doi 10.18699/PlantGen-2025-287

Features of functioning of multigenic protein families under different stresses in some fruit crops

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Motivation and aim: The multigene protein family of class III peroxidase (POD) is the key antioxidant enzyme, which responds to different negative biotic and abiotic factors as well as involved into the plant development and growth. The number of encoding genes varies in different species and could be up to 100 and more. For example, has 138 and 124 POD genes were detected in *Oryza sativa japonica* and *Glycine max* respectively, whereas only 73 PODs were found in *Arabidopsis thaliana*. So, the evolution of this superfamily is inextricably linked to doublings and duplications. Such gene paralogisation could be resulted in the gene subfunctionalisation and influence on the plant resistance to stress factors. The aim of this study, therefore, was to evaluate the expression of POD genes in two fruit crops (*Prunus persica* and *Vitis vinifera*) using transcriptomics techniques and determine the possible correlation between the gene's expression, stresses and cultivars with different tolerance.

Materials and methods: To identify and analyse PODs we used the genome assembly of *V. vinifera* cv. Pinot Noir cl. PN40024 and the genome assembly of *P. persica* cv. Lovell from NCBI. The identification of POD genes was based on finding the peroxidase domain using InterProScan software (accession number IPR000823 according to the database). The exon-intron structure was detected using the CDS description and genome annotation. A motif analysis was performed using the MEME online tool in classic mode. A phylogenetic analysis was conducted using IQ-TREE software. The multiple alignment of protein sequences was performed using the MAFFT. The best amino acid substitution model was estimated with ModelFinder. To analyse the expression of the POD family in the peach, a transcriptome analysis of the available RNA-seq data in NCBI was performed. The first analysed experiment (PRJNA610066) was dedicated to the expression of this gene family upon infection with the fungus *M. laxa* in the mature and immature fruits of *P. persica* cv. Venus. The second analysed experiment was dedicated to study the effect of cold stress on peroxidase gene expression in the peach (PRJNA784945). Experiments *in vitro* were carried out to evaluate the expression of peroxidase genes in grapes under different stresses. Chloride and sulphate salinisation, by adding NaCl and Na_2SO_4 to the culture media, and induction of drought conditions, by adding mannitol to the culture media, were used as stresses. The 40 transcriptomes of several grape cultivars were analysed: Ferkal, BBB, Aligote, Podarok Magaracha, and Saperavi. Sequencing was performed by 150 bp paired-end reads using BGI platform. All transcriptomes were analysed using the same pipeline: quality assessment, filtering and trimming, mapping to the peach and grape peroxidase database respectively, and calculating the scores to estimate the expression level of each POD gene. Quality and length trimming were conducted using fastp. Mapping to the peroxidase database was carried out using a pseudo-alignment algorithm implemented in Kallisto v0.48.0. TPM (transcripts per million) was used for the comparative estimation of POD gene expression. Also, we investigate the POD expression in flower buds of *P. persica* under the cold stress in the laboratory condition. We selected to type of cultivars: medium tolerant (Loadel and Spring Gold) and high tolerant (Podarok Like and Temisovskij) and evaluate the gene expression for four POD genes by qPCR.

Results: Peroxidase genes form a diverse family with different exon-intron structure, and limited variation in the composition of conserved motifs. No significant association between gene structure (homology level, number of introns, composition of conserved motifs) and expression level under different stresses was established. Peroxidase genes could be clustered into low-expressed and high-expressed genes. The total peroxidase expression often increases under the stress, but individual POD genes could react in different way. Also, the various response of the same genes we observed in peach and grape cultivars with different tolerance to stress. For example, a correlation between the overall peroxidase expression and the cold tolerance of different peach varieties was demonstrated. Therefore, the expression pattern of POD may indicate the stress tolerance of cultivars of fruit crops.

Conclusions: Class III peroxidase is a large family of genes, some of them have functional specificity. The distinct genes exhibit increased and decreased expression depending on the type of stress. Thus, peroxidase gene duplications can be considered as one of the mechanisms of adaptive evolution that leads to more efficient functioning of the antioxidant system of plants.

Funding: The study is supported by the Russian Science Foundation Grant No. 23-76-10013.