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Review

Fungal Necrotrophic Interaction: A Case Study of Seed Immune Response to a Seed-Borne Pathogen

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Abstract: Seeds play a vital role in the perpetuation of plant species, both in natural environments and agriculture. However, they often face challenges from biotic stresses, such as seed-borne pathogenic fungi. The transgenerational transmission of these seed-borne fungi, along with their dissemination during seed commercialization, can contribute to the emergence of global epidemic diseases, resulting in substantial economic losses. Despite the recognized impact of seed-borne pathogens on agriculture, our understanding of seed–pathogen interactions remains limited. This review establishes parallels between the current state of knowledge regarding seed responses to pathogen interactions and well-established plant defense models, primarily derived from typical physiological conditions observed during leaf infections. Examining fragmented results from various pathosystems, this review seeks to offer a comprehensive overview of our current understanding of interactions during seed development and germination. The necrotrophic interactions in *Brassicaceae* are described using recent transcriptomic and genetic studies focused on the *Arabidopsis*/*Alternaria* pathosystem, which illustrates original response pathways in germinating seeds that markedly differ from the general concept of plant–pathogen interactions. The co-existence of regulatory mechanisms affecting both seed resistance and susceptibility, potentially promoting fungal colonization, is examined. The vulnerable response during germination emerges as a crucial consideration in the context of sustainable plant health management in agriculture.

Keywords: seed-borne pathogens; *Alternaria brassicicola*; seed defense; susceptible response; transcriptomics; *Arabidopsis*

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1. Introduction

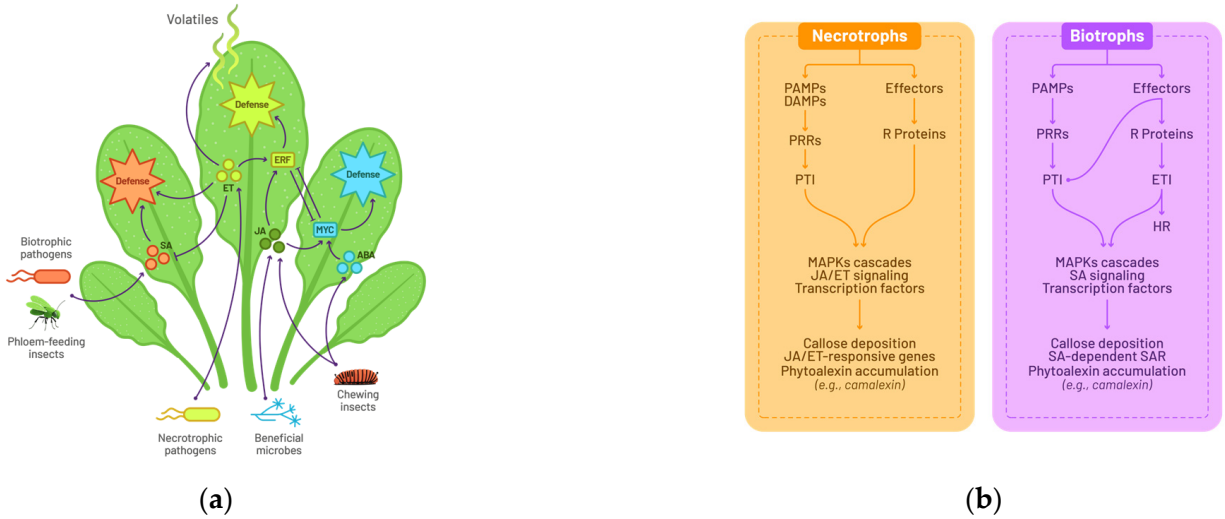
Seeds are the fundamental reproductive structure and play a crucial role in the propagation and survival of flowering plants and gymnosperms, including many economically important species [1–3]. The seed is composed of an embryo (sporophyte), reserve tissues (cotyledons, endosperm, or perisperm), and often a covering tissue, known as the testa [1,2]. After seed development, the mature seed is metabolically equipped to optimize germination and seedling establishment upon dissemination in a stressful environment [1]. Seed germination is controlled by complex regulation integrating environmental signals, such as light, temperature, nitrate sources, and endogenous hormonal signals, such as the well-described abscisic acid and gibberellin pathways [1,3,4]. Germination begins with water imbibition that induces a burst of respiratory and energetic metabolism, activating

translation from stored RNA and RNA neo-syntheses. Environmental factors will interfere with hormonal metabolism and seed responsiveness, controlling embryo growth potential and radicle protrusion through weakened endosperm and testa rupture. Hydrolysis and mobilization of the storage nutriment are concomitant with this step of germination completion to support the transition to an autotrophic development phase signaling seedling establishment [1,5].

In addition to its physical and chemical attributes, the seed also hosts a diverse microbial community, called a microbiome, which can influence its ecological and biological functions. This seed-associated microbiota is acquired during various stages of the seed's life cycle [6,7]. The community of seed-borne microorganisms, including bacteria, fungi, viruses, and oomycetes, which can colonize the surface of the seed, are referred to as the epiphytic microbiota. Microorganisms colonizing internal tissues, such as the embryo and endosperm, are known as the endophytic microbiota. The microbiota can be transmitted from the mother plant to the seedling through seed germination. This phenomenon is known as vertical transmission and provides a way for the microbiome to spread into plant communities [6,7]. The beneficial microbiota contributes significantly to seed nutrient uptake, disease resistance, and adaptation to environmental changes [7]. Moreover, some seed-borne microbial agents are pathogenic and can affect seed viability and germination vigor. Their transmission to seedlings is a major cause of disease outbreaks and crop yield loss [8,9]. This is particularly critical in the case of endophytic localization of pathogens because seed treatments are not effective. Moreover, the increasingly restrictive ban on the use of pesticides makes it necessary to seek alternative solutions to control the sanitary quality of the seed [8–11].

A wide variety of plant microorganisms, including bacteria, nematodes, viruses, and fungi, are described as plant pathogenic agents [12,13] (Figure 1a). These cause significant losses in valuable crops, such as brassicas [14]. An important group of phytopathogens that strongly affect agricultural production are fungi [11,15]. According to their nutritional strategies and host interactions, fungi can be classified as biotrophs, necrotrophs, and hemibiotrophs [16]. Biotrophs rely on living host cells for nutrition, necrotrophs use hydrolytic enzymes and toxins to induce host cell death and subsequently feed on dead tissues, while hemibiotrophs alternate between biotrophic and necrotrophic phases [16–18]. Plants have developed complex defense responses to protect against these pathogens. Defense mechanisms differ according to pathogen lifestyles [12,13]. They involve different physical barriers, such as cell wall appositions, hormonal signaling, and antimicrobial metabolites, such as phytoalexins or reactive oxygen species (ROS) and proteins [12,13,19,20] (Figure 1b). While the significance of seed pathogen transmission in crop diseases is acknowledged and it is hypothesized that seed pathogen interactions differ markedly from models commonly described in pathosystems using leaf infections [21], mechanisms of seed response to a pathogenic agent remain poorly documented. Recent studies employing the pathosystem *Arabidopsis thaliana*/*Alternaria brassicicola* [22] have detailed non-canonical defense responses in germinating seeds [23]. This is in contrast to the established model describing defense mechanisms against necrotrophic agents [20,24] based on the physiological model of rosette leaf inoculation [21]. Notably, the response of the germinating seed not only expressed resistance mechanisms but also identified a susceptible response that could potentially facilitate the spread of the pathogen [23].

This review assesses general models of pathogenic plant interactions, encompassing plant defense mechanisms and providing examples to illustrate susceptible responses. The aim is to contextualize recent knowledge pertaining to seed immune responses [23]. Regarding seed responses, predominant insights have been gained from studying necrotrophic interactions [22,23,25,26], primarily using the *Arabidopsis thaliana*/*Alternaria brassicicola* pathosystem. However, due to the fragmentary knowledge of interactions controlling seed pathogen transmission, this perspective is complemented by examples borrowed from other pathosystems. These novel working hypotheses of biotic interactions during germination open avenues for considering innovative crop protection strategies.



(**a**) (**b**)

Figure 1. General model of plant defense signaling and immune responses. (**a**) Overview of the plant's signal detection mechanisms involving interactions with herbivorous, beneficial, and pathogenic microorganisms. (**b**) The plant immune response against necrotrophs and biotrophs involves diverse defenses, including physical barriers, hormonal signaling, and antimicrobial metabolites. Swift responses, initiated by Pathogen Recognition Receptors (PRRs), progress from P/MAMP-triggered Immunity (PTI) to Effector-Triggered Immunity (ETI) upon evasion by pathogens.

2. Plant Immune Response

2.1. A General Model for Plant–Pathogen Interactions

To safeguard themselves against attack from phytopathogens, plants have developed a sophisticated immune system. This can be classified as either innate immunity or induced immunity [12,13,27]. In host plants, the innate immune response is the first line of defense. Upon contact with external pathogens, the plant utilizes specialized Pathogen Recognition Receptors (PRRs) to identify specific molecules from the infectious agent, known as Pathogen/Microbe-Associated Molecular Patterns (P/MAMPs), as well as damage-associated molecular patterns (DAMPs). This process initiates a swift response designed to impede the pathogen's proliferation, referred to as P/MAMP-triggered Immunity (PTI) [12,13]. However, if the pathogen manages to evade PTI through the deployment of virulent effector molecules, an induced immune response is activated. This response involves the recognition of the pathogen's effector molecules by plant resistance proteins, such as nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins. Subsequently, the Effector-Triggered Immunity (ETI) mechanism is activated to effectively detect and target the pathogen [12,18,19].

Once plant immunity is triggered, physical barriers are reinforced [12,20,24], such as callose deposition or cuticular wax accumulation. Subsequently, a complex network of signaling pathways orchestrated by the phytohormones salicylic acid, jasmonic acid, and ethylene comes into play [16,19,28]. These signaling pathways lead to an oxidative burst [16,19], the production of antimicrobial peptides (AMPs) [29], and the biosynthesis of secondary metabolites, such as indole-derived metabolites, including phytoalexins [19,30] (Figure 1b). The salicylic acid (SA) pathway has been associated with plant response to biotrophic microorganisms [16,31] (Figure 1b). The accumulation of SA induces ROS production, molecules that usually trigger the hypersensitive response (HR), a type of programmed cell death targeting infected cells, thus limiting the spread of pathogens [19,31,32]. At this stage, when a plant successfully overcomes an infection, it could develop systemic acquired resistance (SAR), making the plant ready to induce a resistance mechanism in case of a pathogenic attack. This phenomenon works like a memory, providing heightened resistance to subsequent attacks and ensuring extended and more robust protection against phytopathogens [33,34].

Other hormones such as ethylene and jasmonic acid, along with secondary metabolites, such as camalexin, brassinin, and glucosinolates (GSL), are produced in response to infection by necrotrophic and hemibiotrophic pathogens. These hormones act as signaling molecules to activate plant defenses, while the secondary metabolites directly inhibit pathogens or produce toxic derivatives crucial for combating the pathogens [18,28,30,35] (Figure 1b).

To gain a comprehensive understanding of plant defense mechanisms against microbial pathogens, the research emphasis has predominantly centered on plant susceptibility [12,13]. However, the intricate defense mechanisms implicated in a non-host plant remain unknown until now [27,36]. The crucial distinction in these mechanisms lies in the specificity of resistance, where host resistance is specifically tailored to particular pathogens, while non-host resistances (NHR) provide a more generalized defense. Although they share common mechanisms, such as the activation of signaling pathways and the production of antimicrobial compounds, host resistance is highly adaptive, providing specific immune responses, while NHR acts using broader defenses against various threats [36,37]. NHR comes down to a capability to recognize diverse microbial agents as non-hosts, granting plants broad and persistent resistance against a wide range of pathogens [27,36]. This phenomenon is believed to originate from a coevolutionary dynamic between plants and pathogens [13]. Plant immune resistance is influenced by a combination of abiotic factors, pathogen characteristics, host physiology, and plant tissues under attack. Certain studies proposed that this event arises either due to the inability of pathogens to suppress the PTI [13] or because one of the plant's NB-LRR receptors recognizes the effector molecules by the pathogen, thus triggering ETI [13,27,37,38]. However, detailed NHR mechanisms are still not fully understood. Consequently, it is imperative to conduct further research to elucidate the molecular foundations of plant resistance and achieve a more comprehensive grasp of plant defense mechanisms [36].

2.2. Susceptible Response and Plant Tolerance

Furthermore, the endophytic condition assumed by specific pathogenic agents in non-host plants engenders considerable scientific interest, given its potential association with the elicitation of susceptible responses (SRs) [27]. This scenario is marked by the host's constrained capacity to counteract infection, fostering an environment conducive to pathogen proliferation and establishment inside the plant.

A pathogen strategy encompasses the manipulation of plant defenses and metabolic processes to its advantage. The pathogen must employ factors, such as effectors, phytohormones, and phytotoxins, to activate the so-called SR genes within the plant. This enables the pathogen to control both the plant's defense mechanisms (physical and physiological) and metabolic processes, such as the reallocation of energy-rich compounds and the regulation of metal equilibrium, for its own benefit. Particularly in the case of phytopathogenic fungi, it is well documented that they have evolved not only to counteract the plant's immune response but also to manipulate it for their own benefit. This includes their capacity to recognize plant-derived factors, such as chemical signals in the cuticle, leaf topology, and trichomes, thereby facilitating their successful colonization of the host [20]. Furthermore, certain seed-borne necrotrophic fungi, like *Botrytis cinerea* (*B. cinerea*), employ sophisticated systems to elude host recognition. They achieve this by hijacking or disrupting plant defense signaling and disabling defense mechanisms through the secretion of proteinaceous effectors and sRNA effectors, all geared towards promoting their own benefit (Table 1) [39,40]. In particular, *B. cinerea* adopts a multifaceted approach in hosts like tomato, tobacco, and *Arabidopsis*. It deploys effectors, such as BcSpl, from the Cerato-platanin protein family, and BcGs1, a glycoproteinaceous elicitor, to induce necrosis and programmed cell death (PCD) [41,42] with the purpose of generating dead tissue to grow through its necrotrophic properties. It is also described in the tomato host that *B. cinerea* manipulates SA accumulation via β-(1,3)(1,6)-d-glucan exopolysaccharide, suppressing JA-mediated signaling pathways and promoting disease development [43]. Moreover, it is

documented in *Arabidopsis* that *B. cinerea* hijacks auxin metabolism through GH3.2 expression and Auxin Indole-3-Acetic Acid–Aspartic Acid–IAA–Aspartate (IAA-Asp) production, altering hormone dynamics to favor colonization and virulence [44]. In a strategic extension of its arsenal, *B. cinerea* also utilizes small RNAs to manipulate the RNA interference (RNAi) system in *Arabidopsis* and tomato. This tactic specifically targets key immune defense genes, such as peroxiredoxin (associated with oxidative stress), mitogen-activated protein kinases (MPK1, MPK2, MPKKK4), and a cell wall-associated kinase (WAK), silencing genes critical for immune defense and further enhancing its pathogenic capabilities [45].

Table 1. Plant responses induced by *Botrytis cinerea* that illustrate susceptible response.

Host	Pathogen-Induced Plant Response Pathway	Reference
Solanum lycopersicum (tomato), *Nicotiana tabacum* (tobacco), and *Arabidopsis thaliana* (*Arabidopsis*)	Programmed cell death (PCD)	Frías et al. [41] Zhang et al. [42]
Solanum lycopersicum (tomato)	SA pathway	El Oirdi M et al. [43]
Arabidopsis thaliana (*Arabidopsis*)	Auxin pathway	González-Lamothe R et al. [44]
Solanum lycopersicum (tomato) and *Arabidopsis thaliana* (*Arabidopsis*)	Peroxiredoxin (oxidative stress-related gene), mitogen-activated protein kinases (MPK1, MPK2, MPKKK4), and a cell wall-associated kinase (WAK)	Weiberg et al. [45]

Identifying genes that facilitate pathogen proliferation contributing to disease development is crucial for implementing genetic strategies focused on suppressing symptom-associated genes, leading to plants being better adapted to infections. Tolerant plants would be able to maintain their productivity levels, remain symptom-free, and potentially even benefit from the presence of endogenous pathogens [46]. Nevertheless, it is important to acknowledge that immunity defense mechanisms remain effective, and their efficacy may vary depending on the plant and pathogen species, as well as other environmental factors.

3. *Alternaria brassicicola*: An Important Seed-Borne Pathogen in Brassicaceae and a Model for Studying Pathogen Interactions with Seeds

Brassicaceae (Cruciferae) comprise approximately 4140 species, including the model species *Arabidopsis thaliana* (*Arabidopsis*) and economically important brassica vegetables, such as *Brassica oleracea* (broccoli, cauliflower, kale, brussels sprouts, and cabbage), *Brassica rapa* (vegetables, oilseeds, and forage), *Brassica juncea* (vegetables and mustard seeds), and *Brassica napus* (oilseeds) [47,48]. Brassicaceae species are susceptible to the seed-borne necrotrophic fungi *Alternaria brassicicola* (Berk.) Sacc. (referred to as *Alternaria* hereinafter), the causal agent of black spot disease. This seed-borne pathogen *Alternaria* (Ascomycota, Dothideomycetes, Pleosporales, and Pleosporaceae) is a common genus that frequently occurs as a saprotroph of decaying organic matter, but few species are plant pathogens [49,50]. Diseases caused by *Alternaria* species are widespread and can affect other crops, such as tomatoes, apples, carrots, and potatoes [51,52]. The economic impact on agricultural production is significant, as it affects the plant at all stages of development, from seed germination to post-harvest [22,50,53].

The infection process begins with fungal penetration into the plant through wounds or stomata [20]. Once inside the plant, the fungus secretes secondary metabolites, toxins [54,55], and a series of hydrolytic enzymes, such as proteases, lipases, and Cell Wall-Degrading Enzymes (CWDEs), leading to the death of plant tissues and facilitating the entry of the fungus into plant cells [49,51,56]. In leaf tissue, disease symptoms include leaf spots, yellow chlorotic halos, necrotic lesions, and leaf size reduction, resulting in a decrease in leaf photosynthetic capacity. In seedlings, a symptom known as damping off occurs and is

characterized by the rotting of the plant's stem base and roots, wilting, and causing the death of young plants as a consequence of the infection [49,51,56].

Alternaria is transmitted to the seed and is considered a seed-borne pathogen. At the seed level, *Alternaria* infection can adversely affect seed quality, germination rates, and crop yield. Several factors influence disease development and severity, including moisture, low light intensity, temperature, plant age, and conidia density [50,53]. The impact of infection on agricultural production and the search for solutions to reduce its harmful effects motivate the study of the mechanisms of interaction between plants of agronomic interest and *Alternaria*.

4. Seed Defense Mechanisms and Interactions with the Necrotrophic Seed-Borne Fungi *Alternaria brassicicola*

4.1. A silent Interaction with the Seed

Mechanisms of the transgenerational transmission of pathogens are poorly documented because few infection symptoms can be detected in seeds. Many seed-borne pathogens are considered non-hosts during seed development [57,58]. A striking example is the bacterial pathogen *Xanthomonas campestris* pv. campestris, known for attacking cruciferous plants [57]. In beans, this pathogen is transmitted from seed to seedling. Interestingly, its growth in these plants is like how it behaves in plants contaminated with *X. citri* pv. phaseoli var. fuscans (a bean-related pathogen). This does not significantly affect seed germination or seedling development, nor does it cause visible disease symptoms [57].

4.2. Interaction with the Necrotrophic Seed-Borne Fungi Alternaria brassicicola

Because seeds contribute to reproductive success, it is hypothesized that seed protection mechanisms would be part of their adaptive traits [25]. At the time of dissemination, the mature seed has acquired structural and metabolic properties [4,25] that could contribute to non-host resistance. The accumulation of secondary metabolites, such as phenolic compounds, in the mature seed [26] and the development of physical barriers, such as the seed coat [4,25], include some of those mechanisms. Also, pending seed germination and seedling establishment, these properties enable seeds to maintain their survival when challenged by abiotic and biotic pressures in their environment [7,25,26,59,60].

The endosperm acts not only in the control of seed germination but also as a protective barrier during germination [4,61]. Genes encoding detoxifying enzymes, such as glutathione S-transferases and peroxidases, play a crucial role in protecting the embryo against ROS that could result from pathogen interaction during seed germination. Genes related to plant–fungus interactions and hormonal metabolism also hold crucial roles in enhancing immunity and regulating plant growth. A transcriptomic study showed an over-expression of genes responsible for synthesizing salicylic acid (SA), indole-3-acetic acid (IAA), and amino synthetases (GH3) [61]. At the transcriptome level, the activation of secondary metabolites and defense response in the endosperm illustrates the activation of defense pathways during germination [61]. It is quite remarkable that certain mechanisms in seeds seem to differ from those in plants. Nevertheless, there are also instances of similar defense mechanisms being observed. For example, plant defense through polyphenol oxidase (PPO), a group of enzymes that catalyze the oxidation of hydroxy phenols, yielding products with antimicrobial properties. In dormant wild oats (*Avena fatua* L.), seeds attacked by *Fusarium avenaceum* trigger post-translational activation of the PPO, demonstrating the seed's ability to induce enzymatic biochemical defenses against pathogenic fungi. This PPO activity was also found to be induced in non-living caryopsis cover tissues, such as lemma and pale [26].

The mature seed has developed resistance properties to face biotic and abiotic stress after dispersal. However, the immune responses of seeds to biotic interactions during germination have been infrequently studied, although this step is crucial to control seed-borne pathogen transmission to the new plant [22,25]. The model pathosystem *Arabidopsis*

thaliana/*Alternaria brassicicola* has been recently used to describe gene regulation induced by the necrotrophic interaction during germination and seedling establishment [22,23,62].

RNA-seq data from *Alternaria*-infected and healthy *Arabidopsis* seeds were compared at different time points of the germination kinetics until the stage of seedling establishment. Although a transcriptomic study carried out on inoculated rosette leaves [21] highlighted an induction by *Alternaria* of the jasmonate pathway and camalexin metabolism (Figure 1b), the response of the germinating seed rather reflected an activation of the SA and ET pathways to the detriment of the jasmonate pathway [23]. It is documented that this type of unexpected response could constitute a diversion towards inappropriate defense pathways of the plant by the fungus to induce an SR favoring its development [39,40]. Additionally, genes related to response to hypoxia and indole-derived metabolites, such as GSL, were induced by *Alternaria* during germination [23]. Gene ontology enrichment analysis showed a stronger and more significant association among genes related to the response to hypoxia at an early development stage. Their fold enrichment decreased as seedling establishment progressed (3 Days After Sowing (DAS): 7.08, 6 DAS: 4.29, and 10 DAS: 3.07) [23]. This response is surprising, but it illustrates a competition for oxygen, which benefits the physiology of the early imbibed seed. Seed germination is well adapted to low oxygen levels [63], and these low oxygen levels limit the growth of the fungus. The induction at the RNA level of the indole GSL metabolism that was observed in *Arabidopsis* seeds [23] is well described [64].

Phenotyping analyses of mutant seeds deficient in the identified pathways via RNA-seq showed that the mutants deficient in ethylene response *ein2* and *etr1* exhibited a 21.9% and 55.4% lower rate of necrosis than the WT control, respectively. Also, GSL-deficient mutants (*qk0* and *gtr1gtr2*) exhibited a 65.9% and 73.6% lower rate of necrosis than those in the control group, respectively. Noticeably, the GSL-deficient mutants displayed also a reduced *Alternaria* overgrowth [23], indicating that the lack of necrosis, usually mediated by the *Alternaria*-induced GSL pathway in WT, would be limiting colonization of the necrotrophic fungus. In this context, the induction of GSL metabolism could also contribute to the SR of the infected seed. The identified changes in gene regulation induced by *Alternaria* in germinating seeds provide an original working hypothesis where SR and defense mechanisms co-exist (Figure 2).

Scientific evidence reinforces the hypothesis that the seed–pathogen interactions differ from models described for the whole plants. It is notable that the transcriptome of infected tissues differed drastically between 6-day-old seedlings and 10-day-old seedlings [23,62]. Unpublished data obtained in our laboratory have shown that necrosis symptoms (Figure 3) in newly germinated seedlings (4 DAS) exhibit more distinct and localized patterns compared to older seedlings (after 10 days), where necrosis becomes more diffuse and widespread in the infected tissue.

Microscopic view (objective 20×) of necrotic symptoms on *Alternaria*-infected *Arabidopsis* cotyledons in visible and fluorescent light. The necrotic area is shown in red (false color) using a long-pass GFP filter. At an early germinative stage (4 DAS) necrosis occurs in well-defined areas, whereas at later stages (6 and 10 DAS), necrosis is diffuse. GSL mutants show no necrosis at 3 DAS. Scale bar: 50 µm.

It is hypothesized that at the germinative stage, the young seedling reacts to infection with a well-circumscribed HR. GLS-deficient mutants did not show any necrosis symptoms at the young seedling stage (4 DAS), suggesting that the observed HR would be mediated by the activation of the plant's GSL pathway, whereas after 6 and 10 days of development, new necrosis was observed, including GSL mutants. The diffused appearance of the latter is attributed to cell death directly induced by the fungal penetration of plant tissues (unpublished data).

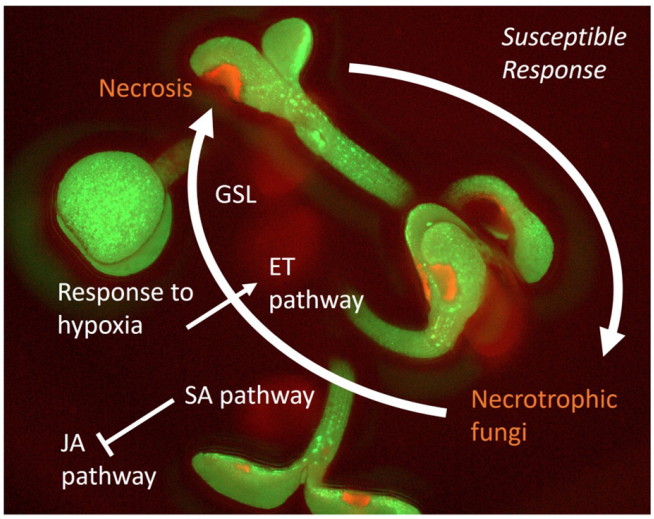


Figure 2. Working model illustrating a susceptible response (SR) to *Alternaria* in germinating seeds. Proposed regulations have been provided by transcriptomic and mutant analyses [1]. *Alternaria* induced at the RNA level salicylic acid (SA) pathway and the indole glucosinolate (GSL) metabolism, as well as ethylene (ET) signaling and the response to hypoxia. The SA pathway is usually induced to the detriment of jasmonic acid (JA) response, which has not been found to be repressed in the transcriptomic data. Mutant analyses illustrated the contribution of GSL and response to ET in the occurrence of necrosis symptoms that are needed for *Alternaria* development. Responses to hypoxia have been also documented to induce ET signaling. The text with white font describes the responses of the seed illustrated by the transcriptomic analysis.

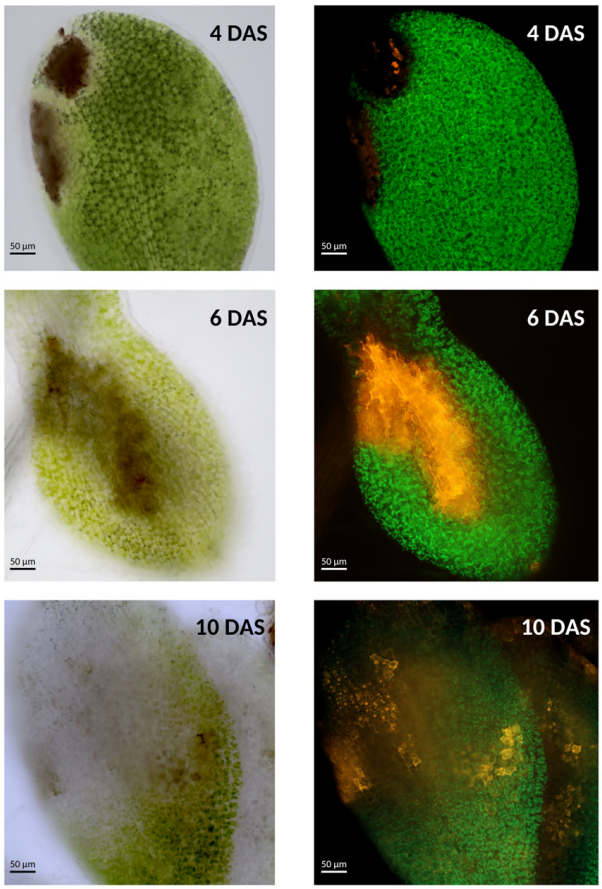


Figure 3. Necrosis profiles at 4, 6, and 10 Days After Sowing (DAS).

5. Conclusions and Future Perspectives

Despite significant progress in seed biology, there are still notable gaps in our understanding of seed immunity. Recent molecular and genetic analyses of *Arabidopsis* interactions with *Alternaria* during germination and seedling emergence have highlighted a particular defense mechanism and adaptive strategies employed when competitiveness is crucial for the survival of both the fungus and the germinating seedling [23]. The *Arabidopsis thaliana*/*Alternaria brassicicola* pathosystem illustrates that the fungus controls the hormonal response, while the seed attempts to kill the fungus by reducing oxygen. This type of model provides a basis for unraveling the intricate molecular dynamics of their interactions. This model not only provides a basis for unraveling the intricate molecular dynamics of their interactions but also represents a valuable framework for understanding the complex mechanisms of pathogen resistance and host response [22,23]. Specifically, further detailing the contribution of GSL metabolism to necrosis symptoms and *Alternaria* colonization in germinating seeds [23] is necessary. This exploration could offer a strategic lever for controlling seed tolerance to necrotrophic attacks and effectively managing transgenerational pathogen propagation.

Arabidopsis is a widely studied model species that presents advantages of a wide diversity, including well-characterized genetic, genomic, and phenotypic resources [65–67]. While *Arabidopsis* may not hold direct economic significance, its close phylogenetic relationship and shared genetic and molecular traits with Brassica species [68] facilitate the transfer of valuable knowledge regarding seed–fungal interactions to benefit Brassica crops. To further explore this, we plan to leverage *Arabidopsis* gene expression profiles using the Weighted Gene Correlation Network Analysis (WGCNA) approach [69]. Future analyses should involve conducting transcriptomic studies on deficient mutants in GSL and camalexin within the context of seeds infected by *Alternaria* during germination. These upcoming investigations aim to unveil co-expression networks, grouping genes based on similar expression patterns and phenotypic contributions. The WGCNA approach would be helpful to identify master regulatory genes controlling seed tolerance and provide candidate genes to search orthologs in brassica crops, which contribute to seed resistance in crops, such *Brassica* vegetables.

The investigation of seed defense mechanisms against pathogenic fungi has become essential in the pursuit of strategies to ensure seed health and mitigate extensive losses in agricultural fields. Comparative genomics will provide deeper insights into plant–pathogen interactions. This knowledge is needed to develop genetic resistance or to better manage diseases in brassica crops.

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