# ABOUT POSSIBLE USE OF Agropyron *Vp-1* (*Viviparous-1*) GENS- HOMOLOG FOR IMPROVEMENT OF SOFT WHEAT

*M.G. Divashuk[1], P.Yu. Krupin[1], I.A. Fesenko[1], V.I. Belov[2], O.V. Razumova[1], A.A. Korotaeva[1], G.I. Karlov[1]*

[1] *Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Moscow 127550, Russia*
*e-mail: divashuk@gmail.com;*
[2] *Department of Distant Hybridization of N.V. Tsitsin State Botanical Garden, RAS,*
*Moscow province, Snegiri-1 143591, Russia*

S u m m a r y

The molecular CAPS-marker was developed which permits to identify the *Viviparous-1* gen of *Agropyron*. The variant of wheat-agropyron hybrid № 209 was revealed, which contain the *Viviparous-1* gen from *Agropyron*. It was shown, that this variant has higher resistance to root sprouting, than other studied variants of wheat-agropyron hybrids. The isolated variant may be used as donor of *Viviparous-1* gene of *Agropyron* for its introgression to soft wheat genome thought suggested molecular marker.

In many countries including Russia, pre-harvest sprouting of grain is a serious problem that strongly limits increase of wheat production today. Seed germination at the root significantly worsens grain quality and, consequently, economical efficiency of its production (1). Germination occurs as the result of dormancy violations in immature seeds, which leads to the activation of physiological processes and early embryo growth. Dormancy of seeds is provided by a combination of many factors: the balance of abscisic and gibberelic acids, α-amylase activity, the presence in glumes and seed shell of water-soluble inhibitors of germination, ear architectonics (2-4). During the evolution, wild species developed dormancy as the mechanism to avoid unfavorable factors and intraspecific competition. The selection of cultivated crops was aimed at simultaneous and fast germination of seeds, which has led to their increased sensitivity to the factors provoking violation of dormancy (5).

An important step in studying the phenomenon of seed germination (and its particular case - pre-harvest sprouting) in cereals is associated with cloning *Viviparous-1* genes. The gene *Viviparous-1* (*Vp-1*) was found to control late embryogenesis in cereals (6) and perform two functions: providing embryo maturation and regulation of seed transition to dormancy by suppressing germination. The analysis and cloning of *Vp-1* genes have shown that they encode in cereals the transcription factors intensely expressed in developing embryos of seeds (6-8). All cloned orthologs of *Vp-1* contain functional domains B1, B2 and B3, which bind to DNA in the area target promoters and activate the expression of corresponding genes (5-11). The structure and expression of three homologous *Vp-1* genes of soft wheat were studied, which revealed that each of them can encode a full-size protein. However, incorrect splicing of the pro-matrix RNA leads to an aberrant translation product. In this case, soft wheat lines in which this gene contains an insertion of 193 bp lenght and deletion of 83 bp demonstrate higher expression level of *Vp-1* product synthesized at the correct splicing, which fact possibly determines in these forms the resistance to pre-harvest germination (12). Incorrect splicing has also been detected in most of *Vp-1* transcripts in diploid and tetraploid ancestors of soft wheat, which suggests that modern wheat has inherited this gene structure from ancestral forms (12).

Incorporation in soft wheat genome of genetically modified construct containing the gene *Vp-1* from *Avena fatua* L. has resulted in increased resistance to pre-harvest sprouting (12). Therefore, it is possible to increase the resistance to pre-harvest germination in soft wheat by interspecific and distant hybridization providing the introduction into its genome of *Vp-1* genes obtained from different species of cereals, the most promising of which is wheatgrass.

Creation of octaploid wheat-wheatgrass hybrids can be the effective approach providing a "selection bridge" for gene transfer from wheatgrass to wheat. Such interspecific hybrids carry 38-42 chromosomes of wheat and 14-18 chromosomes of intermediate wheatgrass. Numerous commercially valuable traits and cytogenetic studies of these octaploid hybrids indicate the presence in these plants of different combinations of wheatgrass chromosomes and, therefore, different *Vp-1* genes originated from this wild species (14).

The purpose of this study was to identify the lines of intermediate octaploid hybrids of wheat and wheatgrass carrying the genes orthologous for *Viviparous-1* and originated from intermediate wheatgrass, and evaluation of these lines' resistance to pre-harvest sprouting.

*Technique.* The object of study were wheat-wheatgrass hybrids №№1779, 1514, 209, 70s, 1744, 80, 186, 5787, 1867, 4044, 1375 and 1754 ($2n = 42W + 14Iw$, where Wh and Iw - chromosomes of wheat and intermediate wheatgrass, respectively) obtained through the stepwise interspecific hybridization between various wheat cultivars and *Thinopyrum intermedium* and further selection of hybrids in the Department of Distant Hybridization of N.V. Tsitsin State Botanical Garden of RAS. Seeds of wheatgrass cv Rostovskii 31 (k-33706; $2n = 42$, SSJJJsJs) were kindly provided by the Department of Fodder Crops of N.I. Vavilov All-Russia Research and Development Institute of Plant Growing (St. Petersburg).

DNA was isolated according to the method of R. Bernatzky and S.D. Tanksley with slight modifications (15). PCR was performed using VP-1BB4F and VP-1BB4R (12). Amplification was carried out under the scheme:  initial denaturation at 94 °C for 1 min and then 35 cycles at the following change of regimes: 95 °C, 1 min; 66 °C, 1 min; 72 °C, 1 min; then final elongation at 72 °C for 10 min. Alignment of sequences was performed using the program GenDoc. Restriction with endonuclease Hae III was performed for 12 h at 37 °C. PCR products were separated in 2% agarose gel with TBE buffer at 6 V/cm field strength. The molecular weight marker of amplification fragments was 100 bp Ladder ("Fermentas", Lithuania).

A seed dormancy index for each sample was determined by calculating a weighted index of germination of threshed grains in Petri dishes using the modified method of M. Walker-Simmons (16) (test for 14 days). The wax-ripe ears were harvested, threshed,

put by hand in Petri dishes (d = 10 cm) ridge down on two layers of filter paper moistened with 10 ml distilled water, and germinated at non-specified temperature (22-26 °C). The weighted index of germination (GI) was calculated under the formula:

$$GI = \frac{(14 \times n_1 + 13 \times n_2 + ... + 1 \times n_{14})}{D \times N},$$

where $n_1$, $n_2$, ... $n_{14}$ - the number of newly germinated seeds on the 1st, 2nd and subsequent days; $N$ - total number of seeds; $D$ - total duration of testing, days; 14, 13, ..., 1 - correction factor.

*Results.* Previously, the authors had performed a direct sequencing of the intron-exon region of *Viviparous-1* genes-orthologs in wild relatives of wheat (17). Using the comparison of sequences and the designed primers Vp1BB4 (17), there was developed a molecular CAPS-marker (cleaved amplified polymorphic sequences) allowing the recognition of *Viviparous-1* genes of wheat and wheatgrass nature. The revealed single nucleotide differences suggested choosing restriction enzyme Hae III providing identification of *Vp-1* genes inherited from wheatgrass and wheat. In the case of a wheatgrass *Vp-1*, amplification and restriction with Hae III results in fragments of 539 and 349 bp lengths; this assumption was confirmed in DNA sample of intermediate wheatgrass *Thinopyrum intermedium*. In the case of wheat *Vp-1*, resulting fragments have the lengths different from those in wheatgrass and depending on allelic state of the gene. There also should be considered the possibility of amplification of restriction fragments and genes-orthologs from the subgenomes A and D of soft wheat. For the subgenome D, results of restriction can't be predicted owing to the absence of its nucleotide sequences in NCBI database (The National Center for Biotechnology Information, USA).

That's why the developed marker was firstly tested on several cultivars of wheat and samples of intermediate wheatgrass (*Th. intermedium*) (Fig. 1). The obtained data coincided with theoretically expected results of restriction. For wheat, electrophoregrams also showed the presence of extra fragments, probably due to amplification in homologous genes located in soft wheat subgenomes A and D, and further restriction. However, none of them had a length characteristic to wheatgrass fragments (539 and 349 bp). Thus, the developed CAPS-marker was proved to be efficient for identification of the wheatgrass gene *Viviparous-1* in the genome of soft wheat.
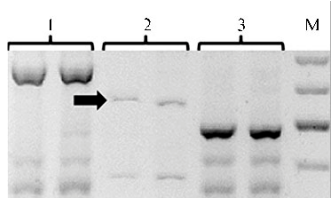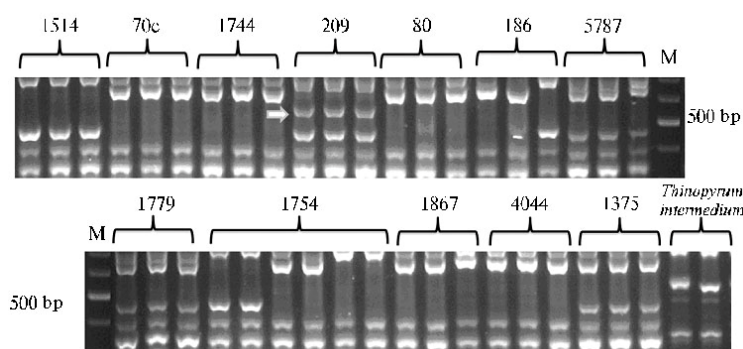


Fig. 1. Electrophoregram showing the products of restriction with Hae III enzyme after their amplification with primers VP-1BB4: 1, 3 — respectively, soft wheat cultivars Nota and Kuma; 2 – intermediate wheatgrass *Thinopyrum intermedium*, arrow shows the marker band specific to the wheatgrass gene *Viviparous-1* (*Vp-1*); M – molecular weight marker 100 bp Ladder, from up to bottom, resp., 700, 600, 500, 400 bp.

The developed molecular CAPS-marker was used for testing the genomes of wheat-wheatgrass hybrids, and the only one of the 12 tested forms (№ 209) was found to carry the wheatgrass gene *Viviparous-1* (Fig. 2). Along with it, the wheat-wheatgrass hybrids differed by alleles of the wheat gene *Vp-1* (see Fig. 2).

Thresholds of pre-harvest sprouting resistance in wheat can be assessed by germination index of a sample: GI <20 - resistant, 20<GI<50 - relatively resistant, GI> 50 - non-resistant (18).



Fig. 2. Electrophoregram showing the products of restriction with Hae III after amplification of DNA samples obtained from 12 lines the wheat-wheatgrass hybrids (№№ 1779, 1514, 209, 70s, 1744, 80, 186, 5787, 1867, 4044, 1375 and 1754) with primers VP-1BB4: M — molecular weight marker 100 bp Ladder, from up to bottom, resp., 700, 600, 500, 400 bp. Arrow shows the band specific to the wheatgrass gene *Viviparous-1* (*Vp-1*).

The evaluation of possible links between *Viviparous-1* gene and pre-harvest sprouting resistance has shown that the hybrid sample № 209 carrying the wheatgrass gene *Viviparous-1* is more resistant than all other investigated forms of wheat-wheatgrass hybrids (GI of the samples №№ 1779, 186, 1514, 5787, 209, 1867, 70s , 4044, 1744, 1375, 80 and 1754 amounted to, respectively, 76, 68, 84, 75, 40, 73, 57, 85, 63, 59 and 52%). This only sample was defined as relatively resistant, while all other hybrid forms were non-resistant.

These data allow to assume: the wheatgrass gene *Viviparous-1* detected in the genome of the sample № 209 contributes to its increased resistance to pre-harvest sprouting. Though, this hypothesis needs to be confirmed by transfer of this gene into the genome of wheat and further assessment of practical suitability of this approach for improving wheat cultivars. The authors believe that the developed molecular CAPS-marker provides real prospects for directed introgression of the wheatgrass gene *Viviparous-1* into wheat genome using marker-assisted selection (MAS).

So, a molecular CAPS-marker has been developed and proved as efficient for separate identification of the wheatgrass gene *Viviparous-1* at presence of the orthologous wheat genes *Viviparous-1*. Among the tested wheat-wheatgrass hybrids, the only sample (№ 209) carrying the wheatgrass gene *Viviparous-1* has been revealed. This sample was found to manifest the increased resistance to pre-harvest sprouting than other studied samples, and it can be used as a donor of the wheatgrass gene *Viviparous-1* for its introgression into the genome of soft wheat using the developed molecular CAPS-marker.

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