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TRANSCRIPTIONAL PROFILE OF SOD GENES IN SUNFLOWER UNDER BROOMRAPE STRESS

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INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is an obligate, chlorophyll-lacking root parasite and one of the most destructive pathogens of sunflower in Eastern Europe and the Mediterranean region, including the Republic of Moldova.

The continuous emergence of new, more virulent races following the cultivation of monogenic resistant varieties (*Or1–Or7*) has shifted research focus toward understanding non-specific, quantitative polygenic resistance mechanisms. Reactive oxygen species (ROS) are inevitable byproducts of cellular metabolism that function both as signaling molecules and as mediators of oxidative damage during plant stress responses. Superoxide dismutases (SODs) represent the first enzymatic defense against ROS, catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. Despite their essential role, the transcriptional regulation of SOD genes during the early stages of broomrape infection remains largely unexplored.

MATERIALS AND METHODS

Plant material and growth conditions: Experiments were performed on root tissues from sunflower (*Helianthus annuus* L.) seedlings of three F_1 hybrids: Favorit and P64LE20 (resistant to broomrape) and Performer (susceptible). Seeds were provided by INCDA Fundulea, Romania. Seedlings were grown on perlite in Petri dishes until the two-true-leaf stage. For biotic stress induction, pre-germinated *O. cumana* seeds were added to the growth substrate. Root samples were collected under two conditions: control (no pathogen) and infected (presence of *O. cumana*), at 2, 6, 12, and 24 hours post-inoculation.

RNA isolation and cDNA synthesis: Total RNA was extracted from frozen root tissues using TRI-zol reagent (Applied Biosystems). RNA purity and integrity were assessed spectrophotometrically and by 1% agarose gel electrophoresis. cDNA was synthesized using RevertAid RT (Fermentas) with Oligo$(dT)_{18}$ and random hexamer primers.

Gene expression analysis: Relative expression of *Mn-SOD I, Mn-SOD II, CuZn-SOD I, CuZn-SOD II* genes was determined by Real-Time PCR (QuantStudio® 5, Applied Biosystems) using SYBR Green and specific primers designed from *Helianthus annuus* NCBI sequences. Actin was used as the reference gene, and relative expression levels were calculated by the $2^{-}\Delta\Delta CT$ method. Three biological replicates were analyzed, and statistically significant differences were considered at $p \leq 0.05$ (ANOVA, Bonferroni test).

RESULTS AND DISCUSSIONS

Comparative analysis of SOD gene expression revealed distinct patterns between resistant and susceptible sunflower hybrids. In the absence of biotic stress, transcriptional activity followed the pattern Cu/Zn-SOD II > Cu/Zn-SOD I > Mn-SOD II > Mn-SOD I, with more pronounced variations in the susceptible Performer. Upon infection, the resistant Favorit showed rapid, oscillating up- and down-regulation of Mn-SOD I, Mn-SOD II, and Cu/Zn-SOD II during the first 12 hours, stabilizing by 24 hours, reflecting fine-tuned early redox control. P64LE25 exhibited a gradual and sustained activation, particularly of Mn-SOD I and Cu/Zn-SOD II, indicative of a delayed but stable oxidative homeostasis. In contrast, the susceptible Performer displayed moderate early activation followed by strong repression of Cu/Zn-SOD isoforms, culminating in a marked reduction of Cu/Zn-SOD II at 24 h, signaling transcriptional loss of control and oxidative overload.

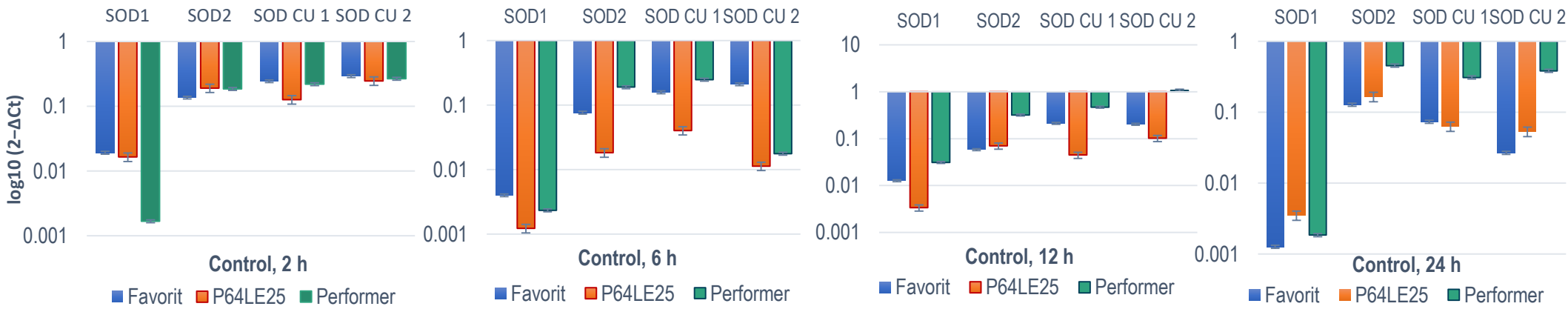

Figure 1. Expression levels of *SOD* genes in the roots of sunflower seedlings (hybrids Favorit, P64LE20, and Performer), cultivated in the absence of the pathogen, analyzed at different time intervals.

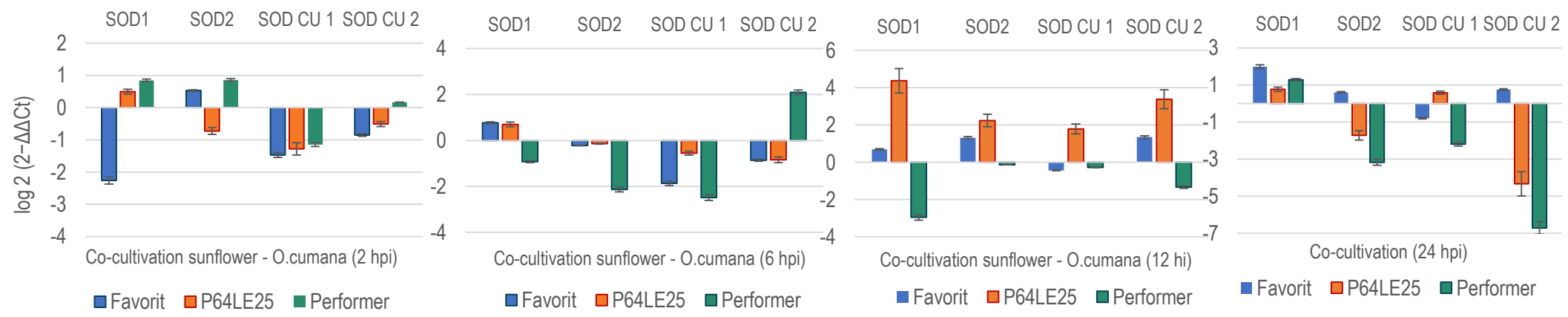

Figure 2. Comparative analysis *SOD* gene expression in the roots of resistant (Favorit, P64LE20) and susceptible (Performer) sunflower hybrids, depending on the duration of co-cultivation with germinated *Orobanche cumana* seeds (pathogen-present vs. control conditions).

Conclusions: The results provide a molecular basis for the use of SOD genes as early markers of resistance to *Orobanche cumana* infestation. Differential expression profiles can be integrated into marker-assisted breeding programs, facilitating the early identification of tolerant genotypes and accelerating the development of resistant sunflower cultivars.

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