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# Comparative Analysis of Powdery Mildew Disease Resistance and Susceptibility in *Brassica* Coenospecies

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**Abstract:** *Erysiphe cruciferarum*, a causative agent of powdery mildew disease, has emerged as a serious threat in *Brassica juncea* and its closely related species. To date, no resistant cultivars have been identified in Brassica species against powdery mildew. Here, we used histopathological, biochemical, and molecular approaches to elucidate the powdery mildew disease progression and host responses in three Brassica cenospecies, namely *B. juncea*, *Camelina sativa*, and *Sinapis alba*. Based on the results of disease progression, *S. alba* was found to be extremely resistant to powdery mildew infection, whereas *B. juncea* and *C. sativa* were highly vulnerable. In addition, the disease spread rate to uninfected parts was comparatively higher in *B. juncea* and *C. sativa.* Histopathological results revealed more pathogen-induced cell death in *B. juncea* and *C. sativa* compared to *S. alba*. We also examined the role of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in *B. juncea*, *C. sativa*, and *S. alba* after powdery mildew infection. Based on our findings, the enzyme activity of SOD, POD, and CAT was relatively higher in *S. alba* then that of *B. juncea* and *C. sativa* after powdery mildew infection. Furthermore, we evaluated the expression levels of salicylic acid (SA) signature genes, including pathogenesis-related protein viz., *PR1*, *PR2*, and *PR5* in *B. juncea*, *C. sativa*, and *S. alba* after *E. cruciferarum* infection. Based on our findings, the expression levels of SA marker genes *PR1*, *PR2*, and *PR5* increased in all three species after infection. However, the fold change was relatively higher in *S. alba* than in *B. juncea* and *C. sativa.* In future, further studies are required to identify the potential candidates in *S. alba* that are involved in powdery mildew disease resistance.

**Keywords:** powdery mildew; *Brassica juncea*; *Sinapis alba*; *Camelina sativa*; PR proteins; salicylic acid



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## 1. Introduction

Plants, being sessile, are frequently challenged by fungal pathogens of different lifestyles, such as biotrophs, necrotrophs, and hemibiotrophs, which jeopardize their survival [1,2]. Necrotrophic pathogens cause host cell death and nourish on nutrients released by dead cells, whereas biotrophic pathogens require colonization and feeding on living host tissue to complete their lifecycle [2]. In contrast, hemibiotrophic pathogens first invade hosts through biotrophic invasion and then transition to necrotrophic growth [3]. Biotrophic pathogens possess specialized structures known as haustoria that assist them with the production of effector proteins in the host cells as well as the absorption of nutrients [4]. There has been significant progress in understanding the pathophysiology of hemibiotrophic or necrotrophic pathogens, and various key players in pre and post infection have been

reported. However, there is a limited understanding about the pathogenesis of biotrophic pathogens due to their obligate parasitic lifestyle and incompetence to thrive on synthetic media [5]. The inability to cultivate or genetically alter obligatory biotrophic fungal infections in vitro makes it difficult to understand the molecular mechanisms behind their pathogenicity. However, with the aid of multiomics and live cell imaging tools, various key effectors or other virulence factors have been identified in different biotrophic fungal pathogens, but they still warrant function validation. Despite having various infection and nutrition acquisition strategies, most of the pathogenic fungi are recognized by the host defense system, which then activates the distinct immune responses. Plant immune systems involve microbe-associated molecular pattern-triggered immunity (PTI) and microbial effector-triggered immunity (ETI) to recognize and defend from pathogens [2]. To combat disease threats, the plants exhibit both innate (structural shields) and induced defense systems (systemic acquired resistance, SAR). However, SAR is significantly more potent and durable and is controlled by defensive hormonal pathways (SA; jasmonic acid, JA; and ethylene, ET) that interact either synergistically or antagonistically [6]. These hormonal-signaling cascades (SA and JA/ET) and their crosstalk display accurate and quick defense response to a single pathogen or multiple pathogens with different lifestyles. Interestingly, SAR is known to provide resistance against a broad range of phytopathogens (bacteria, fungi, and viruses) with different lifestyle and modes of infection. PR proteins are the signatures of the SAR pathway, which possess antifungal, antibacterial, and antiviral activity and protects the host distal or uninfected parts from subsequent pathogenic attack. The primary plant defense against biotrophic pathogens is the activation of the SA pathway and its signatures such as PR proteins. In plants, PR proteins are the important group of diverse proteins which are activated by microbial pathogens, fungal elicitors, and signaling molecules. PR genes display distant expression against biotrophic and necrotrophic pathogens. For instance, *PR1*, *PR2*, and *PR5* are dramatically induced during biotrophic and semi-biotrophic infections [6–8], while as *PR3*, *PR12* and *PR13* are induced by necrotrophic pathogens. The overall rate of transcription of PR genes during biotrophic or necrotrophic infections mainly relies on SA and JA pathways and their crosstalk. For instance, SA biosynthesis mutants lacking SA accumulation severely impairs plant defenses against biotrophic and semi-biotrophic pathogen invasion [9]. In contrast, SA or its analogs applied exogenously is adequate to enhance host defense in response to biotrophic and semi-biotrophic diseases [10]. The initial host response against pathogen attack is the hypersensitive response, which triggers the generation of ROS, predominantly $H_2O_2$, and $O_2$ radicals. These ROS activates plant defense mechanisms, such as programmed cell death, or serves as elegant secondary messengers to induce a number of genes and signaling cascades involved in plant defenses. Interestingly, biotrophic and necrotrophic fungal pathogens distinctly respond to their host's oxidative stress. For example, biotrophic pathogens confine and inhibit the oxidative burst by secreting effectors while necrotrophic pathogens favor oxidative burst due to its reliance on it. In addition to ROS formation, the activation of mitogen-activated protein kinases is another first response to pathogen attack in plants. However, improperly created ROS could deleteriously impact the function of DNA, proteins, and lipids in cell components, leading to cell death. In this regard, ROS-scavenging enzymes such as SOD, POD, and CAT balance the ROS homeostasis and assist plants in fending off pathogen-induced adverse effects [11]. There are many reports on the role of antioxidant enzymes (SOD, POD, and CAT) in alleviating pathogen-induced oxidative detrimental effects in different crops.

Globally, plant diseases reduce crop yield by 11–30% yearly, endangering global food security [12]. Among them, powdery mildews are considered the most serious diseases in many agriculturally important crops. There are about 500 powdery mildew species that can infect diverse plant species, including both monocots and dicots, worldwide [13]. A powdery layer of white spores is a common appearance of most powdery mildews, and some notable examples are gooseberry (*Sphaerotheca morsuvae*), powdery mildew of grasses and cereals (*Erysiphe graminis*) [14], and powdery mildew of Brassica (*E. cruciferarum*) [15].

Crucifer powdery mildew disease is caused by *E. cruciferarum. Erysiphe* spp. which infects all leaves, stems, and siliques, significantly reduces Brassica crop yields by lowering plant growth and seed development [6,16]. There are several reports of outbreaks of powdery mildew in Brassica species in different parts of the world, which caused huge yield losses in India [17], Poland [18], Turkey [19], the United States [20], Korea [21,22], and in Australia [23]. Powdery mildews can develop on plant surfaces as a result of climatic conditions, including temperature and precipitation, which suggests that the impacts of this fungus can differ between habitats and seasons. After soybean and peanut, *B. juncea* is one of the most significant oilseed crops farmed in India. In terms of area and production, Indian mustard, or *B. juncea* L., is the third most significant oil seed crop in the world [24]. However, fungal diseases have become a major cause of its low production and economic losses. Among them, powdery mildew has emerged as one of the most devastating fungal diseases in recent times [25]. Previously, this disease was not acknowledged as a significant problem in *B. juncea*, but, in recent years, it has emerged as a serious issue in most of the states in India. All of the *B. juncea* cultivars that are now cultivated in India are extremely vulnerable to powdery mildew, and, as of yet, no sources of resistance have been identified [25]. To date, no resistant cultivar is available in the Brassica gene pool, but some wild cultivars, such as, *S. alba*, have shown resistance against this pathogen [15,26]. However, what elements could provide some cruciferous crops almost complete resistance or high susceptibility to powdery mildew remains largely unknown. Nevertheless, fungicides are one method for preventing the spread of fungi; however, they are expensive, toxic to the environment, and ineffectual because of weather changes. In this study, we systematically evaluated the molecular, biochemical, and histopathological tools to study the powdery mildew disease in *B. juncea* and its wild relative's *C. sativa* and *S. alba*. Identifying the powdery mildew-resistant cultivar in crucifer can be used for improving resistant traits in *B. juncea* through molecular breeding as well as genetic engineering.

## 2. Materials and Methods

### *2.1. Plant Material and Fungal Infection*

*B. juncea*, *C. sativa*, and *S. alba* seeds were acquired from Department of genetics, IARI, Pusa, New Delhi. Seeds of *B. juncea*, *C. sativa*, and *S. alba* were sown in plastic pots containing a mixture of soil and organic manure (with a ratio of 2:1) during the winter season at Net house facility IARI, New Delhi. After 2 weeks of germination, seedlings were thinned and 3 plants were further grown in each pot with regular watering. *E. cruciferarum* (H.C.I.O-ID: no. 52067) was previously identified in our lab from naturally infected *B. juncea* powdery mildew leaf samples. Forty-five-day-old plants of three Brassica cenospecies were used for the *E. cruciferarum* infection under natural and lab inoculations according to reference [6]. For control, *B. juncea*, *C. sativa*, and *S. alba* plants were treated with sterile, distilled water and kept separately to avoid cross-infection. Furthermore, both control and infected leaves were collected for further analysis.

### *2.2. Disease Scoring*

In this study, a disease-resistance assay was carried out in three Brassica species, *B. juncea*, *C. sativa*, and *S. alba* in three biological replicates. The comparison of powdery mildew disease incidence and resistance in *B. juncea*, *C. sativa*, and *S. alba* plants was conducted using a variety of metrics, including colony appearance and number, disease index, and percentage of disease leaf area (% DLA). A disease index consisting of six grades, 0, 1, 3, 5, 7, and 9, was examined according to reference [27].

### *2.3. Trypan Blue Staining*

To observe the fungal cell death in *B. juncea*, *C. sativa* and *S. alba*, trypan blue staining was carried out according to reference [27]. Briefly, powdery mildew-infected leaves of *B. juncea*, *C. sativa*, and *S. alba* were stained with trypan blue solution consisting of a mixture of trypan blue (40 mg), 10 mL of phenol (pH 7.5–8.0), 10 mL of glycerol (99%), 10 mL of

lactic acid (85% w:w), and 10 mL of water. After 30 min, leaf samples were first rinsed in sterile water to remove extra stain, and then they were submerged in 70% ethanol for an entire night to remove the chlorophyll pigments. Finally, leaves were stored in 60% glycerol and then observed for powdery mildew-induced cell death.

### *2.4. Antioxidant Enzyme Assays in B. juncea, C. sativa, and S. alba after Powdery Mildew Infection*

Antioxidant enzymes play an important role in plant defense response against biotic and abiotic plant stresses by alleviating detrimental effects caused by oxidative stress. In this study, we measured the levels of SOD, POD, and CAT antioxidant enzymes in both control and powdery mildew-infected *B. juncea*, *C. sativa*, and *S. alba* plants according to reference [28]. Leaf samples of *B. juncea*, *C. sativa*, and *S. alba* were collected for different antioxidant assays. Briefly, leaf samples (1 gm) were grinded using a mortar and pestle using liquid nitrogen. The powdered material was mixed with 1 ml of phosphate buffer (100 mM, pH 7) and centrifuged at 14,000 rpm for 20 min at 4 °C. One hundred µL of supernatant was used to determine enzyme activity. The quantification of the same extract was conducted using the Bradford method [29] For the SOD enzyme assay, 100 µL of enzyme extract was added to 1.1 mL of phosphate buffer (0.5 M, pH 7.0), 40 µL 1% (*v*/*v*) of Triton X-100, 75 µL of L-methionine (0.02 M), and 75 µL of hydroxylamine hydrochloride (10 mM), and 80 of µL of riboflavin was finally added to start the reaction. The reaction mixture was maintained under constant illumination. Similarly, a peroxidase assay was performed using 50 mM of a potassium phosphate buffer (pH 7.8) in a 3 mL reaction mixture, and 100 µL of crude enzyme extract was added, followed by 500 µL of 16 mM Guaiacol, and 500 µL of hydrogen peroxide (12.5 mM) was added to start the reaction. The activity of the enzyme was measured at 470 nm (Hitachi U 2000, Japan). The activity for PODwas calculated by using the molar extinction coefficient of tetra-guaiacol (26.6 $mM^{-1}$ $cm^{-1}$) and expressed in a µmol (TG) formed mg $protein^{-1}$ $min^{-1}$. Catalase is an important enzyme in mediating plant response to fungal pathogens. The catalase activity was measured using 100 µL of a leaf extract added to 1.5 mL of a 50 mM phosphate buffer (pH 7.0). To complete the reaction, 500 µL of $H_2O_2$ (12.5 mM) was added, and the activity was measured at 240 nm with intervals of 30/s. Enzyme activity was calculated using the molar extinction coefficient of $H_2O_2$ (39.4 $mM^{-1}$ $cm^{-1}$), and the activity was expressed in µmol $H_2O_2$-reduced mg protein-1 $min^{-1}$. The experiments were carried out in three biological replicates.

### *2.5. cDNA Synthesis and Real-Time-PCR*

In this study, to assess the expression analysis of *PR* genes after powdery mildew infection, the total RNA was extracted from 100 mg of a control and *E. cruciferum*-infected *B. juncea*, *C. sativa*, and *S. alba* leaf samples using the Ambion RNA isolation kit as described by the manufacturer's protocol (Life Technologies). The concentration and purity of the total RNA from the control and infected leaf samples was measured using a Nanodrop spectrophotometer (NanoDrop 2000 Thermo Scientific, Wilmington, DE, USA). cDNA synthesis and RT-PCR was performed as described by the authors of [6] using alpha tubulin as a housekeeping gene. Briefly, cDNA was synthesized from purified RNA (2 µg) using the Superscript III cDNA synthesis kit (Invitrogen, Waltham, MA, USA). For qRT-PCR, 5 µL of SYBR green master mix (Takara, Japan), 2 µL of cDNA, and 0.5 µL (10 picomol) of forward and reverser primer was used. The qRT-PCR conditions were setup as follows: 95 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. In this study, *PR1*, *PR2*, and *PR5* as well as Alpha tubulin primers weredesigned by Oligoanalyzer software (Table 1). The relative expression levels of *PR1*, *PR2*, and *PR5* mRNAs were quantified using the 2^^Ct method [30]. Each reaction consisted of three biological replicates, and fold changes with less than 0.05 *p*-values were considered significant.

**Table 1.** List of primers used in this study.

| Primer Name | Sequence |
|---|---|
| *PR1*_F | 5′ TCGGCAAGTACCATGATGAG 3′ |
| *PR1*_R | 5′ GCATGTTGGTGGCAACG 3′ |
| *PR2*_F | 5′ CGAAGCCGGACCTAATCAAG 3′ |
| *PR2*_R | 5′ GAGAAGCTGCAAGCCACTAA 3′ |
| *PR5*_F | 5′ GCTTTCGCGTTGGCATAATC 3′ |
| *PR5*_R | 5′ GGAGAGCATGGAAGCAAGAA 3′ |
| *Alpha tubulin*_F | 5′GCCTCGTCTCTCAGGTTATTTC3′ |
| *Alpha tubulin*_R | 5′TGAAGTGGATTCTTGGGTATGG3′ |

### *2.6. Statistical Analysis*

Three biological replicates were employed in each experiment, with each one being performed three times. To find out whether there were any notable variations in the expression of the *PR1*, *PR2*, and *PR5* genes between the control and treatment samples, a Student's *t*-test was used. In this study, the differences in data between the two groups used for comparisons were rated as statistically significant (* $p < 0.05$) or extremely significant (** $p < 0.01$).

## 3. Results and Discussion

### *3.1. Powdery Mildew Disease Susceptibility and Resistance Is Cultivar Dependent*

The Brassicaceae family possess around 338 genera with 3709 species that are used as a source of vegetables, oil, mustard sauces, and fodder [31]. However, biotic stressors, such as powdery mildew, pose a severe threat to them; thus, it's crucial to develop elite resistant cultivars that can resist disease outbreaks. Powdery mildew disease in *B. juncea* has emerged as a serious problem across the globe, which infects all aerial parts thereby causing huge yield losses. Although some cruciferous crops, such as *B. napus*, *Eruca sativa*, and *S. alba* have been reported to be powdery mildew resistant, it remains largely unknown what elements could provide some cruciferous crops almost complete resistance or high susceptibility to powdery mildew. The molecular basis of powdery mildew and the Brassicaceae pathosystem is not fully understood. In addition, how some cultivars are more vulnerable or resistant to powdery mildew remains largely enigmatic. Furthermore, owing to their obligate biotrophic lifestyle, the host pathogen interactions of powdery mildew species are not fully understood, which makes it difficult to pinpoint the key players involved in infection and immune suppression. Therefore, it is essential to identify the key players involved in powdery mildew resistance or susceptibility in both cultivated and wild Brassica cultivars so that they can be further explored in developing powdery mildew-resistant cultivars using genome editing or molecular breeding. Additionally, the timely monitoring of newly emerged fungal strains should be top priority to combat future disease outbreaks under changing climatic conditions. In this study, we systematically assessed the powdery mildew disease progression in three different crucifer members, namely, *B. juncea*, *C. sativa*, and *S. alba* in order to identify the susceptible and resistant cultivars. Based on our clinical findings, *B. juncea* and *C. Sativa* develop characteristic symptoms of powdery mildew, such as white, star-shaped mycelia colonies, while there were no such symptoms detected in *S. alba* (Figure 1a–c).

Similar results were also observed by the authors of [26,32,33]. However, the disease outcome and intensity may vary with warm and relatively dry weather conditions. Since several studies have shown that climatic factors such as temperature and precipitation may affect the establishment of powdery mildews on plant surfaces, as a result, the impacts of this fungus would differ between habitats and seasons [26,34]. Furthermore, it is still completely unknown why certain powdery mildew diseases in *B. juncea* are more severe in various regions and during particular seasons. Previous studies have shown that wild varieties of Brassica, such as *C. sativa* and *S. alba*, are resistant to a number of fungal

pathogens. However, in this present study, only *S. alba* was found to be resistant to powdery mildew when compared to *C. sativa*. Furthermore, based on disease scoring such as powdery mildew colony count, higher numbers of colonies were observed in *B. juncea* and *C. sativa*, and no such macroscopic colony was found in *S. alba* (Figure 2a). Additionally, *B. juncea* and *C. sativa* showed *E. cruciferum* infection with a disease scale of 7–8 (70–80%), while *S. alba* showed 0–1 (0%) of disease incidence (Figure 2b). These observations further revealed that *S. alba* is highly resistant to powdery mildew, whereas *B. juncea* and *C. sativa* are more prone to *E. cruciferum* infection.

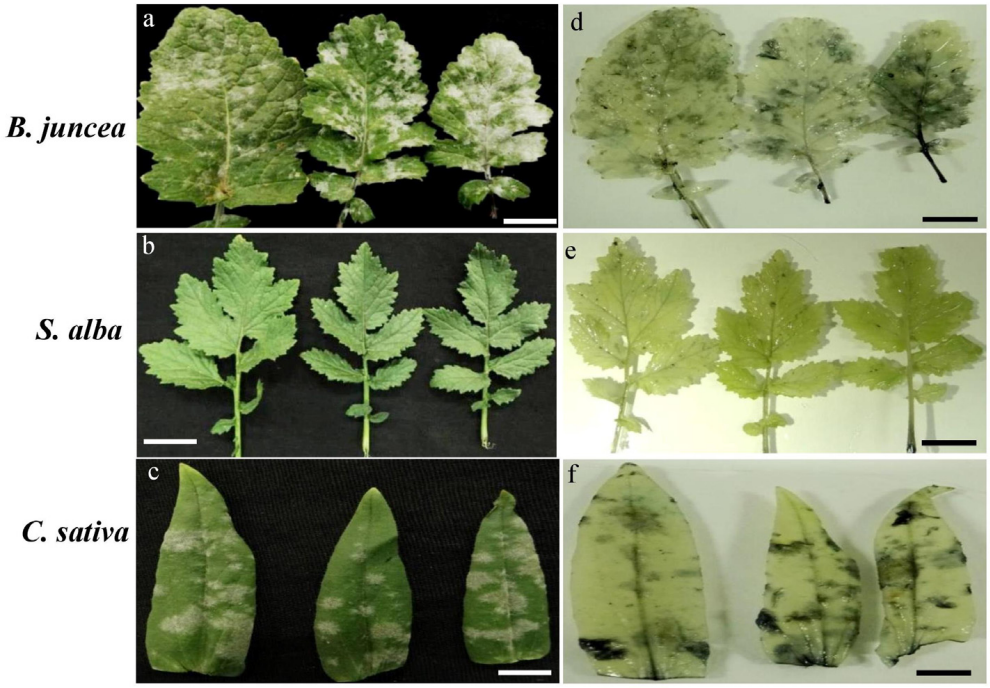


**Figure 1.** Shows powdery mildew infection (**a**) *B. juncea*, (**b**) *S. alba*, and (**c**) *C. Sativa* while as (**d**–**f**) reveals cell death in three Brassica cenospecies using trypan blue staining.

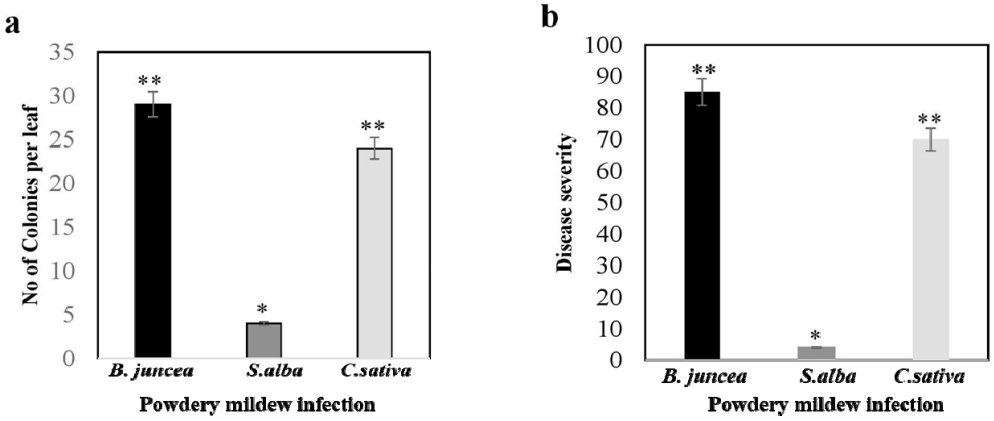


**Figure 2.** Powdery mildew disease scoring in *B. juncea*, *S. alba* and *C. Sativa* plants: (**a**) number of powdery mildew colonies and (**b**) disease severity. Asterisks shows statistically significant (* $p < 0.05$) or extremely significant (** $p < 0.01$).

### *3.2. Powdery Mildew-Mediated Cell Death in B. juncea, C. sativa, and S. alba*

In this study, powdery mildew-mediated cell death in *B. juncea*, *C. sativa*, and *S. alba* after infection was monitored using trypan blue staining. Based on our phenotypic observations, more cell death was found in *B. juncea*, *C. sativa* plants than *S. alba* (Figure 1d–f). A hypersensitive response (HR), or the quick death of plant cells, was frequently noticed at the inoculation

areas during the interactions between plants and powdery mildew. An earlier investigation revealed that powdery causes cell death and systemic PCD in wheat adventitious roots [35]. Earlier studies have demonstrated that biotrophic diseases, including powdery mildew, are successfully restrained in their ability to proliferate by fast, localized cell death [36]. However, plant disease susceptibility and resistance are strongly related to pathogen-induced cell death.

### *3.3. Antioxidant Enzyme Assay in B. juncea, C. sativa, and S. alba Plants after Powdery Mildew Infection*

Powdery mildew-induced cell death is usually linked with reactive oxygen species (ROS) burst, which has both beneficial and detrimental outcomes. For example, ROS can trigger various key defense signaling cascades, such as a hypersensitivity response and localized programmed cell death (PCD), to impair the pathogen establishment and disease development inside the host tissues. It also triggers the expression of defense-related genes which possess broad antimicrobial activities. In contrast, improperly created ROS could deleteriously impact the function of DNA, proteins, and lipids in cell components, leading to cell death [37]. However, plants produce diverse antioxidant enzymes that eliminate ROS ($O_2$ and $H_2O_2$) and to cope with its detrimental effects. Previous studies have shown that the activation of the antioxidant system confers disease resistance against a wide range of pathogens. In light of this, we examined the role of key antioxidant enzymes such as SOD, POD, and CAT in *B. juncea*, *C. sativa*, and *S. alba* after powdery mildew infection. These antioxidant enzymes are crucial for plants' defense mechanisms against biotic and abiotic stressors. Based on our findings, the activity of SOD was relatively higher in *S. alba* than *C. sativa* followed by *B. juncea* (Figure 3a). Similarly, POD activity was also found to be higher in *S. alba* than *C. sativa* and *B. juncea* after powdery mildew infection (Figure 3b). On the other hand, CAT activity was significantly higher in *S. alba* than that of *B. juncea* and *C. sativa* after powdery mildew infection. There is a growing body of evidence that antioxidants such SOD, CAT, and POD play a key role in disease resistance by modulating multifaceted functions, such as the reinforcement of cell wall barriers and protection from oxidative burst, and they also act as signal mediators that in turn leads to oxidative stress tolerance and pathogen resistance [38,39]. Consistent with these reports, the current findings demonstrate that increased SOD, CAT, and POD activity in *S. alba* may be significant contributors to disease resistance against powdery mildew infection.

### *3.4. Deciphering SA Signaling Pathways in B. juncea, C. sativa, and S. alba after Powdery Mildew*

Plant defense against biotrophic and semi-biotrophic diseases depends heavily on the SA defense pathway and its crosstalk with other defense hormones. The host resistance to biotrophic and semi-biotrophic pathogen infection is significantly hampered in SA mutants lacking SA biosynthesis and accumulation [9]. Previous studies have shown that exogenous treatment of SA in plants significantly induced immune responses against biotrophic and semi-biotrophic pathogens. Nevertheless, SA-triggered immunity to biotrophic diseases may be due to the activation of SAR and the induction of PR proteins, which possess strong antifungal activity. In higher plants, PR proteins are broadly distributed and play a critical part in the defensive systems that protect plant cells from a variety of stressors. In light of this, we examined the real-time expression of SA signature genes (*PR1*, *PR2* and *PR5*) in *B. juncea*, *C. sativa*, and *S. alba* after powdery mildew infection. According to our findings, all three species showed an increase in the transcription levels of the SA marker genes *PR1*, *PR2*, and *PR5* after powdery mildew infection. However, the fold change was relatively higher in the powdery mildew-resistant cultivar *S. alba* (Figure 4).

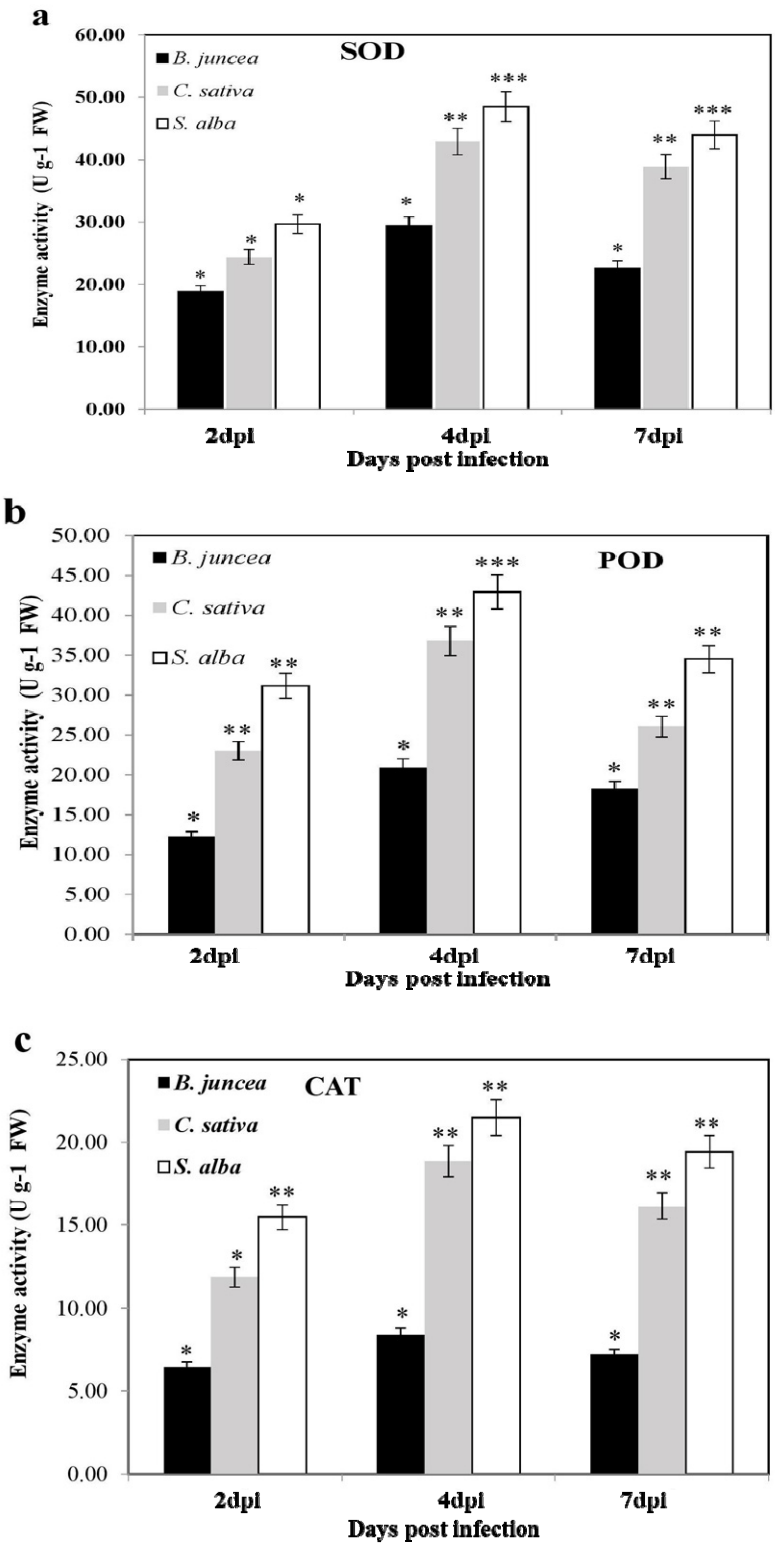


**Figure 3.** Antioxidant enzyme profiling in *B. juncea*, *C. Sativa*, and *S. alba* after powdery mildew infection. (**a**) SOD activity in *B. juncea*, *C. Sativa*, and *S. alba* after powdery mildew infection. (**b**) POD activity in *B. juncea*, *C. Sativa*, and *S. alba* after powdery mildew infection. (**c**) CAT activity in *B. juncea*, *C. Sativa*, and *S. alba* after powdery mildew infection. Asterisks shows statistically significant (* $p < 0.05$) or extremely significant (** $p < 0.01$) or (*** $p < 0.001$).

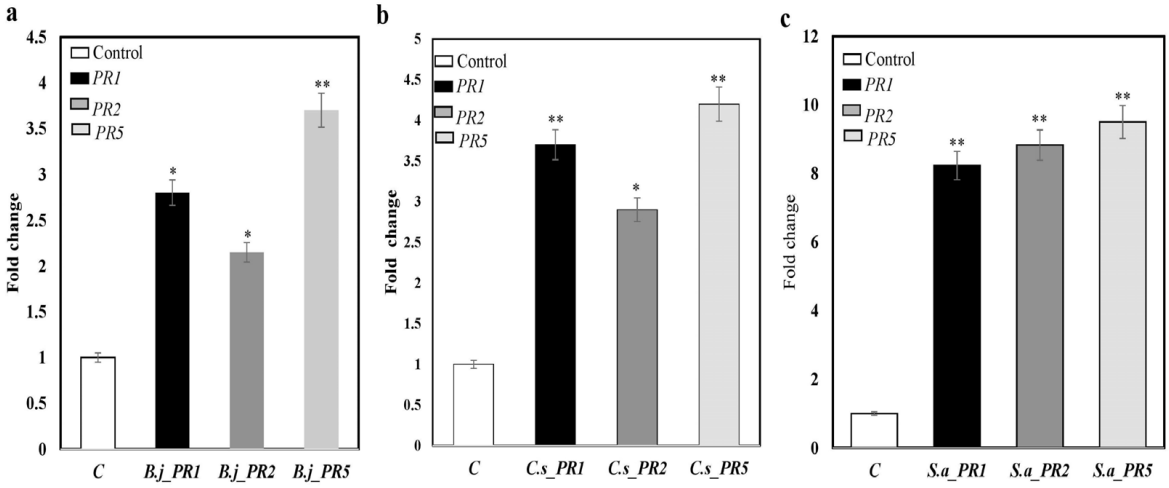


**Figure 4.** Expression analysis of SA marker genes (*PR1*, *PR2*, and *PR5*) in (**a**) *B. juncea*, (**b**) *C. sativa*, and (**c**) *S. alba* after powdery mildew infection. Error bars based on standard error were calculated from three biological replicates (* $p < 0.05$; ** $p < 0.01$). Asterisks shows statistically significant (* $p < 0.05$) or extremely significant (** $p < 0.01$) between control and infected plants.

Similar results were also observed in grapevine upon powdery mildew infection, and they found a significant increase in the expression of β-1,3-glucanases (*PR2*) and a thaumatin-like protein (*PR5*) [40]. Generally, *PR1* is typically seen as an indicator of SA-triggered SAR pathway that inhibits disease progression to non-infected distal parts of host plants [6,41]. In addition, SAR also plays a key role in HR-related cell death. Previous studies have also shown that the constitutive expression of *PR1* gene confers disease resistance to plant pathogens [42]. Hence, a higher induction of the *PR1* gene in *S. alba* might be involved for its disease resistance against powdery mildew infection in both local and distal parts. Our results are consistent with Guo et al. [8], where the expression of the *PR1* gene was significantly increased upon powdery mildew infection in *Cucurbuta moschata*. Similarly, PR2 and PR5 proteins also possess strong antifungal activity. Therefore, elevated transcription levels of *PR2* and *PR5* in *S. alba* could be linked with its disease resistance. PR proteins are well-known players for protecting plants against a variety of microbial diseases and have been utilized for decades to generate resistant plants. Previous research has also demonstrated the antimicrobial activities of PR proteins in different plants, further demonstrating their protective function in disease resistance [2,6,40,43–50]. Overall, the increased expression of *PR1*, *PR2* and *PR5* genes in *S. alba* further supports the notion that these genes may contribute to disease resistance against powdery mildew infection. We speculate that in *S. alba*, SAR limits powdery mildew infection and safeguards the distal, uninfected areas, since these genes are the key player of the SAR pathway. In contrast, many studies have also shown that biotrophic pathogens have evolved intricate strategies for disrupting SA-mediated defense responses in plants. Therefore, it is possible that powdery mildew interferes with SA pathways in *B. juncea* and *C. sativa*, which results in the disease progression. However, to prove this, future studies are required to quantify the SA levels as well as gene knock studies to underpin the mechanism of disease susceptibility in *B. juncea* and *C. sativa* and resistance in *S. alba* after powdery mildew infection. It will be interesting to understand how powdery mildew infection alters plant defense hormonal cascades such as SA/JA, ET, and ABA in both resistant *S. alba* and susceptible *B. juncea*, because there have been reports on hormonal reprograming during powdery mildew in other plants including grapevine. This will provide novel insights on manipulating potential gene networks for improving powdery mildew disease resistance.

## 4. Conclusions

Powdery mildew is becoming a major concern in modern agriculture, as it infects most of the agriculturally important crops, thereby causing huge yield and economic losses. In most places of the world, its frequency has greatly risen due to climate change. Currently, fungicides are used to control powdery mildew disease, but it has many drawbacks because it is detrimental to the environment and because of the emergence of fungal-resistant varieties. Hence, there is a need to find alternatives for the development of powdery mildew-resistant cultivars in sustainable agriculture. In India, powdery mildew disease has emerged as a serious concern in Brassica oil seed crops due to high whether fluctuations, host susceptibility, and a lack of resistant cultivars. In particular, powdery mildew disease outbreaks in *B. juncea* have become major concern as it affects oil seed quality and quantity, leading huge yield and economic losses. At present, all the *B. juncea* cultivars grown in India are highly susceptible to powdery mildew infections. Therefore, identifying resistant cultivars and decoding their molecular mechanism of disease resistance in the Brassica pool will provide novel insights for improving powdery mildew disease resistance in *B. juncea*. Here, we studied powdery mildew disease progression in three Brassica cenospecies and found *S. alba* was highly resistant, and *B. juncea* and *C. sativa* were susceptible to *E. cruciferarum*. Therefore, further studies are needed to unravel the potential candidates and the signaling cascades in *S. alba* that are involved in powdery mildew disease resistance. In the future, the integration of multiomics and gene editing can be utilized for decoding the disease resistance mechanism in *S. alba* against powdery mildew infection, which can serve as an efficient source for developing powdery mildew disease-resistant Brassica cultivars.

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