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# HPPR gene expression in *Salvia sclarea* L. from Republic of Moldova

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**SUMMARY.** *Salvia sclarea* L. has received attention for its broad range of pharmacological activities and usage in cosmetics industry and food as for fragrances. However, little is known about the genetics of the secondary metabolites synthesis in that plant. In this research, the real-time PCR was used to investigate the expression of *HPPR* gene involved into acid rosmarinic metabolic pathway. An increased transcriptional activity of gene in hybrids compared to parental forms was identified.

**Keywords:** Hydroxyphenylpyruvate reductase (HPPR) gene, rosmarinic acid, *Salvia sclarea L*

## Introduction

*Rosmarinic acid* (RA) is a natural phenolic compound widely distributed in the plant kingdom contained in many *Lamiaceae* herbs. This substance has attracted interest due to its biological activities especially concerning its antioxidant, anti-inflammatory, antibacterial properties (Petersen, 2003).

According to EUROPAM, the annual cultivated area of *Salvia sclarea* L. (*Lamiaceae*) in Moldova is arund 2000 ha. The production and secondary metabolites yield is very variable (Dweck, 2000). The success of major compounds content increasing by sage breeding programs could be ensured through ability to regulate the RA synthesis metabolic pathway.

The ability to assess accurately genetic differences between parents and subsequently to predict progeny performance could enhance the efficiency of breeding programs. The investigation of the molecular basis provides opportunities for further research in a wide range of areas. In this study it has been investigated the transcriptional activity of *HPPR (hydroxyphenylpyruvate reductase)* gene governing the RA biosynthesis which includes to involve both the *phenylpropanoid* and a *tyrosine-derived* pathway.

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## Materials and methods

Twenty-eight genotypes of clary sage from *Aromatic and Medicinal Plants Collection* of the Institute of Genetics, Physiology and Plant Protection, ASM, including 13 hybrids and 15 parental forms were evaluated (Martea, 2014). The design of specific primers was performed using ***PRIMER3*** tool.

Total RNA was extracted from a bulk of five plants of each genotype using TriReagent according to the manufacturer's instruction. Real Time PCR analysis was performed with gene-specific primers and Maxima SYBR Green/ROX qPCR Master Mix on a DTprime Real-time cycler. All samples were analyzed in three replicates performed in three different runs. The relative expression was calculated via the $2^{-\Delta Ct}$ method.

## Results and discussion

The analysis of *HPPR* transcriptional activity showed that relative expression ranged from 0,015 to 0,3 conventional units. The highest transcript accumulation level of *HPPR* gene was detected in *[S. s. Turkmen/N $S_7$ x (K-36 x 0-41) $F_2$ x 0-19)$F_1$ x 0-22) $B_4$ x L-15)$F_8$]**$F_1$*** (H1) while the lowest quantities were observed in case of *(K-36 x 0-41) $F_2$ x 0-19)$F_1$ x 0-22) $B_4$ x L-15) $F_8$* (P14) (Fig. 1).

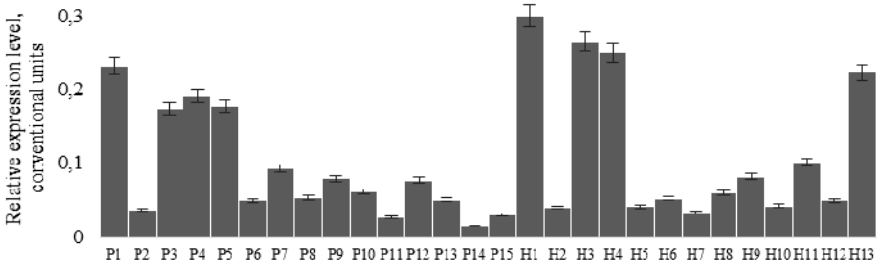


**Figure 1.** Relative expression of *HPPR* gene for clary sage genotypes

The quantity of transcripts in parental forms was relatively lower compared to clary sage hybrids. Results obtained for genetic groups correlated with hybrid vigor in clary sage. Hybrid vigor is substantial and important for most commercial traits in plants. Thus, 7 of the 13 investigated hybrids have shown quantitative values of the transcriptional activity higher than such in parental forms. The characterization of genetic variability and estimation of the genetic relationships among varieties are essential to clary sage breeding programs. Thus, these findings could represent a substantial advantage to predict the heterosis expected from crosses at all levels.

## Conclusions

The level of relative expression of the *HPPR* gene was determined and its involvement in the biosynthesis of acid rosmarinic in *Salvia sclarea* L. was demonstrated.

Our results showed an increased transcriptional activity of HPPR gene in hybrids compared to parental forms, selection of parental forms is an important first step in any breeding programs. At the same time, the generation of genetic information for medicinal plant and application of molecular breeding approaches are necessary for species cultivated in Republic of Moldova.

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