DNA MARKERS TO DETECT POLYMORPHISMS IN STOMATAL BIOGENESIS GENES *EPF1* AND *EPF2*

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INTRODUCTION

EPF1/EPF2 regulatory genes → Encoding secretory peptides EPF1/EPF2 → Regulation of asymmetric cell division → Control of stomatal biogenesis → Control of transpiration / Improving the efficiency of water use → Drought tolerance

The aim of the work was to develop DNA markers for genotyping wheat varieties to find valuable alleles of the stomatal biogenesis genes *EPF1* and *EPF2*.

MATERIALS AND METHODS

The work analyzed a set of 67 bread wheat cultivars of Ukrainian origin, including two control genotypes. For molecular genetic analysis, the total DNA of plants was isolated from three seeds according to a CTAB method. The PCR was followed by electrophoresis in a 1.2% agarose gel.

RESULTS

The nucleotide promoter sequences of the *EPF1* and *EPF2* genes were used to develop four DNA markers, which aim to identify polymorphisms within this region. More information about DNA markers is presented in Table 1.

DNA markers	Gene	Polymorphism	Expected amplified fragment, bp
EPF1-A1	*EPF1-A1*	two SNPs (-1223 A→G; -1221 A→C)	380 / -
EPF1-B1	*EPF1-B1*	the127-bp insertion at the position -1486...-1487	480 / 353
EPF1-D1	*EPF1-D1*	the 11-bp GACCACTACTT insertion at position -1584...-1585	280 / -
EPF2-B1	*EPF2-B1*	the 297-bp insertion (at positions -963...-964)	267 / -

Table 1. DNA markers for determination of the polymorphism of stomatal biogenesis genes EPF1 and EPF2

The EPF1-A1 DNA marker was designed to detect two SNPs in the promoter region of the *EPF1* gene from subgenome A. Its frequency for Ukrainian cultivars was quite high, at 0.88. The EPF1-B1 DNA marker was created to detect variability in the promoter part of the *EPF1* gene from subgenome B. Among Ukrainian varieties, only 2 samples had this insertion (frequency 0.03). However, in 6 varieties, two amplification products were detected at the same time, since different genotypes are used during selection, it can be stated that these varieties are heterozygous for this feature.

EPF1-D1 is designed to detect the in the promoter part of the *EPF1* gene from subgenome D. The frequency of this polymorphism was 0.36 among Ukrainian common wheat varieties. The DNA marker EPF2-B1 was created in order to determine the 297-bp insertion. The size of the expected amplified fragment was 267 bp, indicating an insertion presence (frequency 0.37). The described results are presented in Figure 1

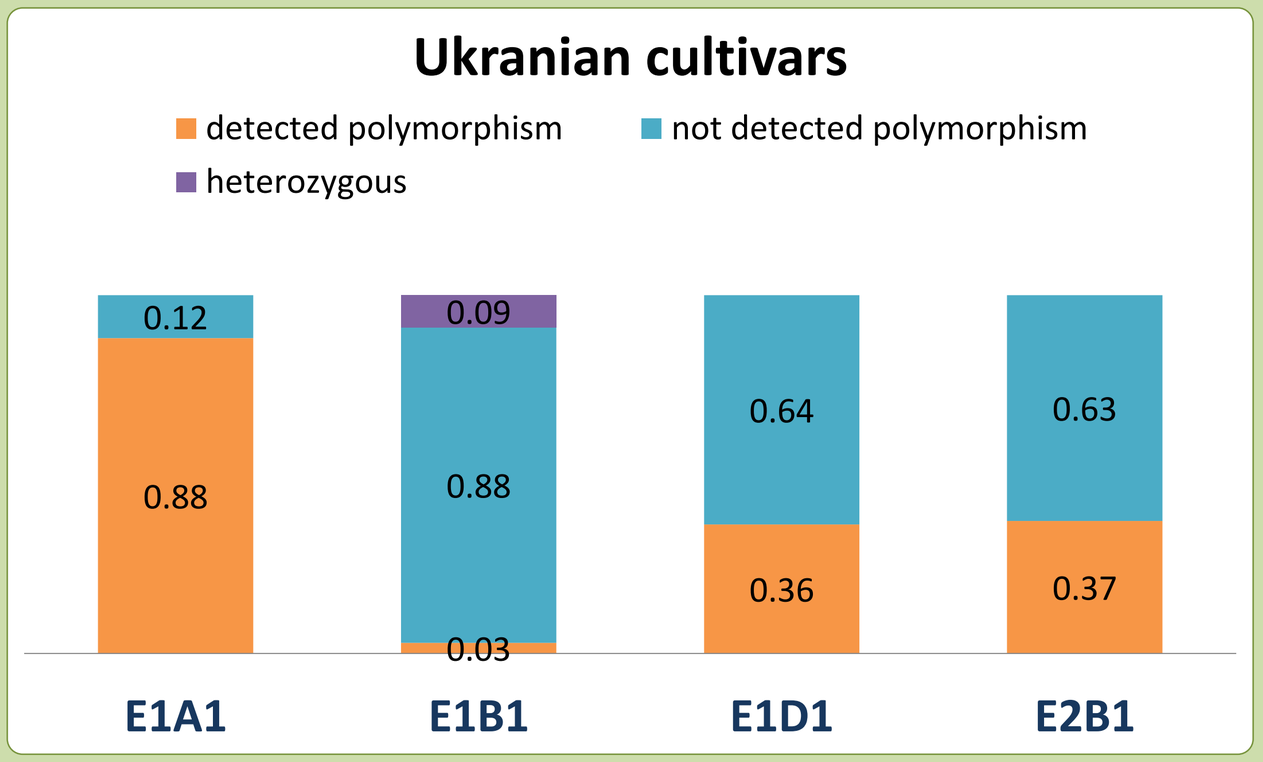

Fig. 1. The frequency of analyzed polymorphisms among a sample of bread wheat varieties of Ukrainian origin.

CONCLUSION

The results show that two SNPs (-1223 A→G; -1221 A→C) of the promoter region of the *EPF1* gene from subgenome A are most common among Ukrainian varieties. The lowest frequency of occurrence among Ukrainian varieties is the insertion in the promoter region of the *EPF1* gene from subgenome B is detected by the EPF1-B1 marker.

Therefore, the developed DNA markers can be utilized for describing diverse wheat samples and screening breeding material for the identification of potential drought tolerance donors.

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