

Progress in the study of pathogenic factors of *Sclerotinia sclerotiorum*

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Abstract. *Sclerotinia sclerotiorum* is a devastating soil-borne plant fungal pathogen that can infect over 600 plant species, including important economic crops such as rapeseed, soybeans, and peanuts. *Sclerotinia*-induced plant diseases are distributed worldwide, and the economic losses caused by *sclerotinia* fruiting bodies are considerable every year. This article combines relevant research results to discuss the research on *Sclerotinia sclerotiorum* from four aspects: the biological characteristics and harm of the fungus, the "two-stage" infection model, the roles of oxalic acid, hydrolytic enzymes, and other five pathogenic factors in the pathogenesis of *Sclerotinia*, and the prevention and control measures of *sclerotinia* fruiting body disease using traditional methods and HIGS technology.

Keywords: *Sclerotinia sclerotiorum*, Pathogenic factor, Prevention and control measures.

1. Introduction

Since the "14th Five-Year Plan", the state has proposed to improve the quality, efficiency and competitiveness of agriculture, and thoroughly implement the action of reducing the amount of pesticides and fertilizers. General Secretary Xi Jinping emphasized at the first session of the 14th National People's Congress: "A strong agricultural country is the foundation of a strong socialist modern country, and meeting the people's needs for a better life cannot be separated from agricultural development."

Sclerotinia sclerotiorum is a devastating soil-borne plant pathogenic fungus that can cause over 60 diseases. Its host range is wide and it can infect over 600 plant species, including important economic crops such as oilseed rape, soybeans, and peanuts[1]. The plant diseases can cause huge economic losses. In the past decade, the infection area of oilseed rape *sclerotinia* disease in China has reached 3.1 million hectares per year, with actual yield loss exceeding 170,000 tons per year, making it the most damaging pest and disease in oilseed rape production[2]. In the United States, direct economic losses from crop diseases caused by *Sclerotinia sclerotiorum* exceed US \$200 million annually[3]. *Sclerotia* has caused huge economic losses in agriculture worldwide and has become an urgent problem to be solved. At present, the pathogenic mechanism of *S. sclerotiorum* has not been fully elucidated, and there are few related studies on the pathogenic factors of *S. sclerotiorum*. Therefore, this paper reviews the pathogenic factors of *S. sclerotiorum*, in order to provide theoretical basis for the prevention and treatment of *S. sclerotiorum*.

2. Biological characteristics of *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary belongs to the phylum Ascomycota, the order Daumomycetes, and the genus *Sclerotium*. *S.sclerotiorum* is a necrotrophic parasitic pathogen, which mainly damages stems, leaves and fruits. *S.sclerotiorum* can have different symptoms in different parts of the plant, but in general it will show highly invasive pathogenic ability after invading the host epidermis, leading to rapid wilting and death of the host plant[4]. The three stages of *S.sclerotiorum* life are composed of ascospores, hyphae, and sclerotia. The vegetative form is white hyphae, germinated from ascospores, with septa, branching and multinucleated, which can be divided into two types: infection hyphae and branching hyphae. The outer layer of the mycelium contains melanin, and when it matures, it forms a black granular sclerotium, through which it can persist for a long time in soil or disease remnants. Under the right conditions, the sclerotium then germinates again, divided into fruiting body germination and mycelium germination. It can germinate into long pedunculated ascus disk, which is composed of colorless rod-shaped ascus and lateral silk fruiting body, ascospores in the ascus, the latter mature from the ascus, attached to the plant surface, germinate into mycelium when conditions are suitable[5].

3. *Sclerotinia sclerotiorum* infection model

Under humid conditions, *S.sclerotiorum* can grow rapidly inside the host tissue, causing necrotic, precocious, or wilting of the host tissue. After killing the host, the fungus can continue saprophytic growth on necrotic plant tissue and form numerous sclerotia in plant residues or soil. Under suitable conditions, they rapidly develop into white dense hyphal masses, and once they stop growing, they mature with dehydration and pigmentation, and the blackened outer surface is resistant to adverse conditions. Therefore, *S.sclerotiorum* can survive in this form for more than 10 years and can cause diseases such as sclerotinia rot, stem rot, and crown rot.

In recent years, the "two-stage" infection model (FIG. 1) was proposed[6], reflecting the complexity of the process of *S.sclerotiorum* infecting plants. *S.sclerotiorum* uses a variety of factors and complex strategies to infect and poison host plants. In the first stage, the tips of complex appressoria release toxins such as oxalate and some hydrolases to digest the epidermis and penetrate the cuticle of plant cells. In the second stage, pathogens rely on toxins (especially oxalic acid) and hydrolytic enzymes to destroy and degrade the cell wall of host cells and other plant tissues, thereby achieving rapid tissue invasion while releasing nutrients to facilitate further pathogen spread[6].

In recent years, Ka Zhang[7] et al. also found that *S.sclerotiorum*, as a dead vegetative parasitic fungus, has a very short vegetative parasitic stage in the early stage of the establishment and colonization of infected plants, which may actually be a semi-living vegetative pathogen. During this period (before the first stage of the "two-stage" infection model), mycelial expansion does not cause host cell necrosis, and transient biotrophic behavior is maintained at the periphery of the colonization site. *S.sclerotiorum* can suppress the host immune response by secreting some non-host-selective toxins and effector proteins to achieve rapid proliferation and prepare for the transition to the necrotrophic parasitic stage. After the successful colonization of the pathogen, the host produces a large amount of reactive oxygen species, and the tissue gradually becomes necrotic, and the pathogen enters the vegetative stage of dead body.

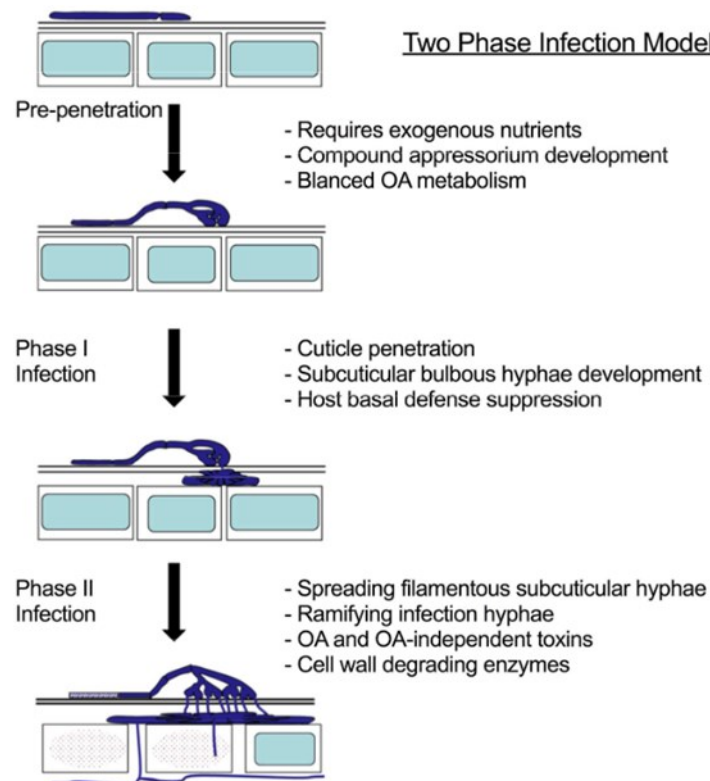


Figure 1. "Two-stage" infection model

4. Pathogenic factors and their pathogenesis

4.1. Oxalic acid

Among all the pathogenic/virulence factors of *S.sclerotiorum*, Oxalic acid (OA) has received the most attention and is considered as a determinant of pathogenicity[8]. OA can play different roles in multiple stages of infection. In the early stage of infection, OA can inhibit oxidative burst and callose deposition, inhibit the burst of reactive oxygen species in host plants, and contribute to the establishment of the vegetative phase of organisms[9]. Subsequently, OA induces the production of reactive oxygen species in the host, which in turn leads to host cell death.

The acidic environment induced by OA accumulation is a key step in the programmed necrosis stage of *S.sclerotiorum*. Oxidoreductase (OAH) catalyzes the oxidation of malonic acid to oxalic acid. The acidic environment generated by OA enhances the activity of hydrolytic enzymes, which weaken the host cell wall structure by chelating calcium ions, reduce the toxicity of calcium ion exposure, trigger cell apoptosis and programmed cell death, and allow the formation of necrotrophic fungal colonies. Additionally, it has been found that OA can manipulate the death fate of host cells, ranging from autophagy related to resistance to apoptosis related to susceptibility.

4.2. Effect proteins

Effect proteins are proteins that have the ability to regulate cell growth, differentiation, and transformation. It can transmit signals through a variety of pathways and activate different intracellular signaling pathways, thereby affecting the physiological and pathological processes of cells. *S.sclerotiorum* can secrete a large number of functionally diverse effector proteins. Some can suppress the immune system of the plant in the early stage of infection, and some can activate the immune response of the host plant and lead to the rapid occurrence of plant tissue necrosis. The symptom is the degradation of the cell wall of the highly active plant, which leads to rapid tissue necrosis, indicating

that the initial osmotic and biotrophic periods are over, and the pathogen then expresses a different set of genes to trigger the programmed cell death of the host cell. For example, effector proteins such as the integrin SsITL and chorismate mutase SsCM1 expressed by *S.sclerotiorum* can help suppress plant defense responses during vegetative growth.

4.3. Hydrolases

Hydrolases are a general term for a class of enzymes that catalyze hydrolysis reactions. Current studies have found that *S.sclerotiorum* infection can induce the expression of a large number of enzyme genes that encode hydrolytic activity, mainly encoding carbohydrate-active enzymes (carbohydrate enzymes). Most of these genes encoding Carbohydrate enzymes are from the Glycoside hydrolases (GH) and Carbohydrate esterases (CE) families, and their expression allows pathogens to infect a wide range of plant hosts. For example, the hydrolytic subtilisin proteinases, which degrade plant cell wall glycoproteins, are thought to play a role in complex apposition penetration and hyphal colonization[10].

4.4. Membrane transport proteins

Plant pathogens must contend with multiple host biochemical defense mechanisms during infection. This can be achieved by avoidance (intracellular growth of pathogens to avoid extracellular phytoalexin), resistance (mutations that alter sensitivity to antimicrobial compounds), or detoxification (modification or degradation of host phytoalexin). Energy-dependent efflux of toxic phytochemicals by membrane-associated transporters is a universal detoxification mechanism that is prevalent in pathogens with a wide host range.

A total of 33 genes encoding ATP-binding cassette (ABC) transporters and 218 Major facilitator superfamily (MFS) transporters were identified in the genome of *S.sclerotiorum*[11]. Extensive studies have proved that ABC transporters are important for the antitoxin ability of pathogenic bacteria to tolerate plants. The Sclerotium homolog protein encoding ABC transporter BcAtr B(SS1G_13659) is up-regulated at the early stage of infection, which reduces the secretion of mycotoxins or promotes the efflux of host phytoprotectin, thereby improving the resistance of plants to Sclerotium.

4.5. Reactive Oxygen Species

Reactive Oxygen Species (ROS) produced by plants are involved in cell proliferation and differentiation, signal transduction and ion transport[12], and are part of the defense response against pathogen attack[13]. *S.sclerotiorum* pathogens induce host production of large amounts of ROS, which can lead to oxidative cell damage of DNA, RNA, proteins, and lipids and trigger programmed cell death. This eventually leads to host necrosis, allowing the pathogen to absorb nutrients from the necrotic tissue. It has been shown that SsNOX1, a gene encoding NADPH oxidase in *S.sclerotiorum*, is expressed during infection, which promotes ROS formation and sclerotium development and is also associated with the production of oxalate, a key pathogenic factor.

5. Measures for the prevention and treatment of sclerotinia caused by *Sclerotinia sclerotiorum*

Sclerotinia rot caused by *Sclerotinia sclerotiorum* is distributed worldwide and occurs from seedling to flowering stage of crops. At present, the control approaches of *S.sclerotiorum* mainly include rational agricultural cultivation and management, pharmaceutical control, breeding disease-resistant varieties, and biological control based on beneficial microorganisms and fungal viruses[14].

5.1. Agricultural control

In the actual control process, agricultural cultivation measures are commonly used by people as the least harmful control method to the environment. Agricultural control measures aim to reduce the number of *S.sclerotiorum* in the soil or to create conditions unfavorable for the development of the disease.

The most common and popular method to control the number of soil sclerotia in agricultural cultivation measures is rotation. Rotating crops susceptible to Sclerotia and non-host crops (not susceptible to Sclerotia) can disrupt the life cycle of Sclerotia, thereby reducing the number of sclerotia

entering the soil every year[15]. Kharbanda[16] et al. showed that tillage measures could reduce the ability of sclerotia to produce ascus disk by burying sclerotia deeply in the soil. Another way to reduce the viability of sclerotia in soil is to control soil water content. Studies have shown that higher soil moisture negatively affects sclerotia survival.

5.2. Chemical control

The application of chemical fungicides plays an important role in the prevention and treatment of sclerotia. Studies have shown that a variety of different types of fungicides can effectively control *S.sclerotiorum*, including aniline pyrimidines, benzimidazoles, bisamides, demethylating inhibitors, quinones exoinhibitors (also known as methoxyacrylate fungicides) and succinate dehydrogenase inhibitors[14].

These fungicides can exhibit different modes of action: aniline pyrimidines can inhibit the reproduction of *Sclerotinia* by interfering with ribosomal RNA synthesis and inhibiting fungal protein biosynthesis. Dimethylamides are thought to inhibit fungal growth by activating the two-component histidine kinase signaling pathway and inhibiting osmotic signal transduction. Benzimidazoles bind tubulin, disrupt microtubule formation and highly inhibit hyphal growth.

But long-term and frequent use of chemical agents can make plants resistant to them. In addition, long-term selective pressure may also lead to resistance in other pathogens. In order to reduce the development of drug resistance and improve the efficacy, different fungicides are usually applied alternately or in combination. Although chemical fungicides can effectively control the mycelial growth of *S.sclerotiorum*, they have little effect on the survival of sclerotia in the soil, and long-term use has caused great pressure on the ecological environment [14].

5.3. Biological control

5.3.1. Biocontrol microorganism

The types of biological control agents mainly include fungi, bacteria, mycoviruses, plant extracts and organic amendments, etc. The main strategies for biological control of sclerotia are to inhibit mycelium growth and sclerotium germination, reduce sclerotium formation, reduce the virality of *Sclerotium* and induce the resistance of host plants[14].

For the biological control of *S.sclerotiorum*, the most commonly used fungus is *Cosp. scutellaria*, which can both colonize the hyphae of *S.sclerotiorum* and destroy its sclerotium. When the fermentation filtrate of ZS-1TN1812 mutant was sprayed on rapeseed leaves, the infection of *S.sclerotiorum* was significantly inhibited, indicating that the antifungal substances (AFS) produced by ZS-1TN1812 strain could be used as potential biopesticides for the control of *S.sclerotiorum* in rape. *Trichoderma* can be heavily parasitized on the hyphae and sclerotium of *S.sclerotiorum*, and it can secrete cell wall degrading enzymes and destroy cell wall structure [17]. Both fungi have the ability to degrade OA, a pathogenic factor of *S.sclerotiorum*. *Pseudomonas*[18] and *Bacillus* are the potential biocontrol bacteria of scleroderma, which mainly inhibit the germination of *Scleroderma* ascospores by producing antibiotic substances or directly parasitizing them.

5.3.2. Biological control agents

Biological control agents refer to the active ingredients with bactericidal and antibacterial activities extracted from some parts of plant tissues, or biological agents that are processed to control plant diseases after separation and purification of secondary metabolites[23]. It has the advantages of low toxicity, low residue, easy degradation, high biological activity, safety against non-target biosafety and not easy to cause pathogen resistance. The active substances (Balansenate I and II) extracted from the seeds of the leguminous plant *Amaranth* and cuminic acid extracted from the seeds of *cumin* showed a good inhibitory effect on *S.sclerotiorum*.

Although biological control agents have many advantages, they are not enough to control the spread of *S.sclerotiorum* to a certain extent. Only when the number of pathogens is controlled within a certain

range can they effectively inhibit the development of the disease. And in the actual promotion, its cost is high, its stability is poor, the cost benefit time is slow and other problems have not been fundamentally solved.

5.4. *Breeding for genetic disease resistance*

Breeding disease-resistant varieties is the most economical way to control sclerotiosis, which can reduce pesticide use and field management costs, thereby improving the economic returns of crops[14]. With the development of molecular biology, transgenic technology has become an effective way to breed disease-resistant varieties. Through genetic engineering technology, the genes related to *S.sclerotiorum* resistance can be found and transferred into target crops, which can improve the resistance level of crops to *S.sclerotiorum*. When resistance genes such as oxalate-degrading enzymes (OxDC), fungal cell wall degrading enzymes (chitinase), and antimicrobial peptides (LTP) were introduced into crops, or genes that prevent host cell apoptosis were overexpressed, the resulting transgenic lines showed significantly increased resistance to *S.sclerotiorum*.

However, *Sclerotinia sclerotiorum* has strong pathogenicity, and crop resistance to *S.sclerotiorum* is mainly controlled by quantitative trait genes with additive effect[24]. At present, resistant varieties are mainly obtained through the study of single gene. There is still a lack of germplasm resources with high resistance to *S.sclerotiorum*, because there is no germplasm resources with complete resistance to *S.sclerotiorum*. Therefore, breeding resistant varieties is still an important way to control this fungus.

Host-induced gene silencing (HIGS) can also be used to construct resistant cultivars. HIGS is a technology based on RNA interference, which can express artificial RNAi vectors targeting important genes of pathogens (e.g., OAH gene of *S.sclerotiorum* in the host. In the process of host-pathogen interaction, small interfering RNA or double-stranded RNA produced by the host is taken up by the pathogen to silence the expression of corresponding genes of the pathogen. Thus, the pathogenic ability of the pathogen is reduced, and the host can acquire resistance to the pathogen. At present, HIGS technology has been successfully used in the control of pathogens, viruses and nematodes. Recently, Xu[25] et al. have shown that RAS signaling genes can be used as host-induced gene silencing targets to control fungal diseases caused by *Scleroderma*.

6. Summary and Prospect

Sclerotinia sclerotiorum has a wide range of host plants. It deploys a variety of factors and complex strategies to establish and infect host plants, which can reduce the yield and quality of a variety of commercial crops. In the past decade, many genes involved in pathogen development and pathogenesis have been identified. In the two-stage infection model, pathogens may inhibit plant defense responses by producing OA, ROS, CWDEs and other pathogenic factors in complex appressorium penetration or primary invasion hyphae, resulting in host cell death and thus achieve the goal of infection. At present, the research on the pathogenic genes of *S.sclerotiorum* is still incomplete, and it is difficult to establish the relationship between the signaling pathways of different special biological processes. At present, the control of *S.sclerotiorum* mainly relies on agricultural cultivation measures and chemical fungicides, but both of these two methods have certain disadvantages. In particular, the long-term abuse of chemical fungicides has made *S.sclerotiorum* resistant to some commonly used fungicides, resulting in increased agricultural production costs, environmental pollution, affecting the health of humans and animals, and killing beneficial microorganisms.

More powerful methods, such as genome-wide CRISPR, should be developed in addition to traditional reverse genetics research analyses during future studies of *S.sclerotiorum* to enhance research on this economically important fungus. Through the application of quantitative resistance breeding and stage-specific defense phenotypic screening or genome editing technology, it is expected to obtain varieties with certain resistance and safely apply to the prevention and control of sclerotia.

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