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Известия ТСХА, выпуск 5, 2014 год

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DEVELOPMENT OF PCR-BASED STS MARKER FOR IDENTIFICATION OF *VIVIPAROUS-1* GENE OF THINOPYRUM SPECIES IN WHEAT BACKGROUND

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The introgression of Viviparous-1 (Vp-1) genes from Triticeae species into wheat genome is one of the possible approaches to improve its preharvest sprouting resistance. One of the promising sources of alien Vp-1 genes is wheatgrass species (Thinopyrum). Aiming at the distinguishing of Thinopyrum and Triticum Vp-1 genes in the same genotype the PCR-based sequence-tagged site (STS) marker was developed. The STS marker can be useful in the studies of the Vp-1 effect on preharvest sprouting in Triticeae, monitoring introgressions in wide hybridization, rapid analysis of allelic variants both in wheat-wheatgrass hybrids and in Thinopyrum, Pseudoroegneria and Dasypyrum species.

Key words: molecular marker, STS, Viviparous-1, Thinopyrum intermedium, Thinopyrum ponticum, Thinopyrum bessarabicum, Pseudoroegneria stipifolia, Dasypyrum villosum.

Preharvest sprouting (PHS) in cereals refers to germination of physiologically mature grains in the head prior to harvest. PHS reduces grain yield and quality of wheat, particularly in the countries with high humidity during the harvest season [2]. PHS usually occurs due to early break of seed dormancy that leads to activation of physiological processes and embryo growth. Seed dormancy is a complex trait affected by abscisic and gibberellic acids, alpha-amylose activity, water-soluble inhibitors of germination in glumes and seed coat, head architectonics [10, 16]. Evolutionary, seed dormancy evolved as a mechanism to avoid adverse environmental factors and intraspecific competition. The domestication of plants was accompanied by selection in favour of uniform and rapid germination that resulted in higher sensibility of seeds to the factors breaking dormancy [8].

Transcription factors play an important role in regulation of gene expression in response to abiotic stresses including prolonged wet weather during harvesting [9]. *Viviparous-1* gene (*Vp-1*) coding for transcription factor in plants is expressed in the cells of developing seed and involved in seed dormancy regulation [14].

Wide hybridization is an approach to improve the PHS resistance of wheat. Transgenic wheat grains expressing *Avena fatua Vp-1* gene showed enhanced sensitivity to abscisic acid and were less susceptible to PHS compared with the control [11, 15]. Therefore, the

tertiary gene pool for wheat can be useful to increase PHS resistance of wheat. One of the most promising sources of alien genes for wheat improvement is wheatgrass species such as *Thinopyrum intermedium* (2n=42)*, Th. ponticum* (2n=70)*, Th. bessarabicum* (2n=14).

Partial wheat-wheatgrass hybrids (2n=56) can be used as an intermediate step in wheat breeding to transfer genetic material form wheatgrass to wheat. Such partial amphiploids contain 42 (38-42) wheat and 14 (14-18) wheatgrass chromosomes [7, 13]. A variety of valuable traits and cytogenetic research of octoploid hybrids indicates different combination of wheatgrass chromosomes in particular lines and, therefore, different Thinopyrum *Vp-1* genes.

The identification of Thinopyrum genes, their allelic variants and study of their expression in wheat-wheatgrass amphiploids is accomplished by the presence of wheat homologues genes *Vp-1A*, *Vp-1B* and *Vp-1D*. The problem may be solved by the development of specific polymerase chain reaction (PCR)-based DNA markers for *Thinopyrum* genes. Such markers can be applied not only for estimation allelic polymorphism of *Thinopyrum Vp-1* gene but also for introgression of the *Thinopyrum Vp-1* to wheat using molecular-assisted selection.

The aim of this paper is to develop a PCR-based marker to distinguish *Thinopyrum Vp-1* genes from wheat *Vp-1* genes in the same genotype.

Materials and methods

Plant materials. The following accessions of wild relatives of wheat were obtained from Germplasm Resources International Network (GRIN): *Thinopyrum intermedium* (PI 547312), *Th. ponticum* (PI 401200), *Th. bessarabicum* (PI 531711), *Pseudoroegneria stipifolia* (W621759), *Dasypyrum villosum* (W621717). Wheat cultivars Bezostaya 1 and Mironovskaya 808 were used as controls of wheat *Vp-1* and wheat-wheatgrass amphiplods (2n=56) (accession number 1872 and 1876) were used as genotypes containing both wheat and Thinopyrum genomes.

DNA extraction and molecular analysis. Genomic DNA was isolated from etiolated leaves as described by Bernatzky and Tanksley [1].

PCR. The PCR with the species-specific STS-primers sequences designed in this paper VivipF and VivipR was performed using the following conditions: an initial denaturation at 95°C for 7 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min and 72°C for 1 min, with the final extension at 72°C for 10 min.

The following primers were used for PCR amplification:

VivipF 5’- GGGTGATTTCATCGTGCTT -3’

VivipR 5’- TCTCCAACACTTGATTTTGACC -3’

Results and discussion

Our previous studies have explored the DNA sequences of different regions of *Vp-1* genes in wild relatives of wheat, namely: *Thinopyrum intermedium*, *Th. ponticum*, *Th. bessarabicum*, *Pseudoroegneria stipifolia*, *Dasypyrum villosum*. The *Vp-1* gene of *Thinopyrum* species was designated *ThVp-1* [3, 4, 5, 12]. The obtained sequences are polymorphic in introns and highly conservative in exons [3, 5, 12]. They showed high homology to wheat genes *Vp-1A*, *Vp-1B* and *Vp-1D* of A, B and D genomes, respectively (85–91%). To design species-specific primers the most polymorphic region IV flanked with Vp-1BB4F and Vp-1BB4R primers was used [3, 5]. The alignment

of *Vp-1* sequences of wheat and wild relatives species submitted to GenBank is shown in Figure 1 (Genbank: Vp-1A (AJ400712.1), Vp-1B (AJ400713.1), Vp-1D (AJ400714.1), Vp-1 *Thinopyrum intermedium*, (KC788534.1), Vp-2 *Th. intermedium* (KC788535.1), Vp-3 *Th. intermedium* (KC788536.1), Vp-1 *Thinopyrum ponticum* (KC788550.1), Vp-2 *Th. ponticum* (KC788551.1), Vp-3 *Th. ponticum* (KC788552.1), Vp-1 *Thinopyrum bessarabicum* (KC788537.1), Vp-1 *Pseudoroegneria stipifolia* (KC788524.1), Vp-1 of *Dasypyrum villosum* (KC788529.1), Vp-2 of *D. villosum* (KC788530.1)). The sequences differ not only in single nucleotide polymorphisms (SNPs) but also in numerous indels (Fig. 1). The primers VivipF and VivipR were designed for the most polymorphic regions (Fig. 2). They would enable amplification of only one product from *Vp-1A*, *Vp-1B* and *Vp-1D* of wheat and distinguishing the PCR product of *Thinopyrum* from them. The expected size of the PCR products were as follows: *Vp-1A* — 541 bp, *Vp-1B* — 383 bp, *Vp-1D* — 399 bp, *Th. intermedium* — 386, 374 and 360 bp, *Th. ponticum* — 400, 377 and 366 bp, *Th. bessarabicum* — 396 bp, *P. stipifolia* — 370 bp, *D. villosum* — 350 and 633 bp. At the same time, a huge genetic variety of wild relatives of wheat should be taken into account and the application of the designed primers in other accessions of the studied species may result in somewhat different amplification products. However, *Vp-1* genes of wheat are well characterized and the expected sizes of PCR-products may be used for preliminary interpretation of the results.

The developed molecular STS marker (designated Vivip) was verified on wheat cultivars and different wheatgrass species accessions of wild relatives of wheat: *Th. intermedium*, *Th. ponticum*, *Th. bessarabicum*, *P. stipifolia*, *D. villosum* (Fig. 3).

After PCR amplification with the primers VivipF and VivipR on DNA of all studied species the DNA fragments of the expected size was obtained. At the same time, in *D. villosum* the PCR product was amplified from 633 bp allele only.

The developed STS marker Vivip enables distinguishing *Vp-1* of the studied wild species (*Th. intermedium*, *Th. ponticum*, *Th. bessarabicum*, *P. stipifolia*, *D.villosum*) from wheat when DNA of these species is used as a template separately. Therefore, it helps to distinguish *Vp-1* genes of different species in hybrids such as amphiploid, addition, substitution, translocated or introgression lines of wheat as well. But if different *Vp-1* genes are in the same genotype the preferential amplification from one of the targets may occur. At the same time, there may be weak or no amplification from other targets. As a consequence, it may lead to false negative results. To estimate the simultaneous amplification from different genomes with the Vivip marker it was tested the use of wheat-wheatgrass amphiploid lines carrying genomes of wheat and *Thinopyrum* chromosomes (Fig. 4).

As a result of testing the Vivip marker on the lines of wheat-wheatgrass amphiploid lines, the amplification products from wheat genes *Vp-1B* (385 bp) and *Vp-1D* (399 bp) as well as distinct bands amplified from *ThVp-1* gene of *Thinopyrum* were shown. Amphiploid 1872 carried 377 bp allele (*ThVp-1a*) presumably from *Th. ponticum* and 1876–360 bp (*ThVp-1b*) allele presumably from *Th. intermedium* (Fig. 4). The band from *Vp-1A* gene of wheat is weak due to its relatively big size and competitive amplification from other DNA targets.

In conclusion, the developed PCR-based STS maker Vivip allows identification of *ThVp-1* gene of wheatgrass on the background of three *Vp-1* genes of wheat *Vp-1A*, *Vp-1B* and *Vp-1D*. The STS marker can be useful in the studies of the *Vp-1* effect on preharvest sprouting in *Triticeae*, monitoring introgressions in wide hybridization, rapid analysis of allelic variants both in wheat-wheatgrass hybrids and in *Thinopyrum*, *Pseudoroegneria* and *Dasypyrum* species.

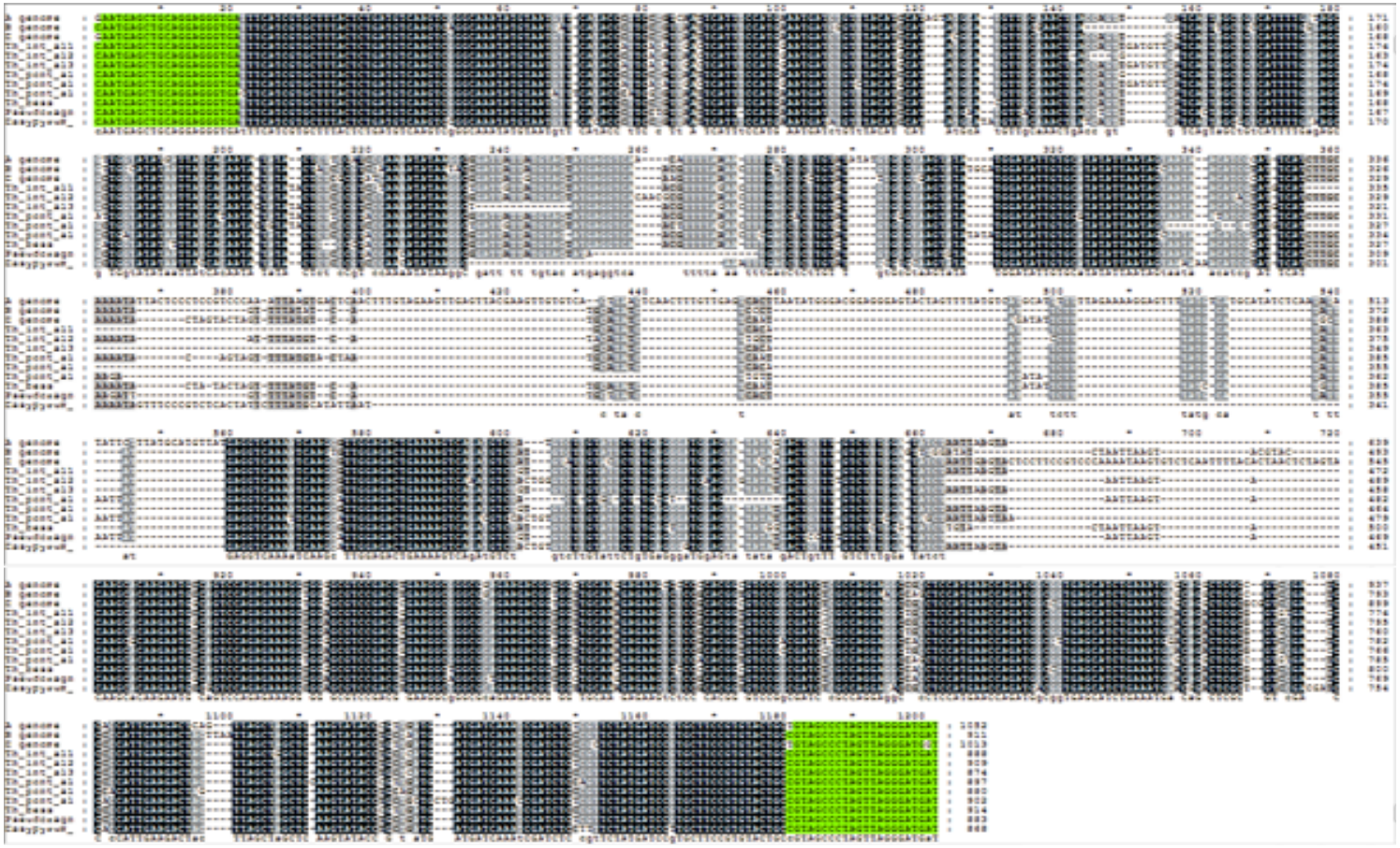

Fig. 1. Alignment of *Vp-1* sequences between Vp-1BB4Fand Vp-1BB4R from top to bottom: Vp-1A (AJ400712.1), Vp-1B (AJ400713.1), Vp-1D (AJ400714.1), Vp-1 *Thinopyrum intermedium*, (KC788534.1), Vp-2 *Th. intermedium* (KC788535.1), Vp-3 *Th. intermedium* (KC788536.1), Vp-1 *Thinopyrum ponticum* (KC788550.1), Vp-2 *Th. ponticum* (KC788551.1), Vp-3 *Th. ponticum* (KC788552.1), Vp-1 *Thinopyrum bessarabicum* (KC788537.1), Vp-1 *Pseudoroegneria stipifolia* (KC788524.1), Vp-1 of *Dasypyrum villosum* (KC788529.1), Vp-2 of *D. villosum* (KC788530.1)

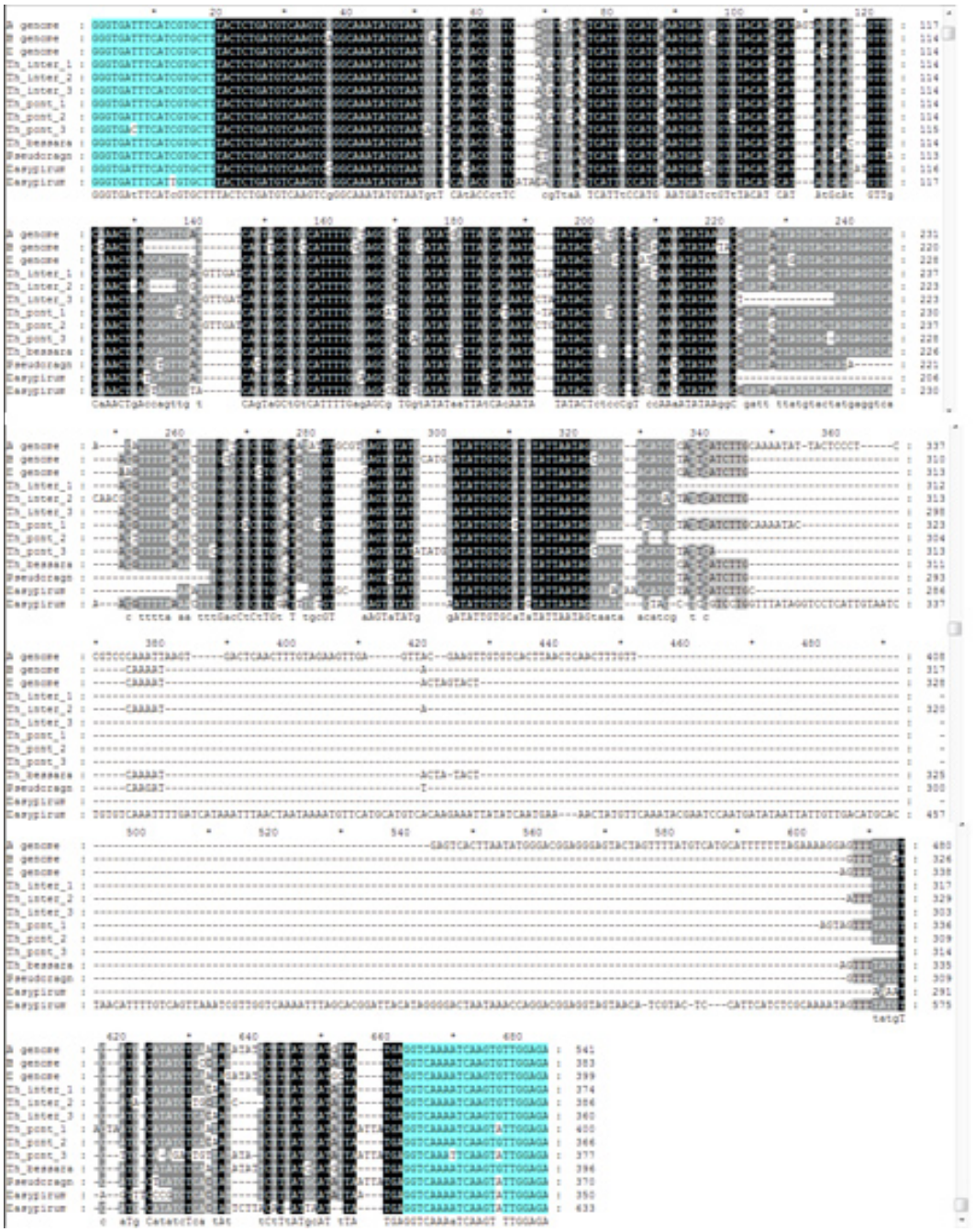

Fig. 2. Alignment of Vp-1 nucleotide sequences between primers VivipF and VivipR of the following species (from top to bottom): *T. aestium*: Vp-1A (541 bp), Vp-1B (383 bp), Vp-1D (399 bp); *Th. intermedium* (386 bp, 374 bp, 360 bp), *Th. ponticum* (400 bp, 377 bp, 366 bp), *Th. bessarabicum* (396 bp), *P. stipifolia* (370 bp), *D. villosum* (350 bp, 633 bp)

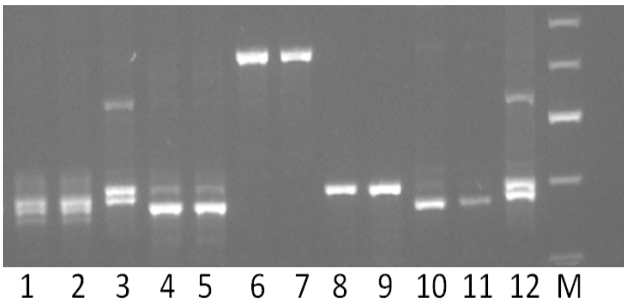

Fig. 3. PCR amplification products obtained using primers VivipF and VivipR 1, 2 — *Th. intermedium;* 3 — *T. aestium* (Bezostaya 1); 4, 5 — *Th. ponticum;* 6, 7 — *D. villosum*; 8, 9 — *Th. bessarabicum*; 10, 11 — *P. stipifolia*;12 — *T. aestium* (Mironovskaya 808); M — DNA size standard (100 bp Ladder)

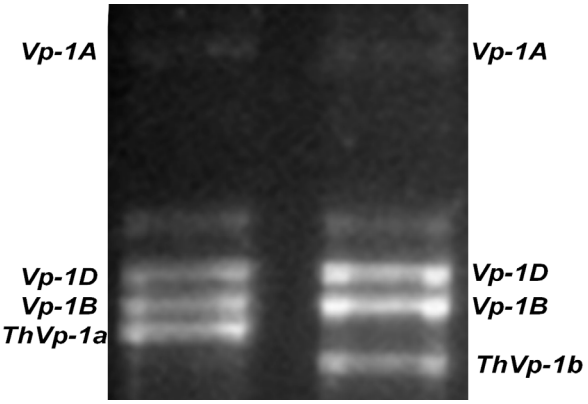

Fig. 4. PCR amplification products obtained using primers VivipF and VivipR; the DNA of wheat-wheatgrass, amphiploids 1872 (left lane) and 1876 (right lane) were used as a template

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СОЗДАНИЕ STS-МАРКЕРА ДЛЯ ПЦР-ИДЕНТИФИКАЦИИ ГЕНА VIVIPAROUS-1 У РАЗЛИЧНЫХ ВИДОВ ПЫРЕЯ В ГЕНЕТИЧЕСКОМ ОКРУЖЕНИИ ПШЕНИЦЫ

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Одним из возможных способов повышения устойчивости к предуборочному прорастанию может быть внедрение в геном пшеницы генов Viviparous-1 (Vp-1) из различных видов трибы пшеницевых (Triticeae). Одним из перспективных источников чужеродных генов Vp-1 являются виды пырея (Thinopyrum). Нами был разработан STS-маркер, позволяющий с помощью полимеразной цепной реакции различить гены Vp-1 пырея (Thinopyrum) и пшеницы (Triticum) при их одновременном присутствии в геноме. Данный маркер может быть использован в работах по изучению влияния генов Vp-1 на устойчивость к прорастанию на корню у пшеницевых, контроле интрогрессии генов при отдаленной гибридизации, а также для быстрого анализа аллельных вариантов Vp-1 как у пшенично-пырейных гибридов, так и у различных видов Thinopyrum, Pseudoroegneria и Dasypyrum.

Ключевые слова: молекулярный маркер, STS, ген Viviparous-1, Thinopyrum intermedium, Thinopyrum ponticum, Thinopyrum bessarabicum, Pseudoroegneria stipifolia, Dasypyrum villosum.

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