrn-D2*, *Vrn-A3*, *Vrn-B3*, *Vrn-D3*, ja kolmes *PPD* lookuses: *Ppd-A1*, *Ppd-B1*, *Ppd-D1*. 47 sorti kandsid ainult suvinisule omast *Vrn-A1a* alleeli , 26 neist sisaldasid lisaks *Vrn-B1* alleeli, ja ainult üks, sort Hiie, sisaldas kõiki kolme dominantset *Vrn1* alleeli. 40 sorti sisaldasid *VRN-1* lookuses ainult retsessiivseid alleele, kõik nad olid talinisud. *VRN-A2* ja *VRN-D2* geenides varieeruvust ei täheldatud ehk kõik analüüsitud sordid sisaldasid dominantseid allele. *VRN-A2* and *VRN-D2*. 21 analüüsitud 87-st sordist kandsid null *vrn-B2* alleeli. *VRN-A3*, *VRN-B3* ja *VRN-D3* lookustes alleelset varieeruvust ei leitud, nimetatud lookused kandsid retsessiivseid alleele.

Samuti ei leitud alleelset varieeruvust *PPD-A1* ja *PPD-B1* lookustes. Kõik 87 genotüüpi kandsid fotoperiodi-mittetundulikku *Ppd-A1b.1* ja fotoperiodi-tundulikku *Ppd-B1a.2* alleele. Fotoperiodi-tundulik *Ppd-D1b* alleel leiti 83-s sordis, vaid fotoperiodi-mittetundulik *Ppd-D1b* leiti nejas sordis.

Kui võrrelda *VRN* ja *PPD* geenide alleelset koostist Maa erinevates regioonides (Kanada , USA, Brasilia, Hiina, Eesti) kasvatatavate nisu sortidega, siis selgub, et erinevates regioonides prevaleerivd (>90) erinevad alleelide kombinatsioonid.

Teiseks käesoleva töö ülesandeks oli tuvastada, kas *VRN* ja *PPD* geenide alleelne koostis mõjutab nisu õitsemise algust. Saadud andmetest nähtub, et suvinisu puhul aleelne varieeruvus *VRN-B1* *VRN-B2* lookustes ei mõjuta õitsemise algust, küll aga mõjutab viimast valgustugevus. Talinisu puhul seisund oli vastupidine ehk allelne variatsioon *VRN-B2* lookuses mõjutas õitsemise algust.

Tulemusi on võimalik kasutada sordiaretuses.

List of abbreviations

ANOVA – Analysis of variance

AP1 gene - *Arabidopsis thaliana APETALA1* gene

bp – base pair

CTAB – hexadecyltrimethylammonium bromide

CO genes - *CONSTANS* genes

DNA - deoxyribonucleic acid

EDTA - ethylenediamine tetraacetic acid

EPS gene - earliness per se gene

FM - functional markers

Gb - Giga base pairs

HDD - host direct duplication

MQ H_2O - Milli-Q water

NIL - near-isogenic lines

PCR - polymerase chain reaction

pH - potential of hydrogen

PI - photoperiod insensitive

PS - photoperiod sensitive

PPD – photoperiod response

RPM – rotation per minute

RT – room temperature

SNP - single nucleotide polymorphisms

TAE - Tris-acetate-EDTA

TE - Tris-EDTA buffer

Tris - tris(hydroxymethyl)aminomethane; 2-amino-2-hydroxymethyl-propane-1,3-diol

VRN – vernalization

Introduction

Common wheat (*Triticum aestivum* L.) is an essential food source for many countries. The global wheat consumption was equal to 783 million metric tons in the previous year (FAOSTAT, 2021). Nowadays, wheat grows on every continent, and its fields cover around 218 million hectares worldwide (Giraldo *et al.,* 2019). Wheat also takes an important place in Estonian agriculture. In 2021, Estonia produced 841 thousand tons and exported 581 600 tons (FAOSTAT, 2021).

Common wheat is categorized into two types: winter and spring. Winter wheat requires vernalization and photoperiod response to ensure the floral transition. It means that, for a certain period of seedling growth, plants need to be exposed to low temperature accompanied by short daylength, otherwise they will not flower (review: Kamran *et al.,* 2014). Vernalization is controlled by *VRN* genes, while photoperiod response is controlled by *PPD* genes. *VRN* genes control plants' sensitivity to temperature, while *PPD* genes control sensitivity to day length (Kamran *et al.,* 2014; Kiss *et al.,* 2014). To date, three major *VRN* genes (*VRN1*, *VRN2* and *VRN3*) have been detected in common wheat. Each of them includes three homeologous loci (*VRN-A1*, *VRN-B1*, *VRN-D1*, *VRN-A2*, *VRN-B2*, *VRN-D2*, *VRN-A3*, *VRN-B3*, *VRN-D3*) in A, B and D genomes of the hexaploid wheat (Yan *et al.,* 2006). The *PPD* gene is also represented by homeologous loci in each genome: *PPD-A1*, *PPD-B1*, *PPD-D1* (Chen *et al.,* 2013; Kippes *et al.,* 2016).

In general, it is suggested that high adaptability to various environments (Trevaskis *et al.,* 2007; Zhao *et al.,* 2016) could be a response to allelic variation in *VRN* and *PPD* genes. If wheat is adapted to local environments, it will flower at the right time and produce a rich yield. Although there are numerous data concerning the natural allelic variation of the vernalization and photoperiod response genes in wheat, there is no information about the genetic diversity of these genes in cultivars grown in Estonia (Zhang *et al.,* 2008; Milec *et al.,* 2013; Chen *et al.,* 2013; Kiss *et al.,* 2014; Zhang *et al.,* 2015).

The aim of this study was to investigate the natural allelic variation of the vernalization and photoperiod response genes in wheat cultivars grown in Estonia, and to evaluate possible effect of the allelic composition on the flowering time. Using molecular markers to identify allelic composition of *VRN* and *PPD* genes, 87 common wheat cultivars registered in the Estonian Variety List were analyzed. Additionally, the heading time of winter and spring wheat cultivars was evaluated under controlled environmental conditions.

The literature review provides a general overview of the common wheat plant, vernalization and photoperiod response impact on flowering time, and how allelic variation and mutations in *VRN* and *PPD* genes could affect the flowering time.

The experimental part gives information about methods used to identify allelic variation and phenotype of common wheat cultivars and to analyze the data.

1. Literature Review

1.1. *Triticum aestivum* L. plant

Common wheat (*Triticum aestivum* L.) is an annual plant, which, along with maize, rice and sorghum, belongs to the grass family (*Poaceae*) (Kumar and Sharma, 2011). In 2019, grass family species provided almost one-third of the total crop production worldwide (FAOSTAT, 2021). The global production volume of common wheat was estimated to be almost 775 million metric tons in the previous marketing year (Tiseo, 2021). Wheat yield is primarily used for food, in particular, to produce flour and breadstuff. Bran from flour milling is a source of livestock feed. Furthermore, wheat grains are used in the production of alcoholic beverages, starch and industrial alcohol. It is noteworthy, that a significant number of grains is saved for further plantings (Shiferaw *et al.,* 2013).

Nowadays common wheat varieties are classified as spring wheat and winter wheat. Spring wheat is planted in early spring and harvested in late summer, while winter wheat is planted in autumn and harvested in summer (review: Hyles *et al.,* 2020). Nowadays, winter wheat varieties are predominantly grown in Estonia (FAOSTAT, 2021). The optimal growth conditions for wheat include 21-24°C temperature, relative humidity of 50-60% and a long (16 h) photoperiod (Strelec *et al.,* 2010).

The life cycle of common wheat consists of 2 phases: vegetative and generative. The vegetative phase is the period of plant development between seed germination and flowering. During this phase, the energy resources of the plant are directed primarily to the growth of leaves and roots. Wheat starts its life as a seed, which germinates in a week into a seedling. As soon as seedling form the primary root system and produce a stem with at least two leaves, the tillering stage begins (Figure 1) (Hyles *et al.,*2020). Tillers are the branches, which grow from the base of the main stem. In the future tillers could grow their own stem with seed head, which leads to higher grain yield per plant. The number of tillers depends on the wheat variety and environmental conditions such as water, nutrients and light (Bowden *et al.,*2008). After the plant finishes forming tillers, the stem extension stage starts (Hyles *et al.,*2020). This stage of growth comprises two parts: jointing and booting. During jointing, the main stem is stepwise telescoped upwards, followed by leaves formation. The final leaf to develop on the top of the stem is a flag leaf, which indicates that an adult vegetative plant becomes competent to form reproductive organs (Bowden *et al.,*2008).

The subsequent phase of plant development is the generative phase, during which the plant starts to produce kernels instead of leaves (Hyles *et al.,* 2020). The full development of the wheat spike occurs at the booting stage. The spike primordia are located just above the flag leaf by this time. In two weeks, the spike becomes swollen, and therefore visible beneath the sheath on the stem. The next stage is heading, when the spike fully emerges from the stem. Spike consists of spikelets, which, in turn, contain florets. Wheat florets are predominantly self-pollinated. After fertilization, florets ripen to form grain. During grain development, the kernel is filling and increases in size. The matured grain turns brown. Finally, the grain dries down until the moisture content reaches approximately 12%, which is low enough to be harvested and stored (Bowden *et al.,* 2008).

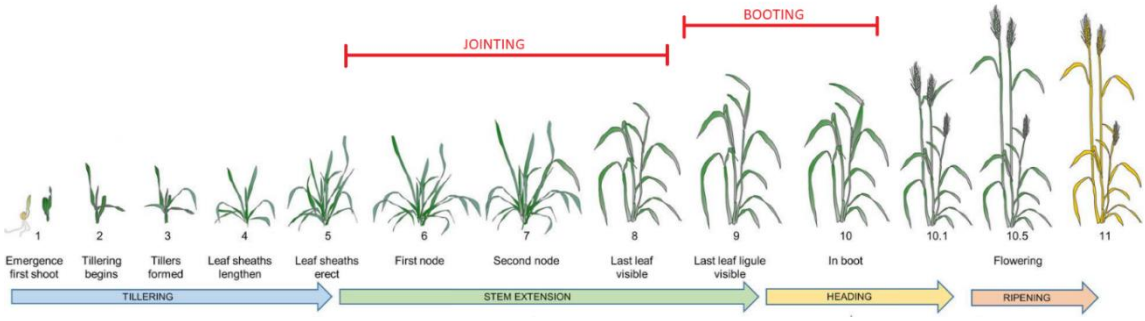

Figure 1. Life cycle of *Triticum aestivum* L. based on Feekes growth scale. Mature plant consists of primary and secondary roots, stem with leaves and spike. Adapted from Hyles *et al.* (2020).

A full life cycle of the spring wheat completes in approximately 120 days from germination to harvesting, and in about 240 days for winter wheat. The mature plants may reach up to 120 cm in height (Acevedo *et al.,* 2009).

It is noteworthy, that the *Triticum aestivum* L. genome was fully sequenced only in 2018 since it is relatively complicated (Guan *et al.,* 2020). Wheat has three closely-related diploid genomes called A, B and D genomes, which make plant hexaploid. Each subgenome consists of seven chromosomes, which gives 42 chromosomes in total. The whole-genome size is 17 Gb (Giga base pairs), containing about 107 891 genes (IWGSC, 2014). Wheat's high adaptivity to various environmental conditions could result from interactions between subgenomes (Dubcovsky and Dvorak, 2007).

Nowadays common wheat is naturalized around the world, but it is believed to be native to Assiros in Greek Macedonia (Figure 2) (Monfreda *et al.,* 2008; Bhogaonkar, 2019). Wheat is adapted to different ecological zones. For instance, wheat varieties grow in the tropical, subtropical, temperate and in the boreal zones (Kiss *et al.,* 2014). Moreover, wheat can grow in strongly acidic soil (Neeman, 1955). However, the plant absolutely does not tolerate heat and flooding, so countries with a warm and damp climate like Turkey, Egypt and Indonesia are dependent on the import of common wheat. In turn, the leading export countries are China, India and Russia. They produce 40 per cent of total wheat production. Estonia is rather self-sufficient in wheat production. According to statistics, it exported 581 600 tons of wheat in 2019, for which 179 998 hectares of agricultural land were used (FAOSTAT, 2021).

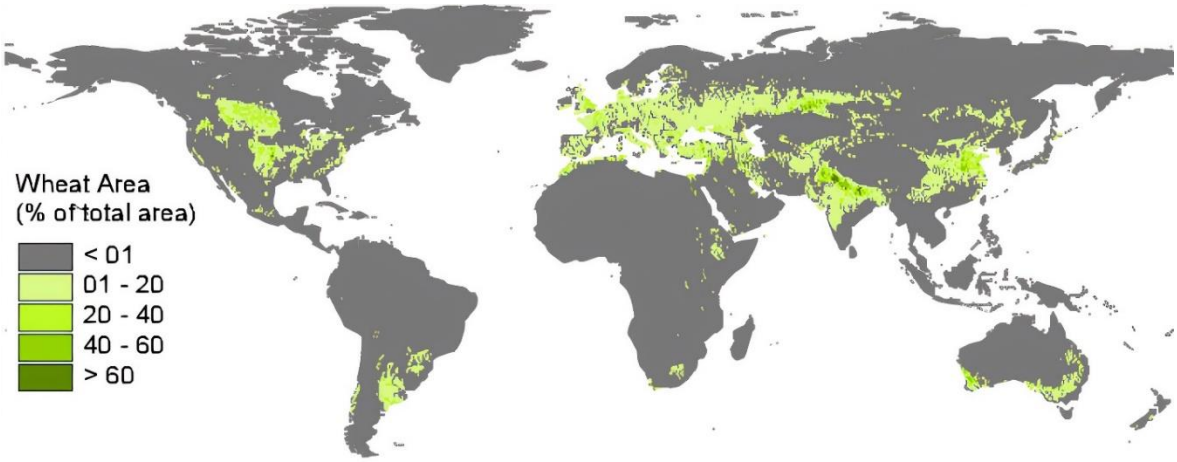

Figure 2. Global distribution of *Triticum aestivum L*. Adopted from Monfreda *et al.* (2000).

As was mentioned earlier, common wheat is widely distributed around the world (Monfreda *et al.,* 2008). Its varieties grow in complex environments, filled with various biotic and abiotic stressors. One of the reasons for such high adaptivity might be the naturally occurring allelic variation of vernalization (*VRN*) and photoperiod (*PPD*) genes. Together these genes determine whether plant could survive the drought, low or high temperatures, and give a rich yield (Whittal *et al.,* 2018).

1.2. Regulation of flowering time

The time of flowering is controlled by three systems. These are vernalization, photoperiod response and earliness per se (Kamran *et al.,* 2014).

Vernalization is a process, which stimulates the plant transition from vegetative to the generative stage after an extended period of exposure to low temperatures (Amasino, 2005). Those wheat varieties that require several weeks of cold treatment to flower are called winter wheat. They are sown in autumn, are vernalized during winter and then harvested in summer. The necessity of vernalization prevents them from flowering in winter thus protecting the plant's reproductive structures from frost damage. Although plants can respond to vernalization at any growth stage, the best effect is accomplished when plants are exposed to low temperature while germinating (Kamran *et al.,* 2014). Plant response to vernalization depends on the intensity of temperature and the duration of exposure (Rawson *et al.,* 1998). Spring wheat, in contrast, does not need vernalization to flower and can be sown at different times of the year. However, some cultivars could flower early when exposed to cold temperatures (Kamran *et al.,* 2014).

The second factor that regulates the flowering time in wheat is the photoperiod response. Photoperiod is the ratio of light and dark halves of the day necessary for a plant to flower. Wheat cultivars can be classified into photoperiod insensitive (PI) or photoperiod sensitive (PS) types (Distelfeld *et al.,* 2009; Zhao *et al.,* 2016). The PS plants are divided into three groups, depending on the required conditions to flower: long-day (24 h light), moderate-day (12 h light) and short-day (6 h light) cultivars (Zhao *et al.,* 2016). Long-day plants predominate in temperate zones, while short-day plants are prevalent in the south. Usually, winter wheat cultivars need a long day for flower transition, which is typical of spring. Whereas spring wheat cultivars in the south are mostly insensitive to day length and can flower even with short daylight (Hyles *et al.,* 2020). Thus, there is a direct relationship between the geographic origin of plants and the requirement in photoperiod (Kamran *et al.,* 2014).

The suggested third factor that regulates the flowering time in wheat, is *earliness per se gene* (*Eps*). Compared to the previous two, this factor has not been fully investigated, but it is assumed that *Eps* regulates flowering independently of external stimuli (Kamran *et al.,* 2014). *Eps* represents differences in time to heading that remains after photoperiod and vernalization are satisfied (Ochagavía *et al.,* 2019). Recent studies have proposed that *Eps* has an impact on the size and number of spikelets (Zikhali and Griffiths, 2015).

All these systems have a genetic basis. Vernalization, photoperiod response and earliness per se are mediated by *vernalization* (*VRN*), *photoperiod* (*PPD*) and *earliness per se genes* (*EPS*). Allelic variability resulting from insertions, deletions, and mutations in the promoter region, is present in all these systems (Dowla *et al.,* 2020). However, the *VRN* genes accounts for 70–75% of genetic

variability affecting flowering time, the *PPD* system for 20–25%, and only 5% of *earliness per se genes* (Stelmakh, 1998).

1.3. Vernalization genes

Vernalization is controlled by the *VRN-1*, *VRN-2*, *VRN-3* and *VRN-4* loci (Distelfeld *et al.,* 2009). *VRN1*, upregulated by vernalization, directly regulates the transition from vegetative to reproductive stage and flowering. *VRN2* is downregulated by vernalization and acts as an indirect repressor of the *VRN1* expression level, by repressing *VRN3*. Upregulated by vernalization *VRN3*, in the case of the dominant allele, further accelerates the development of the reproductive organs but eliminates the requirement for vernalization (Trevaskis *et al.,* 2007; Distelfeld *et al.,* 2009; Chen *et al.,* 2013). Each gene has a variety of alleles. Their different combinations can hasten or delay flowering in response to environmental stimuli (Zhang *et al.,* 2015). The first three loci have been well characterized, but limited information is available for *VRN-4*. It is known that it is located in the centromeric region of chromosome 5D (Yoshida *et al.,* 2010). A report by *Kippes et al., 2015* has discovered that *VRN-4* originated from the insertion of 290kb region of *VRN-A1*, containing distinctive mutations in its coding and regulatory regions, into the proximal region of chromosome arm 5DS. Interestingly, *VRN-4* was essential for the development of spring growth habit in the ancient wheats from South Asia and nowadays is still present with a high frequency in wheat varieties from this region (Kippes *et al.,* 2015).

At least one dominant allele at *VRN-1*, *VRN-3* or *VRN-4* loci is sufficient to determine a spring habit, which leads to partial or full insensitivity to cold treatment. In contrast, winter wheat possesses a dominant allele only at the *VRN-2* locus but recessive alleles at the other three loci, thus requiring exposure to cold temperatures for a certain time period to flower (Kamran *et al.,* 2014).

1.4. *VRN1* genes

Due to their major role in vernalization process, *VRN1* genes are the most extensively studied vernalization genes in wheat genome. By using RNA interference data, it has been shown that reduction of *VRN1* transcription levels resulted in delayed development of generative organs, increased the number of leaves, and extended heading time by 2 to 3 weeks (Loukoianov *et al.,* 2005).

Wheat comprises three orthologous copies of the *VRN1* gene, one in each of the three genomes: *VRN-A1*, *VRN-B1*, and *VRN-D1* located on the long arm of homeologous chromosomes 5A, 5B and 5D, respectively (Pugsley, 1971). The presence of at least one dominant allele at *VRN-A*, *VRN-B* or *VRN-D* loci is enough to confer wheat with a spring growth habit. In spring wheat varieties *VRN1* alleles are usually expressed at high levels. In contrast, the genotype with all recessive alleles (*vrn-A*, *vrn-B*, *vrn-D*) represents winter wheat and requires exposure to low temperatures to flower. In winter wheat varieties the expression level of *VRN1* is initially low but increases during vernalization and remains high through subsequent stages of spike development (Yan *et al.,* 2004a; Loukoianov *et al.,* 2005; Trevaskis *et al.,* 2007).

The recessive winter allele is believed to be the wild-type allele since most of the wild *Triticeae* species have a winter growth habit, so the genetic characteristics are described by comparison with the *vrn-1* gene (Shcherban, 2015). All dominant alleles are considered to be originated from recessive alleles through insertions, deletions or mutations in the promotor or first intron (Loukoianov *et al.,* 2005; Fu *et al.,* 2005)

It is noteworthy, that the *VRN-1* locus is ortholog of the *Arabidopsis thaliana* meristem identity gene, called *APETALA1* (*AP1*) (Trevaskis *et al.,* 2003). Both of them encode transcription factor that regulates the spike's meristem identity. However, *AP1* is not directly regulated by vernalization, and no natural or induced allelic variation at this locus has been found to be associated with differences in vernalization requirement in *Arabidopsis thaliana* (Trevaskis *et al.,* 2003; Loukoianov *et al.,* 2005).

1.4.1. *VRN-A1* alleles

One recessive (*vrn-A1*) and three dominant alleles were identified at *VRN-A1* locus in common wheat: *Vrn-A1a*, *Vrn-A1b* and *Vrn-A1c* (Figure 3) (Yan *et al.,* 2004a). *VRN-A1* alleles vary considerably. Differences were found in the promoter, exon, and intron regions (Yan *et al.,* 2004a; Fu *et al.,* 2005).

Vrn-A1a allele is characterized by duplicated promoter region. Each copy contains the insertion of 43 bp and 131 bp foldback repetitive elements at the same location (Yan *et al.,* 2004a; Zhang *et al.,* 2015). Wheat varieties, having this allele, demonstrate absolute elimination of the vernalization requirement (Kiss *et al.,* 2014). The *Vrn-A1b* allele has several single nucleotide polymorphisms (SNPs) in the host direct duplication (HDD) region and 20 bp deletion in the promoter region. The final *Vrn-A1c* allele has been described as having a large 5,504 bp deletion in intron 1 (Yan *et al.,* 2004a; Fu *et al.,* 2005; Zhang *et al.,* 2015).

In case of diploid and tetraploid wheat, *Vrn-A1d*, *Vrn-A1e* and *Vrn-A1f* alleles were also presented. These alleles are characterized, respectively, by 32 bp, 54 bp and 50 bp deletion in the promoter region (Ivaničova *et al.,* 2016).

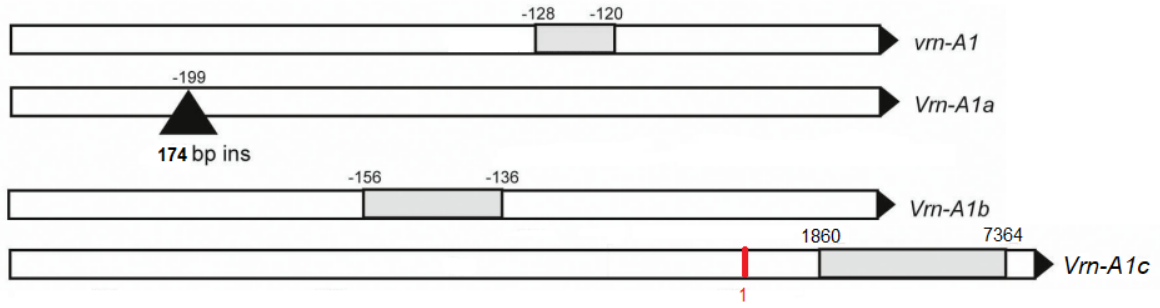

Figure 3. Allelic variation of the *VRN-A1* gene due to deletions/insertions in intron 1 and promoter region. White rectangles correspond to nucleotide sequences. Grey rectangles represent deletions, while black triangle represents insertions. The red vertical line with the number 1 shows the start point of the coding region. Adapted from Golovnina *et al.,* (2010).

1.4.2. *VRN-B1* alleles

In contrast to *VRN-A1* genes, *VRN-B1* genes do not have differences in promoter regions compared to their respective recessive alleles (Yan *et al.,* 2004a; Fu *et al.,* 2005). *Vrn-B1* alleles mostly result from large deletions in their first intron associated with spring growth habit (Fu *et al.,* 2005; Milec *et al.,* 2013).

The allele variants of the *VRN-B1* gene so far detected comprise three dominant (*Vrn-B1a*, *Vrn-B1b*, *Vrn-B1c*) and one recessive allele (*vrn-B1*) (Figure 4). Compared to the *Vrn-A1*, the dominant *Vrn-B1* alleles alone only partially eliminate the vernalization requirement, which manifests in some residual response to cold treatment and late flowering (Stelmakh, 1998).

Vrn-B1a differs from the winter *vrn-B1* allele by a 440 bp deletion in intron 1 (Santra *et al.,* 2009). The same deletion is present in the *Vrn-B1b* allele, which, in addition, has a 36 bp deletion and a single nucleotide polymorphism (G–C) in the first intron (Santra *et al.,* 2009; Milec *et al.,* 2012). However, this SNP is considered a silent mutation, hence it does not have any effect on the vernalization response. The last discovered dominant allele was *Vrn-B1c*, which is characterized by an 817 bp deletion within intron 1 and either a duplication or translocation of a 450 bp fragment into the first intron of *Vrn-B1*. However, effect on flowering time was determined near-isogenic lines (NIL) carrying the *Vrn-B1c* allele were observed to head nearly 14 days earlier than NILs with the *Vrn-B1a* (Milec *et al.,* 2013).

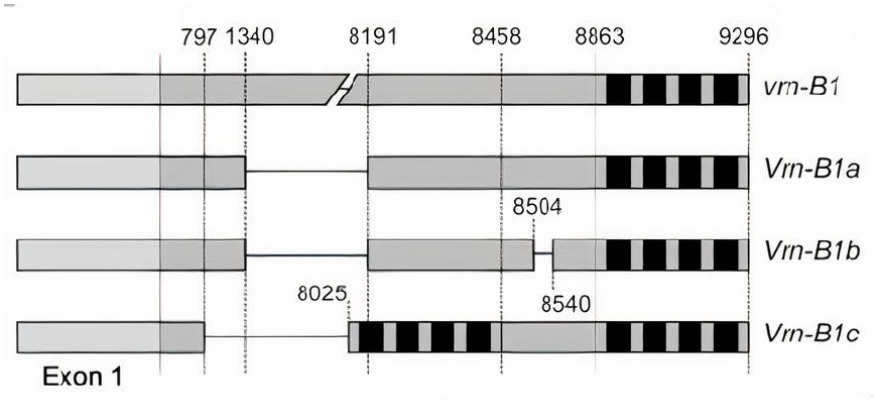

Figure 4. Allelic variation of the *VRN-B1* gene in first intron variation. Alleles variety is characterized by large deletions. Light grey rectangle represent exon 1, while dark grey rectangle represents intron 1. Deletions showed by lines. The rectangle with black vertical stripes represents an identical region duplicated in all alleles. Adapted from Milec *et al*. (2012).

1.4.3. *VRN-D1* alleles

As *VRN-B1* dominant alleles, all cultivars with dominant *VRN-D1* alleles have demonstrated partial sensitivity to vernalization (Fu *et al.,* 2005; Zhang *et al.,* 2008).

VRN-D1 loci include one recessive and four dominant allelic variants (Figure 5). *Vrn-D1a* allele has a 4,235 bp deletion in the first intron. The *Vrn-D1b* allele includes the same deletion and a SNP (C-A) in the promoter region. The plants having the *Vrn-D1b* allele demonstrated 32 days later heading than those with *Vrn-D1a*, which means that *Vrn-D1b* has a lower spring pattern effect (Zhang *et al.,*

2008). The *Vrn-D1c* allele was discovered in 2015 and was characterized by 174 bp insertion in the promoter region of the recessive allele *vrn-D1*. It is proposed that *Vrn-D1c* may increase *Vrn-D1* gene expression, and, therefore, induce early flowering (Zhang *et al.,* 2015). In the same year, the *Vrn-D1s* allele was found, which results from the insertion of an 844 bp DNA transposon into the intron 1 (Muterko *et al.,* 2015).

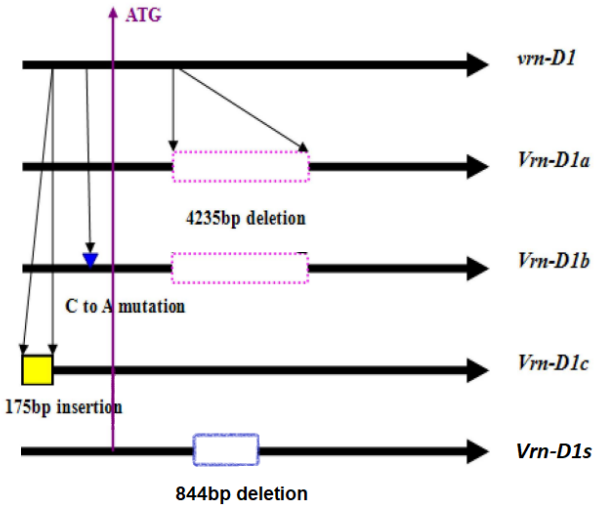

Figure 5. Schematic picture of all *VRN-D1* alleles identified in the wheat cultivars to date. Purple upper arrow means ATG translation initiation codon. Modified from Zhang *et al.,* (2015).

1.5. *VRN2* genes

VRN2 gene is the second major gene responsible for the vernalization response in common wheat. *VRN2* acts as a repressor of VRN3, thereby preventing flowering. RNA interference of the *VRN2* gene demonstrated acceleration of the flowering time in plants by more than a month. The wheat *VRN2* gene has no homologues in Arabidopsis (Yan *et al.,* 2004b).

VRN2 genes are also called *ZCCT* genes, since the proteins that they encode contain 'zinc finger' motive and a CCT domain (Yan *et al.,* 2004b; Zhu *et al.,* 2010). The CCT domain is a 43-amino acid region, which participates in generation of circadian rhythms and photoperiodic sensitive flowering (Wenkel *et al.,* 2006). Each *VRN-2* locus includes two repeated genes named *ZCCT1* and *ZCCT2* (Yan *et al.,* 2004b).

In common wheat, *VRN-A2*, *VRN-B2* and *VRN-D2* loci are located on chromosomes 5AL, 4B and 4D, respectively (Tan and Yan, 2015). Interestingly, *VRN-A2* was translocated from the long arm of chromosome 4 to the long arm of chromosome 5. The same translocation was found in the diploid and tetraploid wheat genomes (Dubcovsky *et al.,* 1998; Yan *et al.,* 2004b).

VRN-2 has three homeologous loci: *VRN-A2*, *VRN-B2* and *VRN-D2*, each of them can be represented by dominant or recessive alleles (Yan *et al.,* 2004b). The dominant allele is considered a wild type. Recessive allele differs from dominant alleles by complete allele deletion (null allele) (Zhu *et al.,* 2010) or point mutations (Distelfeld *et al.,* 2009). Any winter wheat cultivar has at least one dominant allele in some *VRN-2* locus, and a recessive *vrn1* and *vrn3* alleles in all three homologous genomes. Spring wheat cultivars, in turn, carry recessive *vrn2*, but dominant *Vrn1* and *Vrn3* alleles (Yan *et al.,* 2004b).

Until recently natural variation in *VRN-2* has been only detected in diploid and tetraploid wheats (Yan *et al.,* 2004b; Distelfeld *et al.,* 2009). Currently the null alleles at each *VRN-A2*, *VRN-B2* and *VRN-D2* were found in breeding lines and landraces from different region of the USA and China (Zhu *et al.,* 2010). Additionally, it was found that *VRN-B2* can be represented by two allelic forms: *Vrn-B2a-1* and *Vrn-B2a-2*. In contrast to *Vrn-B2a-1*, the sequence of *Vrn-B2a-2* contains a "CAC" motif at positions 136-138 bp from the start codons and 5 additional SNPs (Tan and Yan, 2016).

Despite the fact that the nonfunctional allele of the *VRN2* gene was found in each of the *ZCCT-A1*, *ZCCT-B1* and *ZCCT-D1* genes, no exaploidy wheat cultivar with natural null alleles in all three genes has been found. In order to investigate the effect of the absolutely nonfunctional *VRN2* gene, a *VRN-B2-null* plant containing three null alleles, was developed (Kippes *et al.,* 2016). *VRN-B2-null* plants demonstrated spring growth habit and limited vernalization response.

1.6. *VRN3* genes

The last but not least vernalization gene, *VRN3*, functions as a flowering promoter by upregulating *VRN1* genes (Yan *et al.,* 2006; Distelfeld *et al.,* 2009). *VRN3* gene is represented by three homeologous loci: *VRN-A3*, *VRN-B3*, and *VRN-D3*, located on 7A, 7B and 7D chromosomes, respectively. The presence of at least one *Vrn-3* allele results in spring growth habit, while a set of *vrn-3* alleles causes winter growth habit. Recessive *vrn-3* is also a wild type (Bonin *et al.,* 2008).

Only two allelic forms of *VRN-A3* have been discovered so far in tetraploid wheat, differing by a SNP polymorphism identified in exon 3 (Bonnin *et al.,* 2008).

Allelic varieties of *VRN-B3* gene include one recessive and five dominant alleles (Figure 6). *Vrn-B3a*, in comparison to *vrn-B3*, contains a 5,295 bp retrotransposon in the promoter region (Yan *et al.,* 2006; Bonnin *et al.,* 2008). *Vrn-B3b* also has an 890 bp insertion in the promoter region, which significantly decreases gene transcription and causes plants' later flowering (Yan *et al.,* 2006; Chen *et al.,* 2013). Notably, *Vrn-B3b* is a rare allele, which was found only in one variety in China. The promoter region of *Vrn-B3c* has a 5,300 bp insertion with a 20 and 4 bp deletion (Chen *et al.,* 2013). Furthermore, two dominant alleles have been recently discovered: *Vrn-B3d*, having 1,617 bp insertion, and *Vrn-B3e* with 160 bp insertion in the promoter region (Berezhnaya *et al.,* 2021). Both of them flower later than wild types. The *VRN-B3* gene is an ortholog of the *Arabidopsis FLOWERING TIME1* gene (Yan *et al.,* 2006).

Two recessive alleles have been found for *VRN-D3* gene: *vrn-D3a* and *vrn-D3b*. An SNP in the *vrn-D3b* allele creates an 81 amino acid frameshift, which leads to a later heading date compared to plants carrying the *vrn-D3a* allele (Bonnin *et al.,* 2008; Li *et al.,* 2017).

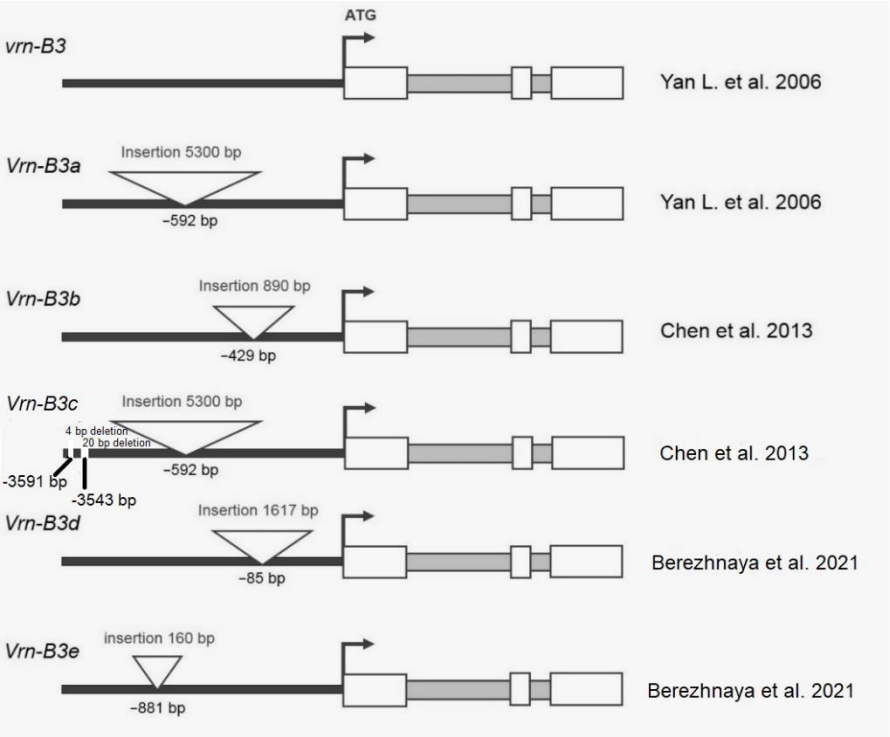

Figure 6. Possible allele varieties at *VRN-B3* loci. The white triangles represent insertions, the white rectangles represent exons, and the black arrows represent the transcriptional start. Adapted from Berezhnaya *et al.* (2021).

1.7. Photoperiod genes

Photoperiodism is important for plant survival and environmental adaptation. Along with vernalization genes, photoperiod response (*PPD*) genes play a key role in the control of the transition of wheat from vegetative to the generative stage, thus determining the most appropriate time for flowering (Stelmakh *et al.,* 1998). The *PPD* expression level relies on daylength and amount of consistent sunny days. Plants can be long-day, short-day, or day-neutral. Wheat wild type is a long-day plant, which means that flowering is accelerated when days are long, but delayed during short days (Kumar *et al.,* 2011). Some wheat varieties carry a photoperiod insensitive genotype, which means that plants have a short delay in flowering in short day conditions compared to long day conditions and can flower even with short daylight (Hyles *et al.,* 2020). These insensitive cultivars are beneficial in lower latitudes, where day length does not change much, such as near the equator and in areas where high summer temperatures and drought become an issue. However, plants growing in temperate climate are mostly photoperiod sensitive, in order to produce a harvest in a short growing season and avoid abiotic stresses (Kiss *et al.,* 2014; Zhao *et al.,* 2016).

Photoperiod-response locus in wheat comprises *PPD-A1*, *PPD-B1* and *PPD-D1* alleles, which are located on chromosomes 2A, 2B, and 2D, respectively (Pugsley, 1971). Different allelic variations have been shown within the photoperiod genes that have an effect on flowering time (Kiss *et al.,* 2014).

Two allelic forms of *PPD-A1* have been reported in common wheat varieties (Beales *et al.,* 2007). The allelic sensitive form, called *Ppd-A1b.1*, was found in Chinese Spring and has a 1.2 kb insertion

in intron 5. The insensitive allele *Ppd-A1* was identified in the Japanese cultivar Chihokukomugi. It differs from *Ppd-A1b.2* by a 1,085 bp deletion in the 5' upstream region (Nishida *et al.,* 2013). Interestingly, *Ppd-A1a* of durum wheat has a deletion in the same region, so it was proposed that regulatory elements are required in this region for precise diurnal expression patterns and for photoperiod response (Bentley, *et al.,* 2011; Wilhelm *et al.,* 2009).

PPD-B1 locus also has two alleles. The insensitive *Ppd-B1a*, identified from Winter-Abukumawase, has an insertion of 308 bp, in contrast to the full sequence of photoperiod-sensitive *Ppd-B1b* allele from Chihokukomugi (Nishida *et al.,* 2013). Furthermore, *Beales et al., (2007)* found several SNPs and a retrotransposon insertion in *Ppd-B1* genes of hexaploid wheat, however, none affects the photoperiodic response.

PPD-D1 is the most significant photoperiod response locus. *PPD-D1* has two alleles that determine photoperiod response. *Ppd-D1a* demonstrated the insensitivity to photoperiod. The only polymorphism found to confer photoperiod insensitivity of the *Ppd-D1a* allele is the 2,089 bp deletion in the promoter region. Probably photoperiod insensitivity was caused by misexpression of the *PPD-D1* gene and, therefore, increased expression of *VRN3* under short and long days (Beales *et al.,* 2007). *Ppd-D1b* is sensitive to daylength and is considered to be the ancestral form of the *PPD-D1* (Zhao *et al.,* 2016).

1.8. Regulation of flowering time

In a temperate climate, the winter variety of wheat is planted in late summer or autumn (Trevaskis *et al.,* 2007; Distelfeld *et al.,* 2009). The plant attains the tillering stage before the onset of winter. Gene expression of *VRN1* is low at this moment (Figure 7). However, long autumn days induce high levels of *VRN2*, which suppresses *VRN3* expression, thereby preventing the plant's transition to flowering. Then due to long cold days in winter, vernalization occurs and activates the transcription of *VRN1* in leaves and generative organs, followed by downregulation of *VRN2* (Trevaskis *et al.,* 2007). An increase in daylight hours in spring promotes activation of *PPD* genes, thereby upregulating the expression of *CONSTANS* (*CO*) genes, which integrate light and circadian clock signals to upregulate the *VRN3* (Distelfeld *et al.,* 2009; Jung *et al.,* 2009). Furthermore, the level of *VRN2* is now so low that it no longer interferes with the release of *VRN3*. *VRN3* transcripts are exported to the shoot tip, where *VRN1* is induced above the threshold required to start the plant transition to flowering. The flowering stage usually occurs in late spring or early summer (Trevaskis *et al.,* 2007).

In summer wheat varieties *VRN1* is initially expressed at high basal levels, which directly represses *VRN2*, and permits flowering. In the case of photoperiod-sensitive varieties, long days induce expression of *VRN3* and further accelerate floral development (Trevaskis *et al.,* 2007).

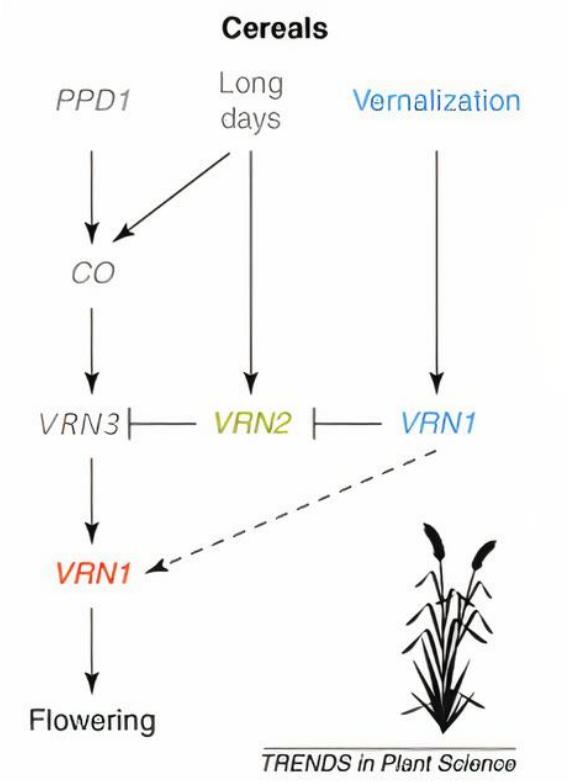

Figure 7. A scheme of regulation flowering in photoperiod-sensitive winter wheat. In particular, vernalization and photoperiod gene expression pathway. The interactions among *VRN1*, *VRN2*, and *VRN3* genes form a feedback regulatory loop, so changes in the transcript levels of any one of these genes affect the transcription of the others. Long sunny days activate the *VRN2* gene suppressing *VRN3*. Vernalization induces *VRN1*, which represses *VRN2*. Low *VRN2* level and long-day induction activate *VRN3*, which in turn, activates *VRN1*. The high VRN1 expression results in flowering. Modified from Trevaskis *et al.* (2007).

1.9. Gene-specific markers

Information about the genetic variation present within or between different populations can be useful in understanding natural breeding (Kage *et al.,* 2016). The tool, by which the existing variations can be detected, is functional markers (FM). Functional markers constitute nucleotide sequences, associated with a certain location within the genome. Using the PCR method followed by electrophoresis, variation or polymorphism in DNA samples for a particular region of the DNA sequence can be identified (Jiang, 2013; Nadeem *et al.,* 2018). To determine the allelic variation of vernalization and photoperiod genes in different wheat varieties, gene-specific primers are constructed. Initially, all analyzed genes have to be sequenced, and then the genome-specific primers can be made (Liu *et al.,* 2012).

Functional markers can accurately distinguish alleles at a single locus (Liu *et al.,* 2012). Nowadays, using FM, about 926 Chinese wheat varieties were characterized for their genotypes at the *Ppd-D1* locus (Yang *et al.,* 2009). Moreover, over two hundred varieties of Chinese wheat have been tested for the *VRN-A1*, *VRN-B1*, *VRN-D1* and *VRN-B3* vernalization genes (Zhang *et al.,* 2008). Functional markers can also be used for analyzing disease resistance locus and the presence of SNPs or insertions/deletions, which can affect gene transcription (Liu *et al.,* 2012).

2. Aims of the Study

The aim of this study was to evaluate allelic variation at *VRN* and *PPD* loci in a set of wheat cultivars cultivated in Estonia.

Specific objectives:

1. To characterize allelic variation at the nine vernalization loci *VRN-A1*, *VRN-B1*, *VRN-D1*, *VRN-A2*, *VRN-B2*, *VRN-D2*, *VRN-A3*, *VRN-B3*, *VRN-D3*, and 3 photoperiod response loci *PPD-A1*, *PPD-B1*, *PPD-D1* in the selected 87 wheat varieties grown in Estonia using molecular markers.
2. Assess the variability in the time of flowering of the analyzed varieties in controlled conditions.
3. To evaluate the possible relationship between genotypes in *VRN* and *PPD* loci and observed flowering data of the cultivars.

3. Experimental part

3.1. Plant material and growth conditions

The 87 *Triticum aestivum* L. cultivars analyzed in this study (Table 1) covered all wheat varieties in the Estonian Variety List (in June 2021). Varieties were included into the List after testing in field trials (Estonian Agricultural Research Centre). The list should represent genotypes, which are well adapted to local environment. The Variety list includes both winter and spring wheat varieties. The seeds were provided by Estonian Agricultural Research Centre.

Table 1. Accession numbers and growth habits of the common wheat cultivars analyzed for *VRN* and *PPD* allelic combinations.

Nr	Name	Accession number	Habit
1	Aateli	Bor 11203	Spring
2	Akvitan	TRI 0902.1	Spring
3	Bailando	STRU093735s5	Spring
4	Berlock	SW 71139	Spring
5	Broca	TRI_S 08259.18-5	Spring
6	Calixo	SEC 426-01-2b	Spring
7	Cornetto	SEC 431-01-9	Spring
8	Daugana	CH 211.13640	Spring
9	Fidibus	STRU 073283k0311	Spring
10	Flippen	SW 11361	Spring
11	Florens	SEC 503-08-3	Spring
12	Happy	SW 91003	Spring
13	Harenda	MHR-KPJ-0611	Spring
14	Herero	STRU 082056K051	Spring
15	Hiie	200	Spring
16	KWS Buran	KW 760-2-08	Spring
17	KWS Collada	LP 588-1-06	Spring
18	KWS Expectum	KW 538-3-14	Spring
19	KWS Mistral	KW 655-3-10	Spring
20	KWS Sharki	KW 535-2-12	Spring
21	KWS Starlight	KW 440-2-14	Spring
22	Leidi	BOR 09243	Spring
23	Levels	SW 131281	Spring
24	Licamero	SEC426-01-1b	Spring
25	Intelligence	TRI_S 08259.6-2	Spring
26	Manu	Hja 23133	Spring
27	Mooni	BOJ 10102	Spring
28	PS Perlicka	PS-30	Spring
29	Quintus	LW04SW744-06	Spring
30	Sibelius	STRU 093754S14	Spring

31	Signal	TRI 0908.4	Spring
32	Sorbas	TRI.000.221	Spring
33	SU Ahab	STRU 093755s15	Spring
34	Tybalt	ZE98-1489	Spring
35	Uffo	Std911331	Spring
36	Wicki	SEC 431-01-3	Spring
37	Vilnius	STRU 073286k011	Spring
38	Voore	186.2.4	Spring
39	WPB Lambada	WPB 11SW250-10	Spring
40	WPB Troy	WPB 09SW064-20	Spring
41	Hymalaya	NOST 11 C 1244	Winter
42	KWS Ahoi	KW 2418-13	Winter
43	KWS Spencer	KW 3807-5-08	Winter
44	Ancher	Sj K0255	Winter
45	Zoltan	LEU 60216	Winter
46	Ruske	90.3.1	Winter
47	Askaban	LEU 50307	Winter
48	SY Landrich	SY 114521	Winter
49	KWS Emil	KW 3844-5-07	Winter
50	Bonanza	BB 732009 W	Winter
51	Ramiro	TAW 1.42918/81	Winter
52	Astronaut	LEU 60613	Winter
53	SU Mendoza	NORD 15/203	Winter
54	Janne	NIC05-4588-A	Winter
55	Davinci	LEU 60305	Winter
56	Balitus	SZD1249	Winter
57	Hellas	SW 15541	Winter
58	Ada	2903-118	Winter
59	Fredis	L193130	Winter
60	Hallfreda	SW 15646	Winter
61	Kena DS	LŽI 5450-1	Winter
62	Nemunas	LIA 0044	Winter
63	Malunas	NOS 709-1494	Winter
64	Rotax	STRU 081966	Winter
65	Creator	SJ 8544003	Winter
66	Lemmy	NORD 08184/096	Winter
67	Johanna	SW 25673	Winter
68	Effekt	SW 85131	Winter
69	Edvins	St.A-1	Winter
70	Etana	LEU 90209	Winter
71	Festival	SW 95594	Winter
72	Ceylon	SW 75107	Winter
73	Skagen	PF798-398 B	Winter

74	Julius	LP03 1056-1	Winter
75	Nordika	HE8352	Winter
76	Architekt	LEU 50218	Winter
77	Talsis	L-96-58	Winter
78	Perenaise	J154.6.1.5	Winter
79	Kallas	LIA 00134	Winter
80	Lutz	TRI_S 08253.5	Spring
81	KWS Meilo	LP1794-4-05	Winter
82	Jasmund	STRU112230.3	Spring
83	Anabel	SG-S1257-09	Spring
84	WPB Match	WPB 12SW484-07	Spring
85	Kajus	STRU 082388k016	Spring
86	Lindras	TRI_S 07251.1-2	Spring
87	Mireete	2.25.1.7.1.16.11.	Spring

Additionally, cultivars Avenju, Genius, RGT Reform and Nuginool were included as perceptively useful for breeding (personal communication Reine Koppel). Cultivars Chinese Spring, Opata, Triso, Granny, Marquise, Thatcher, Tähti and Hope carrying well known alleles of the *VRN* and *PPD* genes were used as controls.

3.2. DNA extraction

All plants were grown in a plant room at 24°C with a 16 hour photoperiod and 70 % humidity. For DNA extraction, leaves from three one-week-old plants were collected and stored at 37°C for two days.

The modified CTAB method was used to isolate plant DNA. The method included physical (lysis) and chemical (precipitation and purification) methods. For the lysis of plant cells, plastic beads were added to the tubes containing plant leaves. Using TissueLyser (QIAGEN) the samples were homogenized by shaking at a high frequency (30/s) for 1 minute. To purify DNA 900 µl of 1.5% CTAB Buffer (Appendix 1) and 2 µl of β-mercaptoethanol were added. Tubes were placed in a 60°C water bath for 30 minutes, and then on ice for 5 minutes. To separate the aqueous and organic phases, 900 µl of chloroform-isoamyl alcohol (24:1) was used. After centrifuging at 8000 rpm for 10 minutes at room temperature (RT), 700 µl of the aqueous phase was transferred into a new 1.5 ml tube. Also, 3 µl of RNAse A was added. Then samples were incubated for 30 minutes at RT. DNA was precipitated with 700 µl cold isopropanol by 30 minutes of incubation at -20°C, followed by centrifugation (13 000 rpm) for 3 minutes at 4°C. The resulting pellets were washed with 1 ml of cold 70 % ethanol by overnight incubation at 4°C. Subsequently, centrifugated (13 000 rpm) for 3 minutes at 4°C and removed ethanol. The previous washing steps were repeated once again. Finally, tubes were incubated for 30 min at 4°C and therefore centrifugated (13 000 rpm) for 3 minutes at 4°C. After removing the supernatant, DNA was air-dried and dissolved in 50 µl TE buffer (Appendix 1). On the next day, 450 µl of MQ H_2O was added.

3.3. PCR amplification of allele-specific markers

DNA fragments, containing required alleles, were amplified using the polymerase chain reaction (PCR) method. The mixture prepared for PCR included 2x DreamTaq-Green PCR Master Mix (Thermo Scientific); 0,35 µmol of each forward and reverse primers and 100 ng of template DNA.

Allelic variation at the *VRN-A1*, *VRN-B1*, *VRN -B3*, *VRN-D1*, *PPD-A1*, *PPD-B1* and *PPD-D1* loci was determined using previously reported allele-specific primers under the same PCR reaction conditions as in in the respective articles (Table 2).

To control whether DNA amplification succeeded, PCR product was loaded onto 1.5 %, 2.5% or 3.5% agarose gel and run in 1xTAE Buffer at 160 V. Amplification products were visualized under UV light. As the size markers, the following representatives were used: 50 bp Gene Ruler, 100 bp plus Gene Ruler, Low Range Gene Ruler and Middle Range FastRuler.

To separate PCR products differing only slightly, fragment analysis (capillary electrophoresis) was applied instead of agarose gel electrophoresis for primers V2ABD-F1 and V2ABD-R2. In this case, amplifications were performed in a 10-µL reaction volume consisting of 10 ng template DNA, 2.5 pmol of forward, 5 pmol of reverse primers, 5 pmol of fluorescent dye and DreamTaq PCR Master Mix (Thermo Scientific). A touch-down program (60°C down to 53°C for annealing temperature) was performed before the regular program was performed.

3.4. Phenotypic evaluation of flowering time in *Triticum aestivum* L. cultivars

To promote synchronized germination, about 20 seeds of each cultivar were soaked in cold water in dark for 5 days at 4°C and transferred to 24°C overnight.

In the experiment, each spring wheat cultivar was represented by four growing pots, containing 3 plantlets each.

Germinated seedlings were planted in soil (Biolan, in plastic pots 15 cm x14 cm, volume 1 L). Plants were grown in a greenroom under controlled environmental conditions (16/8 h day/night, temperature 24°C, humidity 70%. Illuminance varied in different zones of the plant greenroom, and illuminance for a half of the pots (2 pots - 6 plants) with spring wheat cultivars was 110 µE m^{-2} s^{-1}, for a quarter (1 pot – 3 plants), it was 120 µE m^{-2} s^{-1}, and for the last quarter, it was 135 µE m^{-2} s^{-1}.

As for winter wheat, presoaked synchronized seedlings were vernalized for 8 weeks at 4°C. Altogether three pots of each cultivar were grown under three illuminance levels: 110, 120 or 180 µE m^{-2} s^{-1}, respectively.

The appearance of half of the head was tracked by daily observing, and flowering time was defined as the number of days passed after seeds were transferred to the greenroom at 24°C.

Data were analyzed using Microsoft Excel.

Table 2. Primer sequences, annealing temperatures, and expected PCR product sizes.

Locus	Alleles	Primer name	Primer sequence (5'-3')	Expected band size (bp)	Annealing temp. °C	Reference
VRN-A1	*vrn-A1/Vrn-A1a/ Vrn-A1b/ Vrn-A1c*	VRN1AF	GAAAGGAAAAATTCTGCTCG	734/965 + 876/714 / 734	50	Yan *et al.* 2004a
		VRN1-INT1R	GCAGGAAATCGAAATCGAAG			
	vrn-A1	Intr1/C/F	GCACTCCTAACCCACTAACC	1068	58	Fu *et al.* 2005
		Intr1/AB/R	TCATCCATCATCAAGGCAAA			
VRN-B1	*vrn-B1/Vrn-B1a/ Vrn-B1b/Vrn-B1c*	Intr/B/F	CAAGCGGAACGGTTAGGACA	1149/ 709 +1235 / 673 +1199/ 849	56	Milec *et al.* 2012
		Ex1/B/F3	GAAGCGGATCGAGAACAAGA			
		Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA			
		Intr1/B/R4	CAAATGAAAAGGAATGAGAGCA			
VRN-D1	*Vrn-D1*	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	1671	65	Fu *et al.* 2005
		Intr1/D/R3	GGTCACTGGTGGTCTGTGC			
	vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	997	63	Fu *et al.* 2005
		Intr1/D/R4	AAATGAAAAGGAACGAGAGCG			
VRN-2	*Vrn-A2/Vrn-B2/Vrn-D2*	V2ABD-F1	GAAAGAAATCAACGATGGATC	302/ 294/ 320	55	Zhu *et al.* 2010
		V2ABD-R2	ACTGCTAGCTAGCTCCAAGG			
	Vrn-A2/Vrn-B2/Vrn-D2	V2ABD-F2	ATCAACGATGGATCGAGGGA	222 /214/240	55	Zhu et *al*. 2010
		V2ABD-R1	AGGAGAGATGTCGAGGAAGAAG			
	Vrn-B2 (ZCCT-B1)	V2B-F2	ATGTGAGAGAGAGACGCAGTA	1126	57	Zhu *et al.* 2010
		V2B-R1	AAGAGATATGTTATATTATCGAAATT			
VRN-A3	*vrn-A3*	FTex3gAL_F	ATGGTCGGACGAGGGCTCTCA	761	74-64[a]	Bonnin et a*l.* 2008
		FTex1U_R	TCAGGGTGACCTTCGGGAACA			
VRN-B3	*vrn-B3*	FTex3gBL_F	CGCCCGAGCTGCTGGAAGAGT	1031	72-62[a]	Bonnin *et al.* 2008
		FTex1U_R	TCAGGGTGACCTTCGGGAACA			
	Vrn-B3a	FT-B-NOINS-F	ATGCTTTCGCTTGCCATCC	1140	57	Yan *et al.* 2006
		FT-B-NOINS-R	CTATCCCTACCGGCCATTAG			

Table 2. Primer sequences, annealing temperatures, and expected PCR product sizes (continued).

Locus	Alleles	Primer name	Primer sequence (5'-3')	Expected band size (bp)	Annealing temp. °C	Reference
VRN-B3	*Vrn-B3c*	P14-F	GCTTTGAACTCCAAGGAGAA	1401	52	Chen *et al.* 2013
		P14-R	ATAATCAGCAGGTGAACCAG			
	vrn-B3/Vrn-B3d/ Vrn-B3e	FTpr-F	C GAAAGCGGAGGGTATATTAAA	1384/3001/ 1544	60	Berezhnaya *et al.* 2021
		FTpr-R	CCCCGAACATAGAAGAAGCATAG			
VRN-D3	*vrn-D3*	FTex3gDL	AAGAGCACGAGCACGAAGCGA	784	62	Bonnin *et al.* 2008
		FTex1U	TCAGGGTGACCTTCGGGAACA			
PPD-A	*Intact Ppd-A1a/ GS100 1027 bp del/ 1117 bp del*	durum_Ag5del_F1	GTATGCGATTCGCCTGAAGT	452/ 380/ 290	56	Wilhelm *et al.* 2009
		durum_Ag5del_F2	CGTCACCCATGCACTCTGTT			
		durum_Ag5del_R2	CTGGCTCCAAGAGGAAACAC			
	TaPpd-A1a.1/ TaPpd-A1b.1	TaPpd-A1prode _IF	CGTACTCCCTCCGTTTCTTT	338/ 299	57	Nishida *et al.* 2013
		TaPpd-A1prode _IR2	GTTGGGGTCGTTTGGTGGTG			
		TaPpd-A1prode IR3	AATTTACGGGGACCAAATACC			
PPD-B	*Ppd-B1 PRR partly deletion*	Ppd-B1_2ndcopy_F1	TAACTGCTCGTCACAAGTGC	425	55	Zhao *et al.* 2016
		Ppd-B1_2ndcopy_R1	CCGGAACCTGAGGATCATC			
	TaPpd-B1a.1/ TaPpd-B1a.2	TaPpd-B1proinF1	CAGCTCCTCCGTTTGCTTCC	620/312	70-60[a]	Nishida *et al.* 2013
		TaPpd-B1proinR1	CAGAGGAGTAGTCCGCGTGT			
PPD-D	*Ppd-D1a/ Ppd-D1b*	Ppd-D1_F	ACGCCTCCCACTACACTG	288/ 414	54	Beales *et al.* 2007
		Ppd-D1_R1	GTTGGTTCAAACAGAGAGC			
		Ppd-D1_R2	CACTGGTGGTAGCTGAGATT			

[a] - A touch-down program for annealing temperature was performed before the regular program

4. Results and discussion

4.1. Genotyping of common wheat cultivars

87 common wheat cultivars listed in the Estonian Variety List in June 2021, were analyzed in the study. Prior including into the List, adaptation to the environmental conditions in Estonia had been tested and perspective in Estonia cultivars were selected. In particular, 47 of the cultivars were associated with spring growth habit and 40 varieties with winter growth habit.

The first aim of the study was to identify allelic variation at *VRN* and *PPD* loci in Estonian cultivars. Using allele-specific PCR, the DNA fragments were amplified and aligned to reference sequences. The total results of the screening of allelic combinations are presented in Table A.1.

First, all cultivars were tested for the allelic variation in the promoter region of *VRN-A1* loci, using VRN1AF and VRN1-INT1R primers (Table 2). In 47 cultivars, PCR amplicons identical to those in cv. Thatcher (965 bp and 876 bp), and characterizing the dominant *Vrn-A1a* allele (Figure 8) were detected. Meanwhile, none of the analyzed cultivars carried the *Vrn-A1b* allele asides from the control cv. Marquise. In remaining 40 cultivars (according to Variety List all of them had winter growth habit) 734 bp fragment, which may represent both the dominant *Vrn-A1c* and the recessive *vrn-A1* alleles. To distinguish between these two alleles, all cultivars were tested using the primer pair of Intr1/C/F and Intr1/AB/R (Table 2). The 1,068 bp DNA fragment was amplified in all cultivars, indicating the presence of the recessive *vrn-A1* allele (Figure 9).

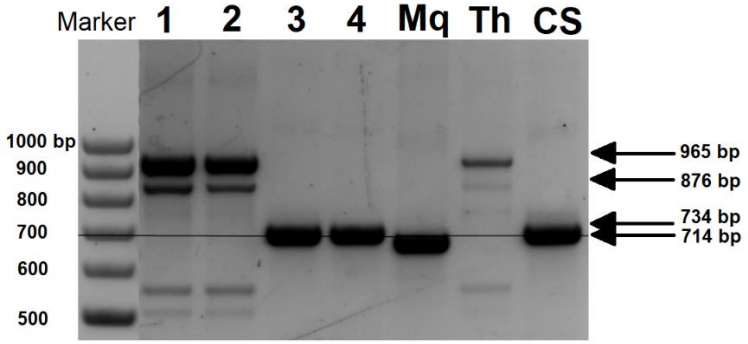

Figure 8. Gel electrophoresis of PCR products, amplified by VRN1AF and VRN1-INT1R primers, on a 2.5% agarose gel. This set of primers can distinguish all four *VRN-A1* alleles in one reaction: *Vrn-A1a* (955 and 876 bp), *Vrn-A1b* (714 bp), *Vrn-A1c* or *vrn-A1* (734 bp). 1, Kajus; 2, Lindras; 3, Opata; 4, Genius. Controls: Mq - Marquise (*Vrn-A1b*), Th - Thatcher (*Vrn-A1a*), CS - Chinese Spring (*vrn-A1*).

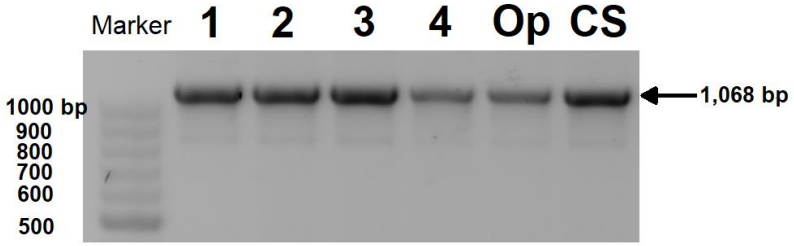

Figure 9. Gel electrophoresis of PCR products, amplified by primer pair of Intr1/C/F and Intr1/AB/R, on a 1% agarose gel. Cultivars carried the *vrn-A1* allele at the *VRN-A1* locus demonstrated a 1,068 bp product. 1, Rotax; 2, Creator; 3, Lemmy; 4, Johanna. Controls: CS - Chinese Spring, Op - Opata.

Then the *VRN-B1* locus was explored. All alleles were identified by using multiplex PCR, including Intr1/B/F, Ex1/B/F3, Intr1/B/R3 and Intr1/B/R4 primers (Table 2). The dominant *Vrn-B1a* allele was presented in 14 cultivars, indicating 1,235 bp and 709 bp amplification fragments, whereas 13 cultivars carried the *Vrn-B1c* allele give the 849 bp fragment (Figure 10). The other 59 cultivars showed an 1,149 bp amplification product, which is characteristic of the recessive *vrn-B1* allele. However, one cultivar, called Malunas, was amplified three times but did not produce any *VRN-B1* PCR product. Probably, the genotype differs from the *Vrn-B1a*, *Vrn-B1b* and *Vrn-B1c* deletion in the intron 1 sequence, to which primers could attach. This cultivar may contain a novel allele at the *VRN-B1* locus.

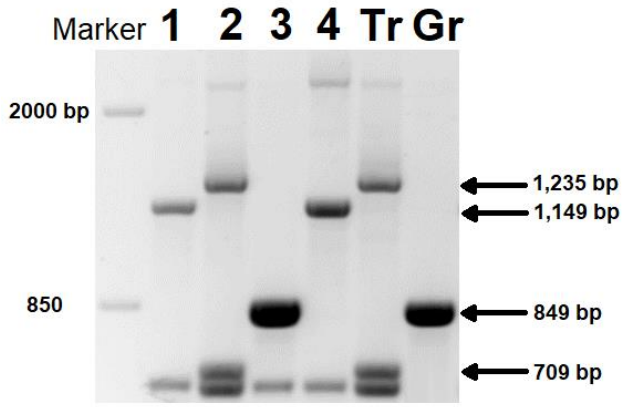

Figure 10. Gel electrophoresis of PCR products, amplified by Intr1/B/F, Ex1/B/F3, Intr1/B/R3 and Intr1/B/R4 primers, on a 1.5% agarose gel. This multiplex PCR can identify all four *VRN-B1* alleles: *Vrn-B1a* (709 + 1,235 bp), *Vrn-B1b* (673 and 1,199 bp), *Vrn-B1c* (849 bp) and *vrn-B1* (1,149 bp). 1, Aateli; 2, Akvitan; 3, Bailando; 4, Broca. Controls: Tr – Triso, Gr - Granny.

The vast majority of cultivars carried the recessive *vrn-D1* allele at the *VRN-D1* locus, while the dominant *Vrn-D1* allele was presented only in one spring wheat cultivar named Hiie (Figure 11). A 1,671 bp fragment, indicating the dominant allele, was amplified using the Intr1/D/F and Intr1/D/R3 primer pair (Table 2). DNA amplification of all the other cultivars by Intr1/D/F and Intr1/D/R4 primers showed a 1,068 bp band characteristic of the recessive allele.

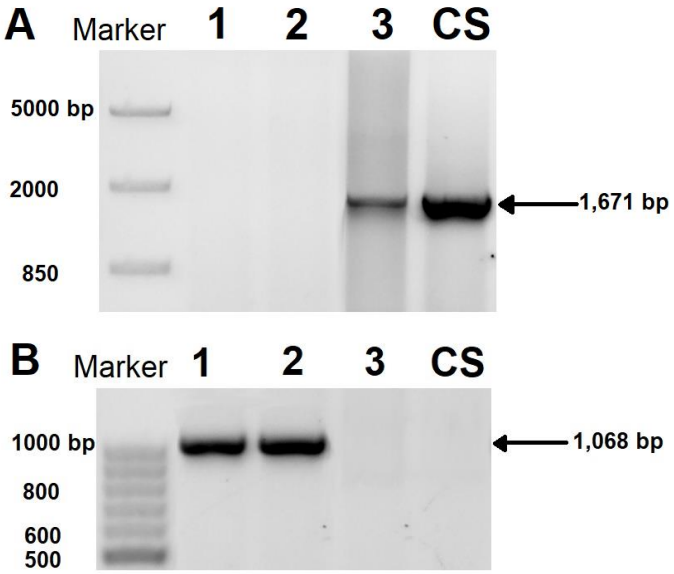

Figure 11. Gel electrophoresis of PCR products on a 1% agarose gel. A: Gel electrophoresis of PCR product (1,671 bp), amplified by Intr1/D/F and Intr1/D/R3 primers, to determine the presence of the *Vrn-D1* allele at the *VRN-D1* locus. 1,

WPB Troy; 2, Hymalaya; 3, Hiie. Control: CS - Chinese Spring (*Vrn-D1*). B: Gel electrophoresis of PCR product, which characterises *vrn-D1* (1,068 bp) allele using Intr1/D/F and Intr1/D/R3 primers. 1, WPB Troy; 2, Hymalaya; 3, Hiie. Control: CS - Chinese Spring.

To determine recessive (null) alleles at the *VRN-2* locus, primer pair of V2ABD-F1 and V2ABD-R2 was used (Table 2). The expected length of PCR products for wild type dominant allele in *VRN-2* locus was 294, 302 and 320 bp for B, A and D genomes, respectively. As amplicons of similar length hardly distinguishable on agarose gels (Figure 12), capillary phoresis was used here.

All cultivars were amplified by primer set of V2ABD-M13-F1, V2AD-R2 and M13(-21) ATTO 565 and analyzed on capillary electrophoresis. The data analysis did not determine any cultivar with full deletion of *VRN-A2* or *VRN-D2* genes, which means that all 87 cultivars carried the dominant *Vrn-A2* and *Vrn-D2* alleles. In contrast, the peak corresponding to the *VRN-B2* locus was absent in some cultivars (Figure 13).

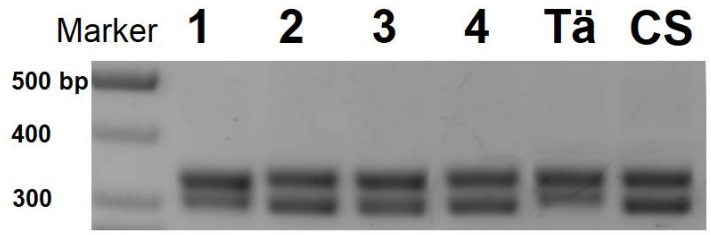

Figure 12. Gel electrophoresis of PCR products, amplified by primer pair of V2ABD-F1 and V2ABD-R2, on a 2% TopVision agarose gel. Cultivars carried the wild type alleles at the *VRN-A2* locus gave a 302 bp PCR product, at *VRN-B2* or *VRN-D2* loci gave a 294 bp or a 320 bp band, respectively. 1, Quintus; 2, Sibelius; 3, Signal; 4, Sorbas. Controls: Tä - Tähti, CS - Chinese Spring.

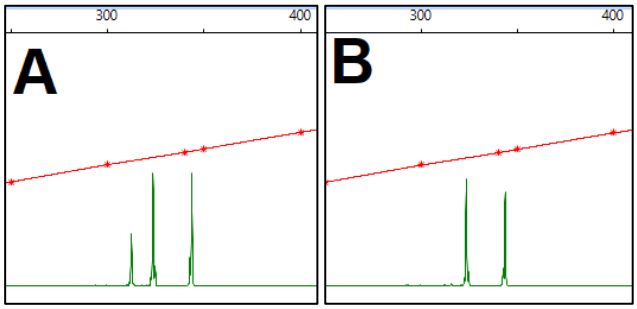

Figure 13. Electropherogram in fragment analysis. A: Peaks correspond to *Vrn-B2*, *Vrn-A2* and *Vrn-D2* alleles in Nemunas' genotype. B: *Vrn-B2* peak is absent, which means the presence of the *vrn-B2* allele at the *VRN-B2* locus, and also *Vrn-A2* and *Vrn-D2* alleles in KWS Spencer' genotype.

Additionally, two pairs of primers (Table 2) were used to confirm the presence of null alleles of the *Vrn-B2* loci. Thus, null *vrn-B2* allele was found in 21 cultivars, which represents almost a quarter of all studied wheat cultivars.

There was no allelic variation in the *VRN-A3* and *VRN-D3* genes, which signifies that all 87 studied cultivars carried the recessive *vrn-A3* and *vrn-D3* alleles (Figure 14). FTex3gAL and FTex1U primers were used, to identify the *vrn-A3* allele, whereas the *vrn-D3* allele was distinguished using FTex3gDL and FTex1U primers (Table 2).

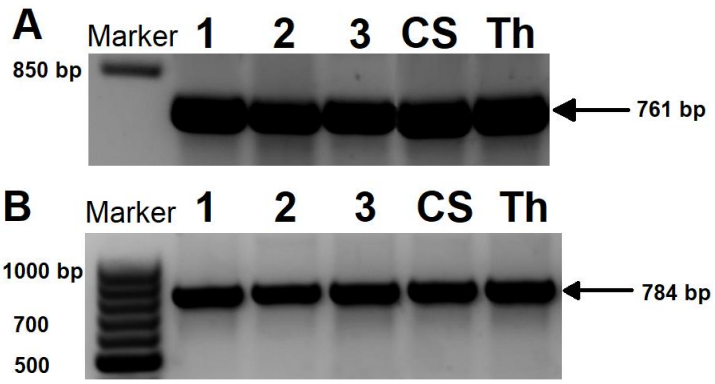

Figure 14. Gel electrophoresis of PCR products on a 1.5% agarose gel. A: Gel electrophoresis of 761 bp PCR product, amplified by FTex3gAL and FTex1U primers, to identify the *vrn-A3* allele. 1, KWS Emil; 2, Bonanza; 3, Ramiro. Control: CS - Chinese Spring, Th - Thacther. B: Gel electrophoresis of 784 bp PCR product, which characterizes *vrn-D3* allele using FTex3gDL and FTex1U primers. 1, KWS Collada; 2, KWS Expectum; 3, KWS Mistral. Control: CS - Chinese Spring, Th - Thacther.

The recessive *vrn-B3* allele, defined by the amplification of a 1031 kb fragment with primers FTex3gBL and FTex1U, was found in 85 cultivars (Figure 15). Alleles of two cultivars did not amplify. Most probably, these cultivars have a dominant allele, which can be amplified by another primer pairs.

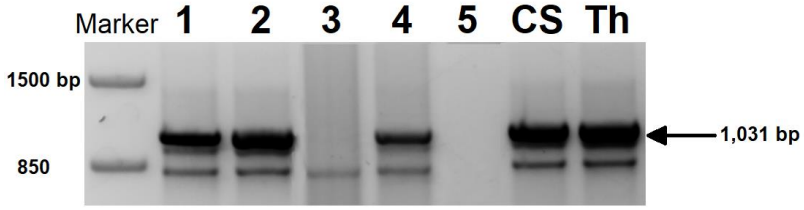

Figure 15. Gel electrophoresis of PCR products, amplified by FTex3gBL and FTex1U primers, on a 1.5% agarose gel. Cultivars carried the *vrn-B3* allele showed a 1,031 bp product. 1, KWS Expectum; 2, Bonanza; 3, Creator; 4, Janne; 5, Aateli. Controls: CS - Chinese Spring, Th - Thatcher.

No allelic variation was detected in *PPD-A1* and *PPD-B1* genes, so we concluded that all 87 cultivars carried 338 bp of *Ppd-A1b.1* and 425 bp of *Ppd-B1a.2* alleles at the A and B genomes, respectively.

Insensitive *Ppd-D1a* allele was not detected in spring wheat cultivars, but presented in 4 winter cultivars. Other 83 cultivars carried sensitive *Ppd-D1b* allele (Figure 16).

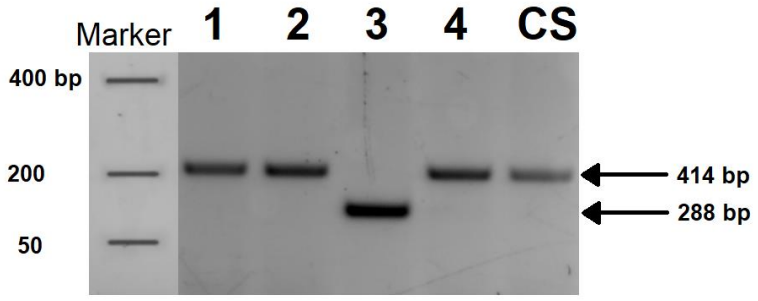

Figure 16. Gel electrophoresis of PCR products, amplified by Ppd-D1_F, Ppd-D1_R1 and Ppd-D1_R2 primers, on a 1.5% agarose gel. Cultivars carried the *Ppd-D1b* allele demonstrated a 414 bp PCR product, while genotypes with the *Ppd-D1a* allele shoowed a 288 bp product. 1, Edvins; 2, Festival; 3, Balitus; 4, Hellas.

Our results demonstrate that all spring wheat cultivars grown in Estonia carry the dominant *Vrn-A1a* allele of the *VRN-A1* gene. In 43% of these cultivars, *Vrn-A1a* was the sole dominant allele of a *VRN1* gene. The *Vrn-A1a* allele has the strongest genetic effect on development of wheat of spring growth habit. Plants carrying this allele in their genome, do not require vernalization to start flowering (Kiss *et al.,* 2014). Also, plants with the dominant Vrn-A1 allele flower earlier than those with dominant Vrn-D1 or Vrn-B1 (Berezhnaya *et al.,* 2021). The highest percentage of the *Vrn-A1a* allele has been detected in spring wheat cultivars from Canada and USA (85-91%) (Fu *et al.,* 2005; Iqbal *et al.,* 2007), while in Argentina, as well as in Estonia, only 50% cultivars carry *Vrn-A1a* allele (Yan *et al.,* 2004a). Dominant *Vrn-A1b* or *Vrn-A1c* alleles were not found in wheats from the Estonian Variety List. 40 out of 87 (46%) cultivars contained the recessive *vrn-A1* allele, which is characteristic for winter wheat cultivars (Kiss *et al.,* 2D014). Plants with this allele prevail among Chinese common wheat cultivars (66%) (Zhang *et al.* 2008).

The highest allelic variability was detected in the *VRN-B1* locus of the spring wheat cultivars. The most frequently identified allele was the recessive *vrn-B1*, which was carried by approximately 70% of Estonian wheat cultivars. This allele is prevailing in Chinese wheat cultivars (Zhang *et al.,* 2008), but in the rest of the world, it is presented by approximately 30 per cent of cultivars (Milec *et al.,* 2013). The *Vrn-B1a* allele was detected in 14 (16%) cultivars, while the *Vrn-B1c* allele was identified in 13 (15%) cultivars from Estonia. Noteworthy, the *Vrn-B1a* allele is the most frequently found allele worldwide. In particular, it is contained in 50% of varieties on all continents, while in Australia the frequency of this allele reaches 70%. The *Vrn-B1c* allele is mainly distributed in Europe (15%), while its frequency does not exceed 5% in the rest of the world (Milec *et al.,* 2013).

None of the cultivars growing in Estonia contained the *Vrn-B1b*, which is not surprising, since this allele is mainly found in wheat cultivars originating from North/South America (59.6 %), and only a small number has been found in Europe (11.2 %) (Milec *et al.,* 2013).

Recessive *vrn-D1* allele was present in 99% of analyzed cultivars. Only cv. Hiie carried the dominant *Vrn-D1* allele. The distribution of *VRN-D1* alleles is largely unexplored. However, it is known that the number of cultivars carrying the dominant *Vrn-D1* allele varies from 14 to 96 per cents in the different climatic zone of China (Zhang *et al.,* 2008). Furthermore, the *Vrn-D1* allele frequency in USA and Canada is low (6,7%) (Fu *et al.,* 2005).

To sum up, single dominant alleles, mediating spring growth habit, were recorded in *Vrn-A1a* allele for 54% cultivars of the Variety List for the *VRN-A1*. We also observed two (*Vrn-A1–VrnB1*) dominant gene combinations in case of 31% cultivars. Combinations of three dominant alleles were found only in cultivar Hiie (1%). All spring wheat cultivars included at least one dominant *VRN1* allele in their genomes, whereas all winter wheat cultivars were characterized by recessive *vrn-A1*, *vrn-B1* and *vrn-D1* alleles.

In our study, the allelic variability was not recorded for the *VRN-A2* and *VRN-2D* genes. However, a perceptible number of cultivars have diversity in the *VRN-B2* gene. Null allele was detected in 14 spring and 8 winter (24%) cultivars. Previously Zhu *et al.* (2010) found a small group of cultivars with a similar genotype in the United States and China. Among 54 Chinese wheat cultivars and landraces, 3 accessions, carrying null alleles in *VRN-A2* and three in *VRN-B2* loci, were identified. Only 3 cultivars with null allele were found between 74 cultivars of USA (Zhu *et al.* 2010).

No allelic variation was also detected in *VRN-A3* and *VRN-D3* genes, which allowed us to assume that recessive *vrn-A3* and *vrn-D3* alleles are present in all analyzed Estonian-grown cultivars. Furthermore, our results demonstrate that 85 (98%) varieties carried the *vrn-B3* allele, except for cv. Aateli and cv. Creator, which did not amplify with the primer pair of FTex3gBL and FTex1U. Most probably, these cultivars have some variation in gene sequences and can be amplified by another primer pairs. Natural variation in the *VRN-B* 3gene is not well studied, but it was shown that the vast majority (98%) of Chinese cultivars have *vrn-B3* allele (Zhang *et al.,* 2015). A similar situation occurs in Russia: only two varieties carried the *Vrn-B3a* allele, and the great majority (86%) of varieties carried the recessive *vrn-B3* allele (Berezhnaya *et al.,* 2021).

Genotyping of *PPD-A1* and *PPD-B1* genes did not reveal allelic variation. All cultivars carried the photoperiod-sensitive *Ppd-A1b.1* allele in A genome and the photoperiod-insensitive *Ppd-B1a.2* in B genome. The small variation was found for the *PPD-D1* gene; thus 83 (95%) cultivars from Estonian Variety List contained the photoperiod-sensitive *Ppd-D1b* allele and only 4 (5%) cultivars contained the photoperiod-insensitive *Ppd-D1a* allele. All cultivars carried *Ppd-D1a* alleles have winter growth habits. Additionally, the photoperiod-insensitive *Ppd-D1a* allele was found in cv. Avenju and cv. Donskaja, (not included into Variety List, but used in breeding). However, we did not find any significant link between genotype in the locus and flowering time in our experiment. Interestingly, in Asia the situation is the opposite, the *Ppd-D1a* is highly presented (98%) among wheat cultivars, but the *Ppd-D1b* allele (2%) is rare (Zang *et al.,* 2015). Frequency of the *Ppd-D1a* allele is 79% in Asia and 58% in Europe (Kiss *et al.,* 2014).

4.2. Phenotypic analysis of wheat varieties in greenroom conditions

The flowering time of 83 wheat cultivars from the Estonian Variety List was evaluated in controlled conditions in the greenroom. Spring wheat cultivar Berlock, and winter wheat cultivars Bonanza, Kena DS and Skagen were excluded from phenotype analysis as seeds of these cultivars were not germinating. Spring and winter wheat cultivars were tested in two independent experiments. The distribution of the average flowering time for 46 spring wheat cultivars and for 37 winter wheat cultivars is presented in Figure 17 and Figure 18, respectively.

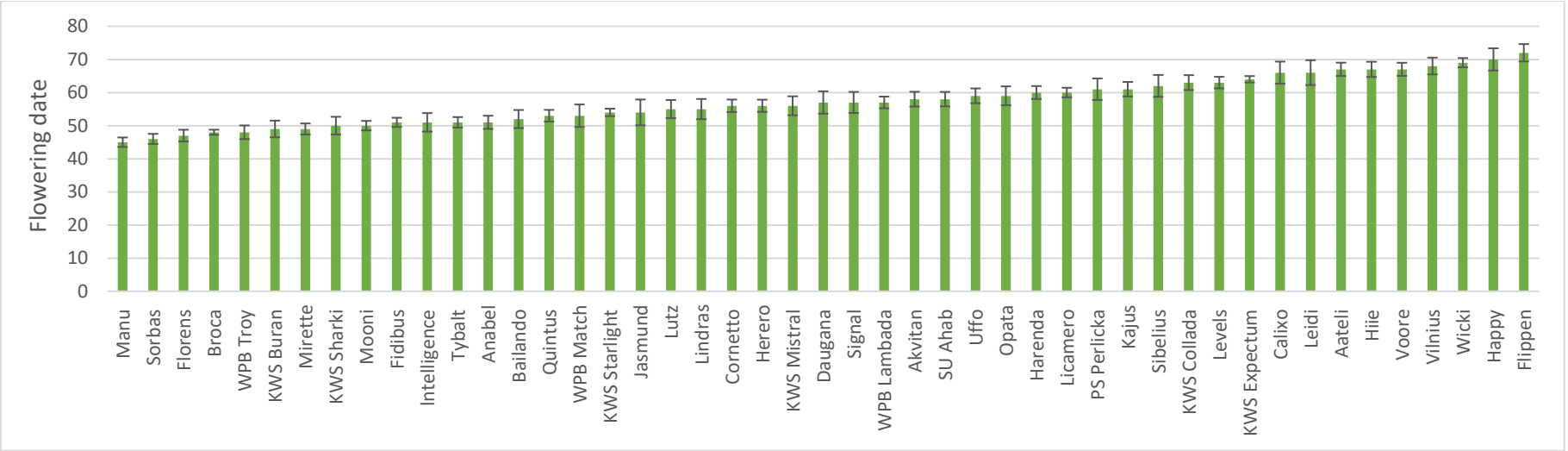

Figure 17. Mean flowering time in greenroom conditions for the studied spring wheat cultivars.

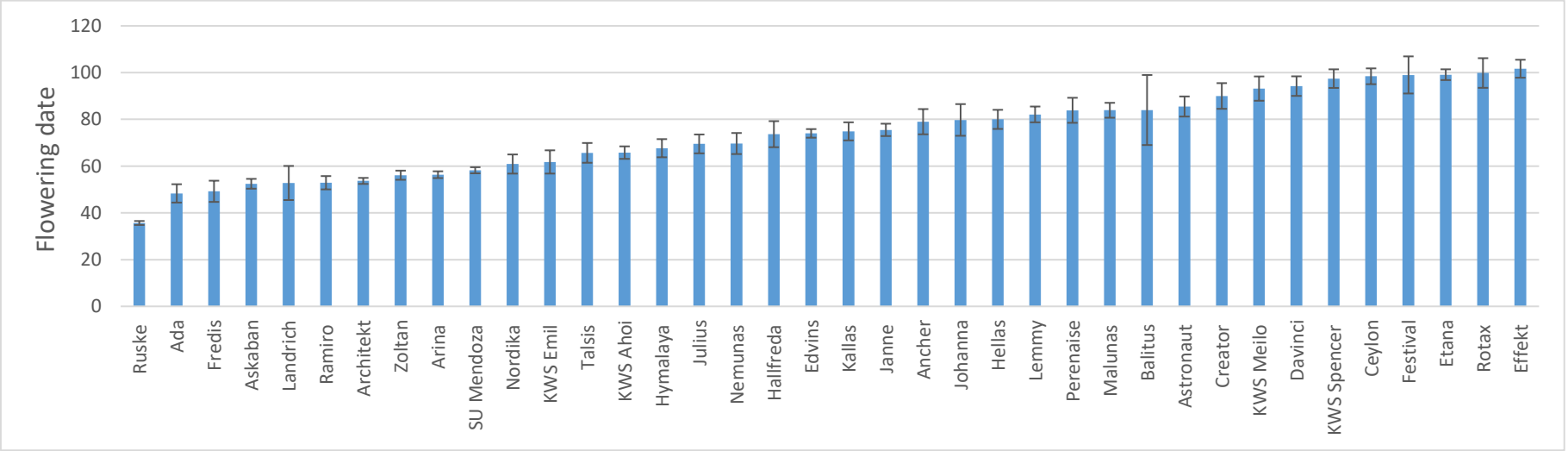

Figure 18. Mean flowering time in greenroom conditions for the studied winter wheat cultivars.

On average, spring wheat plants headed at the 57th day starting from the germination. In winter wheat cultivars, the first spikelet appeared on average on the 74th day after vernalization was finished.

In greenroom conditions, the average flowering time ranged from 45 to 72 days for spring wheat cultivars (Figure 19). Results for winter wheat cultivars demonstrated a broader range of flowering time. The heading dates varied from 36 to 102 days (Figure 20). Thus, a difference of 27 days was observed between the flowering of the earliest and the latest cultivar in the experiment with spring wheat cultivars and up to 66 days when winter wheat cultivars were evaluated. However, in field tests, differences are about 5-8 days (personal communication). Probably, in controlled conditions, we detected additionally phenotypic variation, which is masked usually in the field.

In both experiments, for spring and winter wheat cultivars, plants were grown in three different greenrooms (a full set in each room, with slightly different illumination). Thus, we were able to evaluate the part of the variation which explained genetical differences between cultivars and also effect of illuminance on flowering time. According to results of the one-way ANOVA, the effect of the factor 'cultivar' (genotype in our case) was highly significant in each experiment for both spring (p value < 0.001) and winter (p value < 0.001) cultivars. The genetic variation in our experiments explained 81% of total variation for spring and 75% of total variation for winter wheat cultivars. We conclude that a significant difference between the flowering time among both spring and winter wheat cultivars had a genetic basis.

Two-way ANOVA (the first factor cultivars/genotype, second factor-illuminance) showed that growing conditions highly significantly affected the average flowering time of cultivars (p value < 0,001). As an example, in an experiment with spring wheat cultivars, the plants which have grown at illuminance 135 µE m^{-2} s^{-1} flowered on average up to two weeks later than at 110 µE m^{-2} s^{-1} and 120 µE m^{-2} s^{-1}. However, the average flowering time for cultivars in different trials was correlated (r = 0.55 – 0.89 for spring cultivars; r = 0.74 – 0.83 for winter cultivars, p value < 0,001).

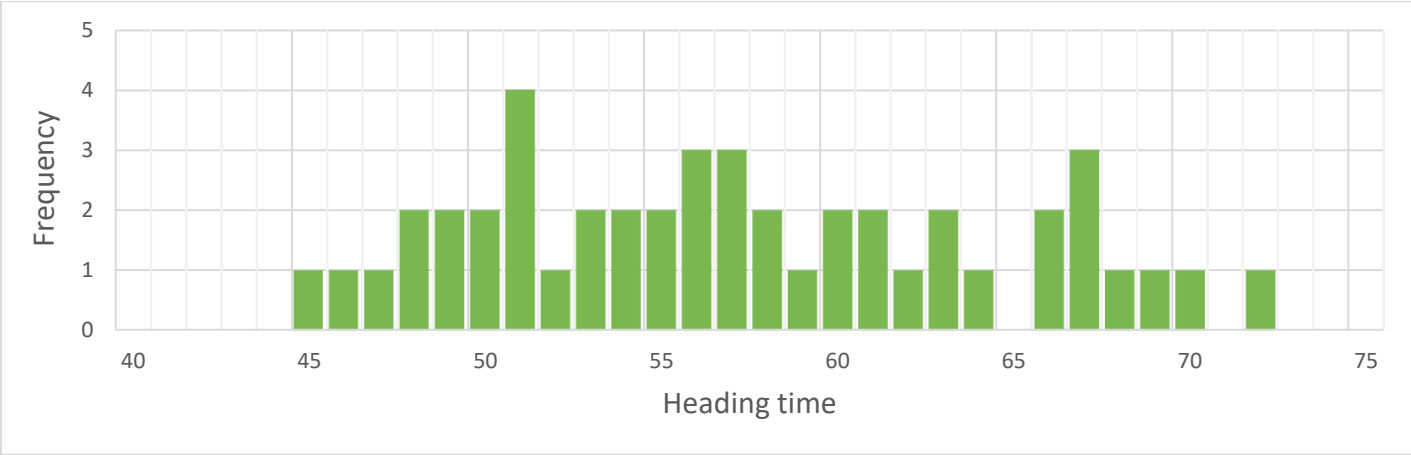

Figure 19. Frequency of mean flowering time in greenroom conditions for the studied spring wheat cultivars.

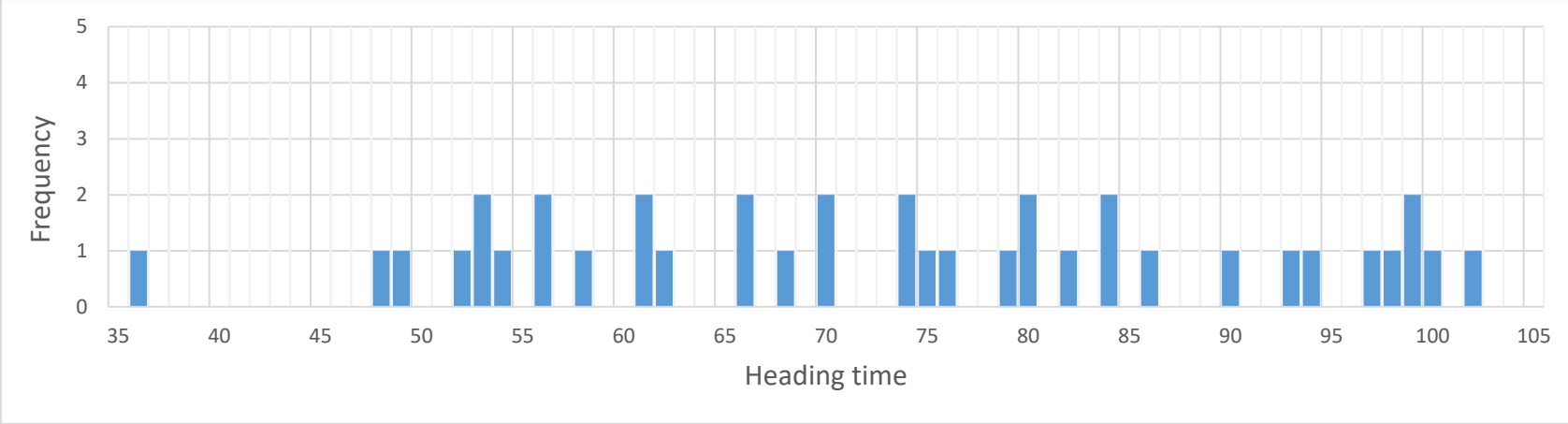

Figure 20. Frequency of mean flowering time in greenroom conditions for the studied winter wheat cultivars.

4.3. Flowering time and genotype in *VRN-B1*, *VRN-B2* and *PPD-D1* loci

Our next goal was to test the effect of allelic combinations in detected polymorphic *VRN* and *PPD* loci on the flowering time. For this purpose, cultivars were divided into groups according to the genotype in detected polymorphic loci. For spring wheat cultivars, it was *Vrn-B1* and *Vrn-B2* loci. We got six different allelic combinations in spring wheat cultivars (Table 3). Winter wheat cultivars were divided according to different genotypes in *Ppd-D1* and *Vrn-B2* loci. We got four such allelic combinations (Table 4).

In order to check whether the difference in the average flowering time resulted from a certain allelic combination, a two-way ANOVA test was used. For spring wheat cultivars, no significant differences between groups with different allelic combinations were detected, but again this test demonstrated that illuminance has a significant (*p* value < 0.0001) effect on the flowering time of the spring wheat (Table 3).

Table 3. Average flowering time in spring wheat cultivars carrying different alleles in *Vrn-B1* and *Vrn-B2* loci.

Locus		Number of cultivars	Illuminance (µE m^{-2} s^{-1})			Average
VRN-B1	*VRN-B2*		110	120	135	
vrn-B1	*Vrn-B2*	12	51,4	55,4	69,4	56,9
Vrn-B1a		10	52,5	56,7	70,3	57,9
Vrn-B1c		10	52,9	56,7	66,6	57,3
		average	**52,3**	**56,3**	**68,8**	**57,4**
vrn-B1	*null*	8	51,2	54,0	64,8	55,3
Vrn-B1a		3	50,3	54,4	61,1	54,1
Vrn-B1c		2	52,0	57,2	61,2	58,1
		average	**51,2**	**55,2**	**62,4**	**55,8**

All our spring wheat cultivars have a dominant *Vrn-A1a* allele. Genetic variation at the *VRN-1* loci is one of the most important factors affecting heading time in wheat (Yan *et al.,* 2004a; Fu *et al.,* 2005). The dominant *Vrn-A1* is epistatic to *Vrn-B1* and *Vrn-D1* (Pygsley, 1971) which may explain why in our study we did not detect any differences in flowering time between spring wheat cultivars differing for alleles at *Vrn-B1* loci.

According to publish data, the dominant *Vrn-A1* is epistatic not only to *Vrn-B1* and *Vrn-D1* (Pygsley, 1971). Furthermore, the dominant *VRN1* alleles for early flowering also are epistatic to *VRN2* gene and can mask their effect (Tranquilli and Dubcovsky, 2000) which can explain why in our study we did not detect any differences in flowering between spring wheat cultivars differing for allelic combinations at *Vrn-B1* and *Vrn-B2* loci.

Two-way ANOVA analysis was also done for winter wheat cultivars. Plants were divided by allelic variability in *Ppd-D1* and *VRN-B2* genes. Three different greenrooms with individual illuminance were also taken into account (Table 4). The differences between genotypes were highly significant comparing groups with normal allele in *Vrn-B2* locus and null allele (*p* value < 0,002). In contrast to spring wheat cultivars, the results demonstrated that illuminance does not have a significant effect

on heading time. Plants carried null allele in *Vrn-B2* locus on average flow up to 23 days early. However, the significant effect on the flowering time for *Ppd-D1* gene was not detected in our experiment.

Table 4. Average flowering time in winter wheat cultivars different alleles in *Ppd-D1* and *Vrn-B2* loci.

Locus		Number of cultivars	Illuminance (µE m^{-2} s^{-1})			Average
Ppd-D1	*VRN-B2*		110	120	180	
Ppd-D1a	*Vrn-B2*	2	84,0	101,7	-	92,8
Ppd-D1b		28	79,1	73,1	72,9	75,2
		average	**81,5**	**87,4**	**72,9**	**84,0**
Ppd-D1a	*null*	5	61,2	61,0	48,4	57,8
Ppd-D1b		3	71,0	67,8	65,7	70,6
		average	**66,1**	**64,4**	**57,1**	**64,2**

In total, we have found that there is a possible bind between allelic composition and *Vrn-B2* locus and flowering time for winter wheat cultivars.

Our study is the first step in finding a genotypic basis for the phenotypic difference in Estonian wheat cultivars, containing various *VRN* and *PPD* allelic combinations. In the future, to accurately prove a link between certain alleles in VRN and PPD genes and common wheat phenotype, a new cultivar can be breaded, which will contain recessive alleles asides from the studied one, and then measure the natural heading times of cultivars in a field experiment. Only in this case, it is possible to approve that a certain allele causes the corresponding plant phenotype.

We can conclude that the genetic polymorphism found in loci *Vrn-B1*, *Vrn-B2* and *Ppd-D1* in our study does not fully explain the phenotypic variation in flowering time detected in our experiments. Thus, unknown genes/alleles, presented in the genomes of cultivars growing in Estonia, can affect the flowering time.

The study provides useful information to improve the adaptability and productivity of wheat, grown in Estonia.

Abstract

Common wheat is an important food crop, production of wheat amounted to 776 million tonnes in 2021 (FAOSTAT, 2021). Wheat is cultivated on all continents besides Antarctica. This broad geographic distribution is explained by effective adaptation to various environments, and, what is especially important for Estonia, to low temperatures. An adaptiveness is a response to allelic variation in *VRN* (vernalization genes) and *PPD* (photoperiod response gene). VRN genes control the start of the vegetative/generative transition, regulating flowering time (FT), and *PPD sensitivity* to daylength. Also, allelic combination of *VRN* and *PPD* genes is the basis for wheat adaptation to a wide range of phenotypic characteristics.

The first aim of this study was to identify allelic variations of vernalization and photoperiod response genes in wheat cultivars cultivated in Estonia. 87 common wheat cultivars were selected based on the Estonian Variety List (June 2021). In the current work, nine *VRN* loci: *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Vrn-A2*, *Vrn-B2*, *Vrn-D2*, *Vrn-A3*, *Vrn-B3*, *Vrn-D3*, and three *PPD* loci: *Ppd-A1*, *Ppd-B1*, *Ppd-D1* were genotyped using allele-specific markers.

It was revealed that 47 cultivars carried the spring *Vrn-A1a* allele. 26 of them also contained the *Vrn-B1* allele, and only one cultivar, Hiie, had all three dominant *Vrn1* alleles. 40 cultivars contained solely recessive alleles in the *VRN-1* locus and were characterized as having winter growth habit. No variation was found in the *VRN-A2* and *VRN-D2* genes, and all analyzed cultivars contained dominant *Vrn-A2* and *Vrn-D2* alleles. However, the null *vrn-B2* allele was detected in 21 out of 87 cultivars. No allelic variation was detected for the *VRN-A3*, *VRN-B3* and *VRN-D3* genes. All of them carried the recessives alleles. The *PPD-A1* and *PPD-B1* loci also did not have any allelic variation. All 87 genotypes carried the photoperiod-sensitive *Ppd-A1b.1* and photoperiod-insensitive *Ppd-B1a.2* alleles. The photoperiod-sensitive allele *Ppd-D1b* was present in 83 out cultivars, while the photoperiod-insensitive *Ppd-D1a* allele carried only 4 cultivars.

Comparison of allelic composition in *VRN* and *PPD* loci in cultivars grown in Estonia with wheat grown in Canada, USA, Brasilia or China revealed that in different regions, different allelic composition in *VRN* and *PPD* loci were prevailing.

The second objective of this research was to determine whether different *VRN* and *PPD* allelic combinations affect flowering time of wheat. In the case of spring wheat cultivars, no genotype effect of allelic compositions was detected, whereas the variability in illuminance (environment) was significant to flowering. Winter wheat demonstrated the opposite situation. In particular, the allelic variation presented *VRN-B2* loci affected the heading time, while illuminance did not show any effect.

In the future, the flowering time of studied cultivars should be tested in field conditions in order to find combinations useful in breeding.

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Appendixes

Appendix 1. Recipes

CTAB Buffer (100 ml) recipe:
1.5 g CTAB
10 ml 1M Tris HCl pH 8.0
4 ml 0.5 EDTA
24 ml 5M NaCl
MQ until 100 ml

TE Buffer (100 ml) recipe:
1 ml 1M Tris-HCl
200 µl 0.5M EDTA
MQ until 100 ml

Appendix 2. Tables

Table A.1. A common table of allelic variations identified in used cultivars.

№	Cultivar	GH	*VRN-A1*	*VRN-B1*	*VRN-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*Vrn-3A*	*Vrn-3B*	*Vrn-3D*	*PPD-A*	*PPD-B*	*PPD-D*
1	Aateli	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3.x*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
2	Akvitan	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
3	Bailando	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
4	Berlock	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
5	Broca	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
6	Calixo	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
7	Cornetto	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
8	Daugana	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
9	Fidibus	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
10	Flippen	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
11	Florens	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
12	Happy	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
13	Harenda	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
14	Herero	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
15	Hiie	SW	*Vrn-A1a*	*Vrn-B1c*	***Vrn-D1***	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
16	KWS Buran	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*

17	KWS Collada	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
18	KWS Expectum	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
19	KWS Mistral	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
20	KWS Sharki	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
21	KWS Starlight	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
22	Leidi	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
23	Levels	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
24	Licamero	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
25	lntelligence	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
26	Manu	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
27	Mooni	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
28	PS Perlicka	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
29	Quintus	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
30	Sibelius	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
31	Signal	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
32	Sorbas	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
33	SU Ahab	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
34	Tybalt	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
35	Uffo	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*

36	Wicki	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
37	Vilnius	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
38	Voore	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
39	WPB Lambada	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
40	WPB Troy	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
80	Lutz	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
82	Jasmund	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
83	Anabel	SW	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
84	WPB Match	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
85	Kajus	SW	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
86	Lindras	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
91	Mireete	SW	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
41	Hymalaya	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
42	KWS Ahoi	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
43	KWS Spencer	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
44	Ancher	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
45	Zoltan	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
46	Ruske	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
47	Askaban	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*

48	SY Landrich	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
49	KWS Emil	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
50	Bonanza	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
51	Ramiro	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
52	Astronaut	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
53	SU Mendoza	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
54	Janne	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
55	Davinci	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
56	Balitus	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	***Ppd-D1a***
57	Hellas	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
58	Ada	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
59	Fredis	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	***Ppd-D1a***
60	Hallfreda	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
61	Kena DS	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
62	Nemunas	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
63	Malunas	WW	*vrn-A1*	***na***	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
64	Rotax	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
65	Creator	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3.x*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
66	Lemmy	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*

67	Johanna	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
68	Effekt	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	***Ppd-D1a***
69	Edvins	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
70	Etana	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
71	Festival	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
72	Ceylon	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
73	Skagen	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
74	Julius	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
75	Nordika	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*null*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	***Ppd-D1a***
76	Architekt	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
77	Talsis	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
78	Perenaise	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
79	Kallas	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*
81	KWS Meilo	WW	*vrn-A1*	*vrn-B1*	*vrn-D1*	*Vrn-A2*	*Vrn-B2*	*Vrn-D2*	*vrn-A3*	*vrn-B3*	*vrn-D3*	*Ppd-A1b.1*	*Ppd-B1a.2*	*Ppd-D1b*

Lisa
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Lihtlitsents lõputöö reprodutseerimiseks ja lõputöö üldsusele kättesaadavaks tegemiseks[1]

Mina Jemilia Tarassova

1. Annan Tallinna Tehnikaülikoolile tasuta loa (lihtlitsentsi) enda loodud teose
Allelic variability by vernalization and photoperiodic sensitivity loci of a number of wheat varieties (*Triticum aestivum* L.) grown in Estonia,

mille juhendaja on Irena Jakobson, PhD

1.1 reprodutseerimiseks lõputöö säilitamise ja elektroonse avaldamise eesmärgil, sh Tallinna Tehnikaülikooli raamatukogu digikogusse lisamise eesmärgil kuni autoriõiguse kehtivuse tähtaja lõppemiseni;

1.2 üldsusele kättesaadavaks tegemiseks Tallinna Tehnikaülikooli veebikeskkonna kaudu, sealhulgas Tallinna Tehnikaülikooli raamatukogu digikogu kaudu kuni autoriõiguse kehtivuse tähtaja lõppemiseni.

2. Olen teadlik, et käesoleva lihtlitsentsi punktis 1 nimetatud õigused jäävad alles ka autorile.

3. Kinnitan, et lihtlitsentsi andmisega ei rikuta teiste isikute intellektuaalomandi ega isikuandmete kaitse seadusest ning muudest õigusaktidest tulenevaid õigusi.

26.05.2022 (kuupäev)

[1] *Lihtlitsents ei kehti juurdepääsupiirangu kehtivuse ajal vastavalt üliõpilase taotlusele lõputööle juurdepääsupiirangu kehtestamiseks, mis on allkirjastatud teaduskonna dekaani poolt, välja arvatud ülikooli õigus lõputööd reprodutseerida üksnes säilitamise eesmärgil. Kui lõputöö on loonud kaks või enam isikut oma ühise loomingulise tegevusega ning lõputöö kaas- või ühisautor(id) ei ole andnud lõputööd kaitsvale üliõpilasele kindlaksmääratud tähtajaks nõusolekut lõputöö reprodutseerimiseks ja avalikustamiseks vastavalt lihtlitsentsi punktidele 1.1. jq 1.2, siis lihtlitsents nimetatud tähtaja jooksul ei kehti.*