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Transcriptional Profile of Sod Genes in Sunflower under Broomrape Stress

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ABSTRACT

This study investigated the transcriptional response of four superoxide dismutase (SOD)-encoding genes (*Mn-SODI, Mn-SODII, Cu/Zn-SODI* and *Cu/Zn-SODII*) in the roots of two resistant (Favorit, P64LE25) and one susceptible (Performer) sunflower hybrids exposed to biotic stress caused by *Orobanche cumana* Wallr. during the pre-attachment stage. Quantitative PCR (qPCR) analysis performed at 2, 6, 12, and 24 hours post-inoculation revealed distinct and genotype-dependent expression patterns.

The resistant hybrid Favorit exhibited a gradual, sustained, and finely coordinated modulation of SOD transcripts, indicative of efficient redox adjustment and controlled reactive oxygen species (ROS) signaling during early defense. P64LE25 showed rapid and relatively strong activation of specific SOD isoforms, suggesting the establishment of an early and effective ROS-scavenging response. In contrast, the susceptible hybrid Performer displayed a disorganized transcriptional profile, characterized by transient activation followed by progressive repression of SOD genes, reflecting impaired regulation of oxidative homeostasis during the critical early phase of parasite interaction.

Overall, the results demonstrate that resistance to *O. cumana* is associated not merely with the magnitude of SOD gene induction, but with the timing, coordination, and compartmentalized activation of SOD isoforms, determined by the genetic background of the host. These findings highlight the potential of SOD transcriptional profiles as early molecular indicators of sunflower resistance to broomrape stress.

Keywords: early host-parasite interaction, *Helianthus annuus* L., *Orobanche cumana* Wallr, qPCR, superoxide dismutases.

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 (Duca et al., 2022; Chachalis et al., 2025). The rapid evolution of new, more virulent races following the cultivation of monogenic resistant varieties (*Or1-Or7*) (Fernandez-Martínez et al., 2015; Fernandez-Melero et al., 2023) has shifted research focus toward understanding non-specific, quantitative resistance mechanisms. In this context, breeding programs developed at the National Agricultural Research and Development Institute Fundulea have generated sunflower genotypes with improved resistance to *Orobanche cumana*, emphasizing the importance of genetic background and the introgression of resistance traits for sustainable crop protection (Sauca et al., 2018). *O. cumana* initiates infection through seed germination stimulated by host root exudates, followed by haustorium formation and attachment to sunflower roots (Port and Duca, 2020). During the early pre-attachment and penetration stages, a complex network of molecular and biochemical responses is activated in the host plant to restrict parasite establishment. Among these responses, oxidative burst and redox signaling play pivotal roles in determining compatibility or incompatibility outcomes (Zhang et al., 2022). However, excessive accumulation of reactive oxygen species (ROS), particularly superoxide radicals ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), can cause oxidative damage to lipids, proteins and nucleic acids (Dumanovic et al., 2021). Therefore, tight regulation of ROS homeostasis is essential during host-parasite interactions.

Superoxide dismutases (SODs; EC

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1.15.1.1) constitute a key enzymatic component of the antioxidant defense system in plants (Duca et al., 2025). These metalloenzymes catalyze the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide, representing the first line of defense against ROS generated under normal metabolic processes as well as under abiotic and biotic stress conditions (Alscher et al., 2002). Plant SODs are classified into three main types according to their prosthetic metal cofactors: Cu/Zn-SOD, Fe-SOD and Mn-SOD. In higher plants, the number, type, gene family organization and subcellular localization of SOD isozymes vary depending on species, developmental stage, tissue specificity and environmental conditions (Gill and Tuteja, 2010).

In most angiosperms, Cu/Zn-SODs are localized predominantly in chloroplasts and cytosol, although additional isoforms have been reported in peroxisomes and the apoplast. Fe-SODs are typically found in chloroplasts and, in some species, in peroxisomes. Mn-SODs are mainly mitochondrial enzymes, with some reports of peroxisomal localization depending on species and stress conditions (Fink and Scandalios, 2002).

Regarding sunflower, have been demonstrated the presence of at least two mitochondrial Mn-SOD isoforms (Mn-SOD I and Mn-SOD II) in seedlings, as well as chloroplastic and cytosolic Cu/Zn-SOD isoforms (Palomo et al., 1999). Early biochemical analyses did not clearly demonstrate Fe-SOD activity in sunflower seedlings (Fernández-Ocaña et al., 2011; Arora and Bhatla, 2017). However, genome-wide analyses following the publication of the Helianthus annuus reference genome suggest the presence of Fe-SOD–like sequences that may be developmentally regulated or stress-inducible (Badouin et al., 2017). In silico annotation indicates that sunflower possesses a multigene SOD family comparable to other dicotyledonous species (approximately 5-8 SOD-encoding genes), including Cu/Zn- and Mn-type genes, while Fe-SOD genes representation appears limited.

Fernández-Ocaña et al. (2011) analyzed the expression of *Mn-SOD* and *Cu/Zn-SOD* genes in sunflower seedlings subjected to abiotic stresses (temperature extremes, mechanical wounding) and biotic stress caused by *Plasmopara halstedii*. Using quantitative RT-PCR, the authors demonstrated that *Cu/Zn-SOD* transcripts accumulated at levels approximately 1000-fold higher than those of mitochondrial *Mn-SOD*. Moreover, Mn-SOD I expression was about 12-fold higher than that of Mn-SOD II and showed significant modulation in response to both biotic and abiotic stimuli, even in the absence of apparent oxidative stress (e.g., low temperature). The authors concluded that mitochondrial *Mn-SOD I* may function as an early sensor of adverse conditions, preventing potential oxidative damage.

The present study investigates the differential transcriptional responses of four SOD-encoding candidate genes: *Mn-SODI* (DQ812551.2), *Mn-SODII* (DQ812552.2), *Cu/Zn-SODI* (AJ786257.1), and *Cu/Zn-SODII* (AJ786258.1) in the roots of three sunflower F_1 hybrids exhibiting contrasting levels of genetic resistance to *Orobanche cumana* during the pre-attachment stage of the host-parasite interaction.

The results contribute to clarifying the involvement of specific SOD isoforms in early host defense against *O. cumana*, providing molecular data for further research on antioxidant regulatory networks, with potential implications for the development of improved broomrape control strategies.

MATERIAL AND METHODS

Plant material and growth conditions. The experiments were performed on root tissue collected from sunflower seedlings (*Helianthus annuus* L.) of three F_1 hybrids. Two of the hybrids, Favorit and P64LE20, carry resistance genes against *Orobanche cumana* attack, whereas Performer lacks resistance genes. Seeds were provided by the National Agricultural Research and Development Institute (NARDI, Fundulea), Romania.

Sunflower seed germination and subsequent growth up to the two true-leaf stage were carried out in Petri dishes on a perlite substrate. To obtain biotic stress variants, pre-germinated *O. cumana* seeds were introduced into the plant growth medium. The parasitic seeds were preconditioned and germinated using root exudates from a susceptible genotype. *O. cumana* seeds were collected from an infested sunflower field in the central region of the Republic of Moldova (Chișinău municipality).

Root tissues were harvested from sunflower seedlings cultivated under two experimental conditions: biotic stress induced by germinated *Orobanche cumana* seeds in the growth medium, and control conditions without pathogen exposure. The host plant gene expression response during the pre-attachment stage of the parasite was analyzed at 2, 6, 12, and 24 h post-inoculation (hpi), with corresponding mock-treated control samples collected at identical time points.

Total RNA Isolation and cDNA Synthesis. Root tissue samples were frozen in liquid nitrogen and stored at -80°C prior to processing. Total RNA was extracted using TRI Reagent (Applied Biosystems) following the manufacturer's standard protocol. RNA quality and quantity were evaluated by spectrophotometry at 260 and 280 nm, and integrity was confirmed by 1% agarose gel electrophoresis. For reverse-transcription reactions the purified RNA samples (1 μg) were treated with DNAse I, RNAse-free (Thermo Scientific). First-strand cDNA was synthesized using RevertAid RT (Thermo Scientific) in the presence of $Oligo(dT)_{18}$ primers and random hexamers, according to the manufacturer's instructions. The experimental design comprised three biological replicates of each experimental variants.

Gene Expression Analysis by Real-Time PCR. Relative expression levels of genes encoding different SOD isoforms were quantified by real-time PCR using the QuantStudio® 5 system (Applied Biosystems). The amplification program consisted of an initial denaturation at 95°C for 10 min, followed by 5 cycles of 95°C for 15 s and 64°C for 20 s, and 40 cycles of 95°C for 15 s and 60°C for 40 s. Each Real-Time PCR reaction (total volume of 20 μl) included: 2 μl of template (1:6 diluted in RNAse free water cDNA), 2 × Maxima SYBR Green/ROX PCR Master Mix (Fermentas), 0.4 μM of each reverse and forward primers.

Gene-specific primers were designed based on the nucleotide sequences of *Helianthus annuus Mn-SODI* (DQ812552.2), *Mn-SODII* (DQ812551.2), *Cu/Zn-SODI* (AJ786258.1) and *Cu/Zn-SODII* (AJ786257.1) available in the NCBI database. The primers used were:

- *Mn-SODI*, F/R: ataaggaaagcccgattttg/ ttgcgattacaaacggtgaa;
- *Mn-SODII*, F/R: tgtgggttctgcaataagtcaa/ acaagcaaaagcaacaacca;
- *Cu/Zn-SODI*, F/R: cctaatgctgtggttggaagag/ agtggagaggctaagttcgtga;
- *Cu/Zn-SODII*, F/R: cactaattggaggtcaatccatc/ caatgataccacatgcaactcttc.

The gene-specific amplification products were assessed by melting curve analysis, followed by agarose gel electrophoresis. All reactions were performed in triplicate, including the non-template controls.

Gene expression levels of the target genes relative to *actin* (AF282624.1) were determined using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008). Expression differences, expressed as fold change (FC), were considered biologically relevant at $FC \geq 1.5$ and statistically significant at $p \leq 0.05$, based on ANOVA followed by the Bonferroni post hoc test. Data presented in the figures represent mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

Expression of SOD genes in sunflower seedling roots under optimal growth conditions

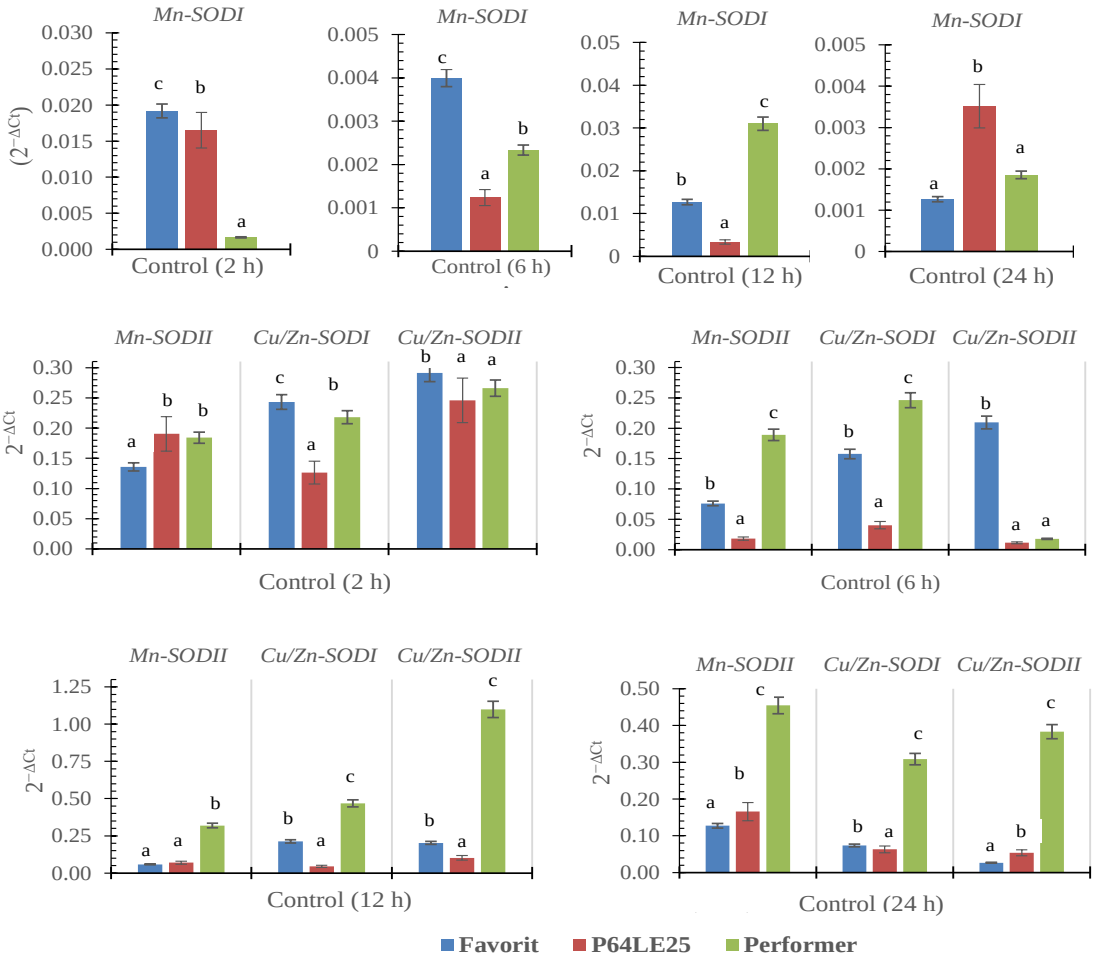
In the roots of sunflower seedlings grown under optimal growth conditions, the four SOD gene transcripts exhibited significant

quantitative variations (Figure 1), reflecting genotype-specific transcriptional profiles among the analyzed hybrids (Favorit, P64LE25, and Performer).

Mn-SODI showed the lowest expression across all genotypes during the 24-hour study period. In the Favorit and P64LE25 hybrids, *Mn-SODI* mRNA levels ranged from 0.001 to 0.019, corresponding to an approximately 19-fold fluctuation. By contrast, the Performer genotype displayed relatively higher expression, with values ranging from 0.002 to 0.031, indicating a 15.5-fold variation.

Compared to *Mn-SODI*, *Mn-SODII* mRNA levels were higher. In the Favorit hybrid, levels ranged from 0.058 to 0.136 (≈2.35-fold), while in P64LE25 they ranged from 0.018 to 0.191 (≈10.60-fold), with peak values observed at 2 and 24 hours. In contrast to the fluctuating dynamics observed in the resistant genotypes, the sensitive genotype Performer exhibited a progressive increase in *Mn-SODII* expression, from 0.184 at 2 hours to 0.455 at 24 hours (≈2.47-fold). Notably, in this genotype, the maximum *Mn-SODII* mRNA level was approximately 2-3 times higher than the maximum observed in the resistant genotypes.

The genes encoding *Cu/Zn-SODI* and *Cu/Zn-SODII* exhibited differential expression among all three analyzed genotypes, including between the two resistant hybrids. Thus, in the Favorit hybrid, *Cu/Zn-SODI* and *Cu/Zn-SODII* reached peak expression at 2 hours (0.243 and 0.291, respectively). Subsequently, the expression of both genes decreased gradually and non-linearly over the 24-hour period, reaching 0.074 for *Cu/Zn-SODI* and 0.027 for *Cu/Zn-SODII*, with distinct expression patterns between the two transcripts.



Note: *Differences between genotypes were assessed by one-way ANOVA followed by the Bonferroni post hoc test. Means followed by different letters indicate significant differences ($p < 0.05$).*

Figure 1. Expression levels of SOD genes in the roots of sunflower seedlings (hybrids Favorit, P64LE20 and Performer) under optimal growth conditions (absence of the pathogen), analyzed at different time intervals (2, 6, 12 and 24 h)

In comparison, the P64LE25 hybrid displayed lower maximum values and lower variability for these transcripts over the 24-hour experimental period. *Cu/Zn-SODI* ranged from 0.127 (2 h) to 0.063 (24 h), while *Cu/Zn-SODII* ranged from 0.246 (2 h) to 0.054 (24 h), showing moderate fluctuations and a less pronounced decline than that observed in hybrid Favorit. This comparison highlights a differential expression pattern of *Cu/Zn-SODI* and *Cu/Zn-SODII* between the two resistant hybrids, both in terms of amplitude and temporal dynamics.

In contrast, the Performer genotype exhibited significantly higher expression levels of *Cu/Zn-SODI* and *Cu/Zn-SODII*, reaching maximum values of 0.468 and 1.078, respectively, at 12 hours.

Comparative analysis of the three genotypes, characterized by different levels of genetic resistance to *Orobanche cumana*, revealed a general decreasing trend in transcript levels in the resistant hybrids (Favorit and P64LE25), with more or less pronounced temporal transitions in the expression of *Cu/Zn-SODI* and *Cu/Zn-SODII* depending on the analyzed time point. In contrast, the sensitive hybrid (Performer) exhibited marked transcript accumulation, particularly for *Mn-SODII*, *Cu/Zn-SODI*, and *Cu/Zn-SODII*, especially at 12-24 hours.

In conclusion, the expression profile of SOD isoform genes in the roots of sunflower seedlings grown under optimal conditions indicates transcriptional activity that varies according to genotype and the time point analyzed. Nevertheless, a general common pattern was observed across the studied hybrid genotypes for the relative expression levels of the genes encoding the different superoxide dismutase isoforms: *Cu/Zn-SODII* > *Cu/Zn-SODI* > *Mn-SODII* > *Mn-SODI*, with more pronounced quantitative variations in the sensitive genotype Performer.

Expression of SOD genes in sunflower seedling roots grown in the presence of germinated *O. cumana* seeds. To assess whether the basal SOD transcriptional profiles observed under optimal growth conditions are modified upon parasite exposure, sunflower seedlings were co-cultivated with germinated *Orobanche cumana* seeds. No visible parasite attachment was detected within 24 h of co-cultivation in any of the analyzed hybrids. However, early host-parasite signaling events are known to precede physical attachment, potentially initiating transcriptional reprogramming during the pre-attachment stage. To characterize this early response, the expression of *Mn-SODI*, *Mn-SODII*, *Cu/Zn-SODI*, and *Cu/Zn-SODII* was analyzed in root tissues at 2, 6, 12, and 24 h post-inoculation (hpi), relative to the corresponding mock-treated controls collected at identical time points under normal growth conditions. Relative transcript accumulation was expressed as $\log_2$ (FC infection/control), allowing quantitative evaluation of SOD gene modulation during early interaction (Figure 2).

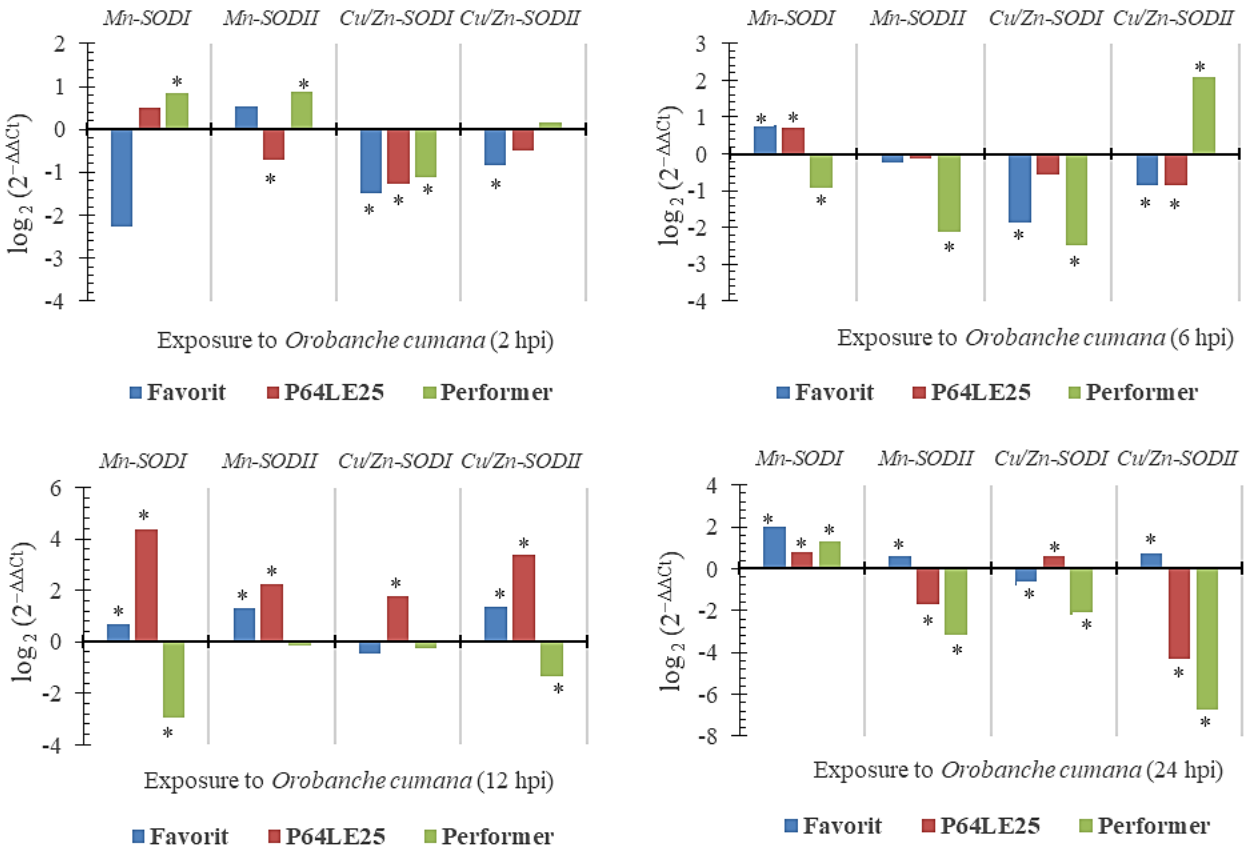
Genotype-specific expression patterns of SOD genes in resistant sunflower hybrids during early interaction with O. cumana

The response of the hybrid Favorit to the presence of *Orobanche cumana* is characterized by an oscillatory gene expression profile, in which SOD isoforms exhibit alternating phases of up- and downregulation. Although the transcriptional dynamics of individual isoforms are distinct, they appear to be functionally interconnected.

Thus, the expression of the *Mn-SODI* gene displayed pronounced oscillations throughout the experiment. After only 2 hours of co-cultivation, *Mn-SODI* was strongly downregulated ($\log_2$FC = -2.26), suggesting a rapid inhibition of transcriptomic activity as an initial response to parasite-derived signals. Subsequently, at 6 and 12 hpi, expression levels recovered and even showed slight upregulation ($\log_2$FC = 0.77 and 0.69, respectively), reaching a maximum at 24 hpi ($\log_2$FC = 1.98, corresponding to approximately 3.96-fold upregulation).

In contrast to *Mn-SODI*, the *Mn-SODII* gene exhibited a moderate and transient response. During the first 2 hpi, *Mn-SODII* was slightly upregulated ($\log_2$FC = 0.53), followed by a non-significant downregulation

at 6 hpi (log_2FC = -0.22). At 12 hpi, a pronounced increase in transcript levels was observed (log_2FC = 1.32, corresponding to approximately 2.50-fold upregulation), and elevated expression (log_2FC = 0.60, ~1.52-fold) was maintained at 24 hpi, confirming sustained activation of this gene during the early pre-attachment phase of the host–parasite interaction.



Note: Expression values are presented as $log_2(FC)$. Asterisks (*) indicate biologically relevant expression differences with FC (pathogen presence/control) ≥ 1.5, corresponding to $|log_2(FC)| \geq 0.58$.

Figure 2. Comparative analysis of SOD gene expression in the roots of resistant (Favorit, P64LE20) and susceptible (Performer) sunflower hybrids during co-cultivation with germinated seeds of *Orobanche cumana* at different time intervals (pathogen presence *vs.* control).

The genes encoding Cu/Zn-dependent isoforms exhibited distinct expression profiles. *Cu/Zn-SODI* was consistently downregulated throughout the experimental period, with the strongest inhibition observed during the first 2 and 6 hpi, corresponding to approximately 2.78-fold (log_2FC = -1.47) and 3.57-fold (log_2FC = -1.86), respectively. In contrast, *Cu/Zn-SODII* displayed a transitional pattern, with initial downregulation during 2-6 hpi (log_2FC = -0.85, ~1.87-fold reduction), followed by upregulation at 12 hpi (log_2FC = 1.35, ~2.55-fold increase), which was maintained at 24 hpi (log_2FC = 0.75, ~1.68-fold increase).

In the case of P64LE25, the second resistant hybrid, the first 2-6 hpi were also characterized by a predominantly downregulatory transcriptional response, although with lower quantitative amplitude compared to the F1 hybrid Favorit. Thus, during the first 2 hpi, among the four analyzed genes, significant decreases in transcript levels were observed only for *Mn-SODII* and *Cu/Zn-SODI*, with log_2FC values of -0.72 (~1.65-fold reduction) and -1.28 (~2.43-fold reduction), respectively. At 6 hpi, the SOD expression profile of P64LE25 remained characterized by low-magnitude variations, which were statistically significant only for Mn-SODI, slightly upregulated (log_2FC = 0.69, ~1.62-fold

increase), and Cu/Zn-SODII, downregulated (log_2FC = -0.84, ~1.79-fold reduction).

At 12 hpi, a clear shift toward SOD gene upregulation was observed in P64LE25, similar to the trend detected in Favorit, but with markedly higher intensity, contrasting with the downregulatory pattern recorded during the first 2-6 hours. A strong accumulation of transcripts was detected, particularly for *Mn-SODI* (log_2FC = 4.37, ~20.7-fold increase) and *Cu/Zn-SODII* (log_2FC = 3.38, ~10.4-fold increase). Pronounced upregulation was also revealed for *Mn-SODII* (log_2FC = 2.24, ~4.72-fold increase) and *Cu/Zn-SODI* (log_2FC = 1.79, ~3.46-fold increase), indicating a high degree of coordinated co-expression of mitochondrial and Cu/Zn-dependent isoforms in response to oxidative stress signals triggered by *O. cumana*.

At 24 hpi, transcript levels of *Mn-SODI* and *Cu/Zn-SODI* remained higher than in the control (log_2FC = 0.77 and 0.58, corresponding to ~1.71-fold and ~1.50-fold increases, respectively). In contrast, transcript abundance of *Mn-SODII* and *Cu/Zn-SODII* decreased by approximately 3.29-fold (log_2FC = -1.72) and 20.1-fold (log_2FC = -4.33), respectively.

In conclusion, both resistant hybrids, Favorit and P64LE25, exhibited time-dependent modulation of the four analyzed SOD genes following exposure to *O. cumana*. In both genotypes, early time points (2–6 hpi) were predominantly characterized by downregulation or low-magnitude changes in transcript abundance, followed by increased expression at 12 hpi.

However, the magnitude, coordination, and persistence of transcriptional variation differed between the two hybrids. Favorit displayed a more oscillatory profile, with alternating up- and downregulation phases and a tendency toward partial stabilization of transcript levels at 24 hpi. In contrast, P64LE25 exhibited a stronger induction at 12 hpi, particularly for *Mn-SODI* and *Cu/Zn-SODII*, followed at 24 hpi by maintenance of elevated transcript levels for some isoforms (e.g., *Mn-SODI* and *Cu/Zn-SODI*) and marked reduction for others (*Mn-SODII* and *Cu/Zn-SODII*).

These data indicate that, although both resistant hybrids respond through coordinated modulation of mitochondrial and Cu/Zn-dependent SOD genes, their temporal transcriptional dynamics and the persistence of expression changes at 24 hpi differ between genotypes.

Expression patterns of SOD genes in the susceptible sunflower hybrid during early interaction with O. cumana

In the case of susceptible hybrid Performer, after only 2 hours of co-cultivation with *O. cumana* seeds, a significant increase in *Mn-SODI* and *Mn-SODII* transcript levels was observed ($log_2FC \approx 0.85$, ~1.8-fold), correlated with a downregulation of *Cu/Zn-SODI* of approximately 2.2-fold (log_2FC = -1.15).

At 6 hpi, differences between the resistant hybrids and the susceptible hybrid became more pronounced, both in the trend of transcript changes (downregulation vs. upregulation) for *Mn-SODI* and *Cu/Zn-SODII* and in the magnitude of expression variation for *Mn-SODII* and *Cu/Zn-SODI*. Specifically, in the susceptible hybrid Performer, *Mn-SODI*, *Mn-SODII*, and *Cu/Zn-SODI* were significantly downregulated (log_2FC = -0.92, -2.13, and -2.48, corresponding to ~1.9-, ~4.4-, and ~5.6-fold, respectively), whereas Cu/Zn-SODII was upregulated (log_2FC = 2.09, ~4.2-fold).

Another difference in SOD gene expression in the susceptible hybrid, compared with the resistant hybrids, became evident after 12 hours of co-cultivation with germinated parasite seeds. This is primarily reflected in the downregulation of *Mn-SODI* and *Cu/Zn-SODII*, in contrast to the upregulation observed in the genetically resistant hybrids at the same time point. While transcript levels of *Mn-SODII* and *Cu/Zn-SODI* in the expression profile of Performer returned to levels similar to the control (these genes having been significantly downregulated at 6 hours), those of *Mn-SODI* and *Cu/Zn-SODII* showed a further decline compared with the control, of approximately 7.7-fold for *Mn-SODI* (log_2FC = -2.95) and

2.5-fold for *Cu/Zn-SODII* (log_2FC = -1.33).

After 24 hours of co-cultivation with *Orobanche cumana*, transcript levels of the three studied genes: *Mn-SODII, Cu/Zn-SODI*, and especially *Cu/Zn-SODII***,** were strongly reduced in the susceptible hybrid Performer. Based on the log_2FC values (-3.17 for *Mn-SODII*, -2.19 for *Cu/Zn-SODI*, and -6.71 for *Cu/Zn-SODII*), the corresponding fold changes indicate approximately 9-fold, 4.5-fold, and 104-fold decreases, respectively, compared with the control.

In contrast, at the same time point, the resistant hybrids maintained elevated transcript levels for two (P64LE25) or three (Favorit) SOD isoforms. For example, in Favorit, positive log_2FC values were observed for *Mn-SODI* (1.98), *Mn-SODII* (0.60), and *Cu/Zn-SODII* (0.75), corresponding to approximately 3.9-fold, 1.5-fold, and 1.7-fold increases, respectively. In P64LE25, elevated transcript levels were detected for *Mn-SODI* (log_2FC = 0.77; ~1.7-fold increase) and *Cu/Zn-SODII* (log_2FC = 0.58; ~1.5-fold increase).

However, it should be noted that the resistant genotype Favorit exhibited moderate downregulation of *Cu/Zn-SODI* (log_2FC = -0.79; ~1.7-fold decrease). In the second resistant hybrid, P64LE25, reductions in transcript levels were also detected for *Mn-SODII* (log_2FC = -0.77; ~1.7-fold decrease) and *Cu/Zn-SODII* (log_2FC = -4.33; ~20-fold decrease), although these changes were still less pronounced than those observed in Performer.

Thus, these results suggest that both resistant hybrids preserved overall SOD gene expression and maintained a more coordinated transcriptional regulation at 24 h, in comparison with the susceptible hybrid Performer, which, despite the upregulation of *Mn-SODI* (log_2FC = 1.28; ~2.4-fold increase), displayed marked downregulation of other three antioxidant transcripts, particularly Cu/Zn-SODII. This pattern highlights genotype-specific vulnerability to *O. cumana*.

Interestingly, in some cases, similar response trends were observed across all three genotypes. For example, downregulation of the gene encoding *Cu/Zn-SODI* was detected at 2 and 6 hours of co-cultivation in the presence of germinated *O. cumana* seeds, whereas upregulation of *Mn-SODI* was observed after 24 hours.

These findings suggest that differential activation of specific SOD isoforms may contribute to the early redox reprogramming that underlies resistance responses.

Reactive oxygen species play a dual and highly dynamic role in host–parasite interactions, being essential for both the host plant and the invading parasite. In the host, the rapid generation of ROS represents one of the earliest responses to parasitic invasion, which contributes to the activation of programmed cell death (PCD), reinforcement of cell walls through oxidative cross-linking, thus restricting parasite penetration and vascular connection. At the same time, ROS function as important signaling molecules that activate plant immune responses (Dumanovic et al., 2021; Shi et al., 2026).

In parasitic plants, however, ROS are equally critical for successful development. Haustorium formation, the specialized organ enabling vascular connection to the host, depends on tightly regulated ROS production. Experimental inhibition of ROS-generating enzymes, such as NADPH oxidases (Wada et al., 2019), has been shown to impair haustorial initiation and differentiation. Moreover, parasitic plants may actively manipulate host redox metabolism, stimulating ROS production to modify host cellular processes and redirect nutrients toward the parasite (Jhu and Sinha, 2022). Thus, ROS-mediated signaling is a complex, bidirectional regulatory network within plant pathosystems, and systemic redox communication between interacting plant species remains an area of active investigation.

Within this context, superoxide dismutases (SODs) represent a central component in modulating ROS homeostasis. By catalyzing the dismutation of superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2), SODs not only prevent excessive oxidative damage but also regulate the balance between toxic and signaling ROS

forms. The resulting H_2O_2 molecule serves as a relatively stable signaling intermediate, capable of diffusing across membranes and activating downstream defense-related pathways. Therefore, SOD activity represents a critical regulatory checkpoint linking oxidative stress detoxification to redox signaling.

In the present study, comparative analysis of SOD genes expression in the roots of sunflower hybrids with contrasting levels of genetic resistance to *Orobanche cumana* revealed differences both between compatible and incompatible host–pathogen interactions and among genotypes, highlighting shared as well as genotype-specific transcriptional response patterns during the pre-attachment stage. Notably, all three hybrids exhibited a transient downregulation of *Cu/Zn-SODI* at early time points (2-6 hpi), followed by upregulation of *Mn-SODI* at 24 hpi, reflecting some general features of the oxidative stress response triggered by parasite perception.

However, the magnitude, direction, and temporal coordination of transcriptional changes clearly distinguished resistant from susceptible genotypes. The susceptible hybrid Performer exhibited an early but unstable activation of Mn-SOD isoforms, followed by a pronounced and progressive downregulation of three SOD genes, particularly *Cu/Zn-SODII* at 24 hpi. This pattern suggests a loss of transcriptional balance and a limited capacity to sustain SOD-mediated antioxidant defense during parasite establishment. In contrast, the resistant hybrids Favorit and P64LE25 maintained a more stable and coordinated expression of SOD isoforms over time. At early time points (2-6 hpi), both genotypes showed predominantly transcriptional downregulation or low-magnitude changes, followed by a pronounced upregulation at 12 hpi, indicative of a biphasic response with an initial adjustment phase and a subsequent activation phase. The coordinated modulation of mitochondrial (*Mn-SODI, Mn-SODII*) and Cu/Zn-dependent (*Cu/Zn-SODI, Cu/Zn-SODII*) isoforms highlights the integrated antioxidant regulation in response to parasitic signals.

Despite these commonalities, the studied hybrids differed in the amplitude, temporal dynamics, and persistence of transcriptional changes, underscoring genotype-specific strategies in resisting *O. cumana*. Favorit exhibited an oscillatory pattern, alternating between up- and down-regulation, with a tendency for partial stabilization of transcript levels at 24 hpi, suggesting fine-tuned redox control. In contrast, P64LE25 displayed a stronger and more sustained induction at 12 hpi, particularly for *Mn-SODI* and *Cu/Zn-SODII*, followed at 24 hpi by persistent elevation of some isoforms (*Mn-SODI*, *Cu/Zn-SODI*) and reduction of others (*Mn-SODII*, *Cu/Zn-SODII*). Although moderate or isoform-specific downregulation was occasionally observed, resistant genotypes generally maintained higher transcript levels and avoided the severe transcriptional suppression observed in the susceptible hybrid. The differences were especially evident at 12 and 24 hpi, when resistant hybrids showed upregulated or maintained expression of SOD genes, whereas Performer displayed marked transcriptional decline.

Taken together, these findings highlight that resistance to *O. cumana* is not merely associated with the activation of antioxidant genes, but with a rapid, coordinated, and reversible regulation of different SOD isoforms, enabling both efficient detoxification of ROS and their use as signaling molecules. In contrast, susceptibility appears to be characterized by dysregulated and progressively suppressed SOD transcriptional responses, which may serve as an indicator of inefficient oxidative stress control and metabolic imbalance during early parasite interaction. Furthermore, the differential involvement of SOD isoforms with known subcellular distributions (Cu/Zn-SODs potentially localized in the cytosol, peroxisomes, and the apoplast, and Mn-SODs primarily mitochondrial) in resistant genotypes suggests that spatial regulation of ROS detoxification is a key determinant of the efficiency of the defensive response.

These observations are consistent with previous reports in sunflower, showing that the enzymatic antioxidant system responds dynamically to biotic stress in both compatible and incompatible interactions between *H. annuus* and *Plasmopara halstedii*.

For example, Herbette et al. (2003) investigated mRNA accumulation of sunflower glutathione peroxidases (*GPXha-1* and *GPXha-2*) and Cu-Zn-dependent superoxide dismutases (*SODha*-1 and *SODha-2*) in response to *Plasmopara halstedii* infection and demonstrated differential transcript accumulation depending on the virulent or an avirulent race of pathogen. Their results showed that *SODha-2*, but not *SODha-1*, mRNA accumulation was induced at 3 and 6 h in incompatible interaction exclusively. Other studies have also shown that, while both virulent and avirulent pathogens can trigger an early, transient oxidative burst, only avirulent strains induce a prolonged and massive ROS accumulation within 3-6 hours post-infection (Levine et al., 1994; Mittler et al., 1999). Taken together, these data indicate that enzymatic components of the antioxidant system, including SOD enzymes, participate in hypersensitive and stress responses, with expression finely regulated according to the interaction type and, potentially, the host genotype.

In line with these findings, similar observations were reported by Letousey et al. (2007), who found a rapid and localized production of H_2O_2 as one of the essential early responses of plants in the *Orobanche cumana Wallr. - Helianthus annuus L.* pathosystem. The *sco* gene, encoding a carbohydrate oxidase that produces H_2O_2 as a by-product, was transiently overexpressed 2 hours after co-cultivation in both resistant and susceptible genotypes, suggesting that the plant triggers an early oxidative stress response during interaction with *Orobanche cumana*. Additionally, differential expression of defense-related genes was observed after 2 and 8 hours of root exposure to the parasite. Genes of the phenylpropanoid pathway (pal, c4h, chs) were rapidly and strongly activated in the resistant genotype, but only transiently in the susceptible one.

The response pattern observed in sunflower plants during the early stages of interaction with *O. cumana* is consistent with results described in other host-parasite systems. In *Arabidopsis thaliana* exposed to *Orobanche ramosa*, rapid H_2O_2 accumulation and transient activation of antioxidant enzymes were detected during the pre-attachment phase, indicating early responses similar to those observed in resistant sunflower genotypes (El-Maarouf-Bouteau et al., 2008).

Thus, in all the examples presented of incompatible host–pathogen systems, early activation and the maintenance of antioxidant enzyme balance, including SOD, ensure the restriction of parasite invasion and the initiation of systemic defense signaling. In contrast, in compatible systems, uncontrolled activation followed by transcriptional repression leads to the failure of antioxidant protection and the successful establishment of the parasite.

In conclusion, the differential expression of SOD genes in sunflower roots reflects distinct strategies for regulating oxidative stress depending on the degree of compatibility with *O. cumana*. Timed and compartmentalized activation of SOD isoforms in resistant genotypes indicates an efficient redox-based early defense mechanism, while loss of transcriptional control in the susceptible genotype highlights failure to restore oxidative homeostasis. Therefore, SOD may serve as a relevant molecular indicator of early resistance to *O. cumana* infestation and a potential marker for the selection of tolerant cultivars in breeding programs.

CONCLUSIONS

The differential expression patterns of the four investigated SOD isoforms (*Mn-SODI*, *Mn-SODII*, *Cu/Zn-SODI*, and *Cu/Zn-SODII*) reflect distinct redox regulatory strategies determined by the genetic background of the sunflower, particularly by the presence or absence of specific resistance genes to *O. cumana*. These strategies ultimately shape the outcome of the host–parasite interaction during the pre-attachment stage.

Comparative analysis of sunflower F_1 hybrids with contrasting resistance levels revealed both shared and genotype-specific transcriptional responses. Importantly, resistance to *O. cumana* was not determined solely by the magnitude of SOD induction, but rather by

the timing, coordination, and compartmentalized activation of specific SOD isoforms. The resistant hybrid Favorit exhibited a gradual, sustained, and finely regulated modulation of SOD transcripts, indicative of efficient redox adjustment and controlled ROS signaling during early defense. Similarly, P64LE25 displayed rapid and relatively strong activation of selected SOD genes, suggesting the establishment of an early ROS-scavenging response. In contrast, the susceptible hybrid Performer showed a disorganized transcriptional pattern characterized by transient activation followed by progressive repression of SOD genes, reflecting impaired redox regulation and failure to restore oxidative homeostasis during the early interaction with the parasite.

From a breeding perspective, particularly in the context of developing quantitative, non-specific resistance to biotic stress factors, a deeper understanding of the regulatory mechanisms controlling SOD gene expression and activity is essential. Such knowledge may provide valuable tools for early screening of crop genotypes basedon characteristic redox responses profiles and for directing host-parasite interactions toward incompatibility rather than compatibility.

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