PCR ANALYSIS OF STOMATA BIOGENESIS GENES IN COMMON WHEAT VARIETIES

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Abstract

The aim of this study was to develop primer systems for precise genotyping of bread wheat cultivars by stomatal biogenesis genes. Seven sets of primers covering different gene regions of EPF1-A1, EPF1-B1, EPF1-D1, EPF2-A1, and MUTE-D1, were designed to annotate genetic diversity among 72 wheat cultivars. The developedDNA markers represent effective, informative means for selecting drought tolerance germplasm donors to promote wheat breeding programs.

Keywords: *EPF1, EPF2, MUTE, DNA markers, genotyping.*

Introduction. Drought tolerance is an extremely complex trait that is regulated at the genetic, physiological, and metabolic levels. Recent world studies point to the stomata a new important factor underlying the fundamental mechanisms of efficient conservation and use of water during drought in wheat. Essential genetic determinants involved in stomata formation are *EPF1*, and *EPF2* genes, which encode secretory polypeptides that control stomatal biogenesis by regulating asymmetric cell division, and the *MUTE* genes, which regulate the transition from proliferation to stomatal cell differentiation [1, 2]. The DNA marker technologies allow us to study the sequence diversity of the target genes among the cultivars and are an efficient molecular genetic tool for germplasm donors selection in conventional breeding. Thus, the aim of the work was to develop primer systems for precise genotyping of bread wheat cultivars by stomatal biogenesis genes.

Materials and methods. Genomic DNA was extracted from 3 kernels for 72 Ukrainian cultivars and purified following the CTAB protocol [3]. Polymerase chain reaction (PCR) of 20 µl included 0,5 µM of forward and reverse primers each (Metabion), Reaction Buffer B (Solis BioDyne) containing 2 mM (1,5 mM for E1D2) $MgCl_2$, 0,2 µM of each deoxyribonucleotide triphosphate (Thermo Fisher Scientific), 1 unit of FIREPol® DNA Polymerase (Solis BioDyne) and 30 ng of total plant DNA. In total 7 primer systems were developed according to our previous researches[4] and used in the study to annotate sequence diversity: E1A1 (+274 SNP in *EPF1-A1* gene, 5'-AGCCGCATGATCTCTACGTG-3', 5'-CCGACACATCCTTCTTCTCC-3'), E1B1 (+273 SNP in *EPF1-B1* gene, 5'-CGTTCACCCCCTTCTTCTCC-3', 5'-CAGGAACCCCTTCTCCTCCA-3'), E1B2 (+273 SNP and +276 6-bp insertion in *EPF1-B1* gene, 5-'ATGATGCTGATGCGAAACCG-3', 5'-AGGAACCCCTTCTCCTCCGT-3'), E1D1 (-9 4-bp deletion in *EPF1-D1* gene, 5'-TACGCGCGCATTCCTGGTCG-3', 5'-GGAACCCCTTCTCCTCCGTC-3'), E1D2 (+250 12-bp insertion in *EPF1-D1* gene, 5'-GGAGAAGGAGGATGGGTCGG-3', 5'-TAGCACTTGCCCTTGCACAT-3'), E2A1 (+451 SNP in *EPF2-A1* gene,5'-

GCCGTGCAAGCGGGTCATGA-3', 5'-GGGGTACGTTGCGGAGCACG-3') MD1 (+652 SNP in *MUTE-D1* gene, 5'-CTCCACGTCAACATCAGCAC-3', 5'-CCAATCTGCAATGCAACAGA-3'). The PCR products were separated by gel electrophoresis using lithium borate buffer (10 mM lithium borate pH 8,5) containing 0,1 μg/ml ethidium bromide. DNA fragments were visualized in UV light. The frequency of polymorphic loci was calculated by dividing the number of polymorphic loci by the total number of loci observed [5].

Results and discussion. Primer system E1A1 amplification of 173 bp with frequency 0.15 denoted SNP T for G at the position +273 from transcription start of *EPF1-A1* gene in the least of cultivars. E1B1 pointed to SNP C for T at the position +273 of *EPF1-B1* gene, generating amplification of 254-bp fragment with frequency 0,46. Another primer system E1B2 for *EPF1-B1* gene annotates +273 SNP T for C and +276 6-bp insertion, producing 88-bp fragment with frequency 0,69. Two systems were developed to genotype *EPF1-D1* gene. E1D1 identifies 4-bp deletion at the position -9 to transcription start generating amplified fragment of 281 bp with frequency 0,74. E1D2 determines 12-bp insertion at the position +250 amplifying 183-bp band with frequency 0,42. One reproducible primer system was developed for *EPF2* and *MUTE* genes. E2A1 was designed to determine the single nucleotide substitution G for A of *EPF2-A1* gene at position +451. Amplification with the system generates 140-bp fragment with frequency 0.06. PCR with MD1 primer system indicates SNP A for T at the +652 position to transcription start producing band of 182 bp with frequency 0,78. As a result, it was shown that the most common polymorphism among the analyzed bread wheat Ukrainian cultivars was SNP A for T at the +652 position of *MUTE-D1* gene. Another common variant was the detected deletion located in *EPF1-D1* gene's promotor (-9 to transcription start). The lowest frequency was SNP G for A (at position +451) of *EPF2-A1* gene.

Conclusions. The combination of developed DNA markers enabled effective and reproducible annotation of cultivars genetic diversity. The developed molecular markers represent effective, informative means for selecting drought tolerance germplasm donors to promote wheat breeding programs.

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