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Organization and evolution of the polyphenol oxidase gene family in barley

Levanova N.M.[1, 2] *, Glagoleva A.Y.[1], Khlestkina E.K.[1, 2, 3], Shoeva O.Y.[1]
[1] *Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia*
[2] *Novosibirsk State University, Novosibirsk, Russia*
[3] *N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg, Russia*
** e-mail: n.levanova@g.nsu.ru*

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Motivation and Aim: Tyrosinases are ubiquitous enzymes among all living organisms. In plants, tyrosinases called polyphenol oxidases (PPO) are involved in the response to abiotic and biotic stresses. In cereals, *Ppo* is predominantly represented by a gene family. Two *Ppo* genes were previously identified in barley: *Ppo1* and *Ppo2*, but additional copies were expected. The aim of the current study was to characterize the *Ppo* gene family in barley, to analyze the functional activity of the *Ppo* genes and their evolutionary relationships with orthologs of other cereals.
Methods and Algorithms: We used the NCBI database, IPK Barley BLAST Server and EnsemblPlants for sequences identification. For sequence alignment and the evolutionary tree construction, MULTALIN and MEGA 7.0 software were used. *Cis*-regulatory elements were analyzed using PLACE database. We developed specific primers for each copy using PrimerQuestTool. We used RT-PCR and qPCR to analyze expression of the *Ppo* genes.
Results: We identified two additional genes *Ppo3* and *Ppo4* based on the previously reported *Ppo1* and *Ppo2* and located them on chromosomes 3H and 4H, respectively. Copies contain the conservative tyrosinase domain, however, have a different intron-exon structure, as well as predicted promoter structure. Expression analysis in various organs (root, coleoptile, leaf, stem, developing spike, pericarp, and hulls) and also under salt stress showed that the *Ppo* genes possess different expression patterns. At the constructed evolutionary tree *Ppo1* and *Ppo2* clustered separately from copies of *Ppo3* and *Ppo4*. Presumably, the formation of the gene cluster comprising the *Ppo1* and *Ppo2* genes occurred as a result of segment duplication in the common ancestor of the Triticeae tribe. Orthologs of the *Ppo3* and *Ppo4* genes of barley were detected in other cereal species.
Conclusion: The *Ppo* gene family in barley contains at least four genes that maintain their functional activity during evolution.
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