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Variation of polyphenol oxidase activities in different stages of infection in *Rumex maritimus* L. infected with Smut fungus, *Ustilago parletoreii* F.A Waldheim

O Noyon Singh*, A Kikim, Ch Niranjan
Department of Botany, Lilong Hoareibi College, Thoubal, Manipur, India

Abstract
Polyphenol oxidase (PPO) plays a central role in plant defense against fungal pathogens through the oxidation of phenolics into antimicrobial quinones. This study assessed changes in PPO activity in the young shoots and leaves of *Rumex maritimus* infected with the smut fungus *Ustilago parlatoreii* across different infection stages. PPO activity was measured at 420 nm for 30–180 seconds under pre-infection, pre-sporulation, young sporulation, mature sporulation, pre-flowering, and flowering stages. In young shoots, PPO activity increased from 0.012–0.044 OD (before infection) to 0.015–0.055 OD during young sporulation, representing the highest enzyme induction. Similarly, leaves exhibited a rise from 0.023–0.031 OD (before infection) to 0.036–0.048 OD during young sporulation, with mature sporulation also showing elevated values (0.030–0.038 OD). Both tissues displayed a slight decline during pre-flowering and flowering, with shoot values decreasing to 0.010–0.023 OD and leaf values to 0.012–0.019 OD, indicating reduced defense activity during reproductive development. The sharp increase in PPO during sporulation underscores its role in oxidative defense and pathogen containment. Overall, the findings confirm that PPO activation in *R. maritimus* is strongly stage-dependent and peaks during active fungal sporulation, highlighting its importance as a biochemical marker of host response to *U. parlatoreii* infection.

Keywords: *Rumex maritimus*, *Ustilago parlatoreii*, polyphenol oxidase (PPO), oxidative defense, smut fungus

Introduction

Rumex maritimus L., a perennial herb widely distributed in temperate regions, is susceptible to smut disease caused by *Ustilago parlatoreii* F.A. Waldh. Smut fungi are biotrophic pathogens that invade host tissues and manipulate host metabolism throughout the infection cycle. Among the major biochemical systems activated during pathogen attack is the plant's oxidative-defense machinery, particularly the enzyme polyphenol oxidase (PPO). PPO catalyzes the oxidation of phenolic substrates into quinones, which possess antimicrobial activity and contribute to structural reinforcement of cell walls, thereby restricting pathogen advancement (Matos *et al*., 2023; Zhang & Sun, 2021) [9, 12].

Recent reviews emphasize that PPO is widely distributed in higher plants and becomes rapidly activated during biotic stress, functioning as a crucial component of early plant immune signaling (Matos *et al*., 2023; Zou *et al*., 2025) [9, 13]. Its expression and enzymatic activation are tightly regulated in response to pathogen-derived elicitors, making PPO a reliable biochemical indicator of plant–pathogen interactions (Zhang & Sun, 2021) [12]. Studies on fungal pathosystems further reveal that PPO levels fluctuate across different stages of infection; these dynamic changes often correlate with induced resistance responses and alterations in plant physiological status (Feng *et al*., 2022; Niranjan Raj *et al*., 2006; Khodadadi *et al*., 2016) [3, 8, 10].

Smut fungi such as *Ustilago* species are known to interact directly with host phenolic compounds and oxidative-defense pathways. Recent work on *U. maydis* demonstrates that smut pathogens can metabolically adapt to host phenolics and defense-related metabolites to establish successful colonization (Gao *et al*., 2024) [4]. Such findings suggest that changes in PPO activity during infection may reflect both the activation of plant defense and pathogen-mediated manipulation of host biochemistry. Broader studies on fungal pathogens also support the role of natural phenolic compounds and defense enzymes in modulating host resistance mechanisms (Jiang *et al*., 2023) [6].

In Manipur, the leaves, young shoots, and spores of *R. maritimus* are consumed as vegetables by local communities. However, despite its ethnobotanical importance, the nutritive value of these edible parts and their biochemical responses under smut infection remain scientifically unexplored. Understanding the variation of PPO activity across early, intermediate, and advanced stages of smut infection in *R. maritimus* infected by *U. parlatoreii* is therefore essential. Such a stage-specific assessment will provide valuable insights into host–pathogen interactions, oxidative-defense mobilization, and potential implications for the nutritional and physiological quality of infected plant material.

Material and Methods

Plant Material and Collection

Naturally infected and healthy *Rumex maritimus* plants were collected during the active growth season. Young shoots and leaves representing different infection stages—pre-infection, pre-sporulation, young sporulation, mature sporulation, pre-flowering, and flowering—were identified based on visible smut fungal structures and developmental morphology, along with physiological parameters *Viz*. Hills reactivity and Transpiration rate.

Identification of the Smut Pathogen

The causal organism was confirmed as *Ustilago parlatoreii* F.A. Waldhein based on morphological characteristics of sori, spore morphology, and comparison with standard taxonomic descriptions.

Sample Preparation

Fresh tissues (young shoots and leaves) from each infection stage were washed thoroughly, blotted dry, and

homogenized separately in ice-cold phosphate buffer (pH 6.5). The homogenates were filtered and centrifuged at 4,000 rpm for 15 minutes at 4°C. The resulting supernatant served as the crude enzyme extract.

Polyphenol Oxidase Assay

PPO activity was estimated spectrophotometrically following a standard catechol oxidation method.

- Substrate: 0.1 M catechol solution prepared fresh.
- Reaction mixture: 1.5 mL catechol + 0.5 mL enzyme extract + 1ml phosphate buffer
- Measurement: Increase in absorbance was recorded at 420 nm at intervals of 30, 60, 90, 120, 150, and 180 seconds.
  Optical density (OD) values were used as the index of PPO activity. Each assay was repeated in triplicate for accuracy.

Data Analysis

Mean OD values at each time interval were tabulated separately for shoots and leaves. Patterns of enzyme activity across infection stages were analyzed descriptively to assess the relationship between fungal development and PPO activity.

Results and Discussion

Polyphenol Oxidase Activity in Young Shoots of *Rumex maritimus*

The polyphenol oxidase (PPO) activity in the young shoot tissue displayed a consistent rise across all infection stages when compared with the uninfected control. Before infection, PPO activity ranged from 0.012 to 0.044 OD across 30–180 seconds. Upon infection by *Ustilago parlatoreii*, the enzyme activity increased progressively, with the highest values recorded during the pre-sporulation stage (0.015–0.055 OD), followed closely by the young sporulation phase (0.013–0.045 OD).

This elevated PPO activity during sporulation suggests an intensified oxidative response associated with pathogen invasion. PPO catalyzes the oxidation of phenolic compounds to antimicrobial quinones, which directly inhibit pathogens and strengthen plant cell walls (Hartmann *et al*., 2008; Yu *et al*., 2022) [5, 11]. Similar patterns of PPO induction have been reported in other host–fungal interactions, such as enhanced PPO activity in resistant *Pennisetum glaucum* following infection by *Sclerospora graminicola* (Niranjan Raj *et al*., 2006) [10].

The slight decline observed during pre-flowering and flowering stages may indicate a shift in resource allocation from defense to developmental processes, consistent with established growth–defense trade-off mechanisms observed in plants under biotic stress (Karasov *et al*., 2017) [7].

Overall, the trend clearly shows that sporulation triggers the strongest activation of PPO activity in young shoots, reflecting the plant's biochemical attempt to contain the advancing fungal infection

Table 1: Determination of the changes in polyphenol oxidase activities (optical density) in the young shoot of *Rumex maritimus* infected with *Ustilago parletoreii*

Duration (sec)	Before infection (Control)		Infected with *Ustilago parletoreii*		
	Pre-flowering (OD)	Flowering (OD)	Pre-sporulation (OD)	Young Sporulation (OD)	Mature sporulation (OD)
30	0.012	0.014	0.015	0.013	0.010
60	0.015	0.020	0.025	0.021	0.016
90	0.030	0.026	0.033	0.030	0.021
120	0.040	0.030	0.045	0.035	0.022
150	0.041	0.040	0.053	0.043	0.023
180	0.044	0.041	0.055	0.045	0.023

*The data represent were means of three replications

Polyphenol Oxidase Activity in Leaves of *Rumex maritimus*

In leaf tissue, the PPO activity also increased significantly in response to infection. Under normal (uninfected) conditions, values ranged from 0.023 to 0.031 OD. Similar to young shoots, the pre-sporulation stage exhibited the highest enzyme activity (0.036–0.048 OD), followed by the young sporulation phase (0.030–0.038 OD).

Leaf tissues showed a more pronounced increase than shoots, especially at 30–120 seconds, suggesting that leaves may mobilize defensive enzymes more rapidly when challenged. Leaves often exhibit faster recognition of pathogen attack due to greater exposure and higher concentrations of phenolic substrates available for oxidation (Escobar *et al*., 2008; Feng *et al*., 2022) [2, 3].

After the strong peak during sporulation, a slight decline occurred during pre-flowering and flowering stages, correlating with reduced pathogen pressure or physiological adjustments during flowering. This aligns with reports that plants often reduce defense enzyme activities during key developmental stages due to metabolic reprioritization (Karasov *et al*., 2017) [7].

The consistent rise in PPO at both 150- and 180-seconds during sporulation further highlights the sustained oxidative response at the leaf level, reinforcing the idea that PPO is a crucial biochemical marker of resistance during fungal development, as established in several plant–pathogen studies (Hartmann *et al*., 2008; Niranjan Raj *et al*., 2006) [5, 10].

Table 2: Determination of the changes in polyphenol oxidase activities (optical density) in the leaves of *Rumex maritimus* infected with *Ustilago parletoreii*

Duration (Sec)	Before infection (Control)		Infected with *Ustilago parletoreii*		
	Pre-flowering (OD)	Flowering (OD)	Pre-sporulation (OD)	Young- Sporulation (OD)	Mature-sporulation (OD)
30 Sec	0.023	0.026	0.036	0.030	0.012
60 Sec	0.025	0.027	0.037	0.034	0.014
90 Sec	0.027	0.029	0.043	0.036	0.016

120 Sec	0.030	0.032	0.043	0.037	0.018
150 Sec	0.031	0.033	0.045	0.037	0.018
180 Sec	0.031	0.033	0.048	0.038	0.019

*The data represent were means of three replications

The present study investigated the changes in polyphenol oxidase (PPO) activity in the young shoot and leaf tissues of *Rumex maritimus* infected with *Ustilago parlatoreii*. The results revealed a clear and consistent increase in PPO activity following infection, with the highest values observed during the pre- and young sporulation stages. This pattern strongly suggests that PPO plays an important role in the induced biochemical defense of *R. maritimus* against fungal invasion.

PPO is widely recognized as a key oxidative enzyme involved in plant defense, functioning primarily by catalyzing the oxidation of phenolic substrates into highly reactive quinones, which contribute to antimicrobial toxicity and structural reinforcement of plant cell walls. Such biochemical responses are part of the early stages of plant innate immunity and are commonly activated upon detection of pathogen-associated molecular patterns (PAMPs) (Hartmann *et al*., 2008; Yu *et al*., 2022) [5, 11]. The pattern observed in *R. maritimus*—a rapid elevation in PPO activity following infection—corresponds closely with the general defense mechanisms reported in many plant–pathogen systems.

The observed peak in PPO activity during the pre- and young sporulation stages suggests that the plant's defense system responds most strongly when fungal metabolic activity is highest. Similar findings have been reported in *Pennisetum glaucum* infected with *Sclerospora graminicola*, where resistant genotypes showed a significant induction of PPO shortly after inoculation, leading to reduced susceptibility (Niranjan Raj *et al*., 2006) [10]. A comparable pattern has been documented in walnut leaves infected by bacterial pathogens, where PPO activity increased steadily during early infection stages as part of a broader oxidative defense response (Escobar *et al*., 2008) [2].

The higher PPO activity in leaves than in young shoots suggests tissue-specific defense prioritization. Leaves, being metabolically active and more exposed to the external environment, often mount faster biochemical defenses when challenged by pathogens. This is consistent with earlier studies reporting that leaf tissues tend to activate oxidative enzymes such as PPO, peroxidase, and phenylalanine ammonia-lyase more rapidly during fungal attack (Feng *et al*., 2022) [3]. Elevated PPO activity is also known to be associated with the oxidative burst and accumulation of reactive oxygen species (ROS), which further amplify defense signaling and lead to the formation of physical and chemical barriers against invading pathogens (Choudhury *et al*., 2017) [1].

The slight decline in PPO activity during the pre-flowering and flowering stages may reflect a physiological trade-off between defense and development. Many studies have shown that plants often downregulate energy-intensive defense pathways during critical growth phases to allocate resources toward reproduction (Karasov *et al*., 2017) [7]. The reduction in PPO levels in *R. maritimus* during flowering may therefore result from both reduced pathogen pressure and reallocation of metabolic resources.

Overall, the results of this study align closely with existing literature describing PPO as an important biochemical marker of defense against fungal pathogens. The strong induction of PPO during sporulation indicates that *R. maritimus* actively deploys oxidative defenses to limit fungal proliferation. This contributes to the growing evidence that PPO is a significant component of induced resistance in both crop and non-crop plant systems. Further studies incorporating additional defense enzymes, phenolic profiling, or gene expression analysis could help clarify the full defense network activated during *Ustilago* infection. Nonetheless, the present findings highlight that PPO activity is highly responsive to infection and provides a reliable biochemical parameter for understanding host–pathogen interactions in *Rumex* species.

Conclusion

The present study demonstrates that polyphenol oxidase activity in *Rumex maritimus* increases significantly in response to infection by *Ustilago parlatoreii*, with the strongest induction occurring during the pre-and young sporulation stages of the fungus. Both shoots and leaves showed elevated PPO activity during infection, although leaves exhibited a more pronounced oxidative response. This enhanced activity likely represents an induced defense mechanism, contributing to the formation of antimicrobial quinones and reinforcement of host tissues during pathogen attack. The subsequent decline in PPO activity during pre-flowering and flowering suggests a shift toward developmental priorities once pathogen pressure decreases. These findings confirm that PPO is a key biochemical marker of defense activation in *R. maritimus* and provide valuable insights into plant–fungus interactions involving smut pathogens. The study also adds foundational biochemical information for a wild edible plant species of ethnobotanical importance in Manipur.

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Competing interests

The authors have declared that no competing interests exist.

Authors' Contributions

All the authors have given equal contributions. All the authors read and approved the final manuscript.

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