Preliminary study on Gramineae resistance to *Sclerotinia sclerotiorum*

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Abstract

Sclerotinia sclerotiorium (Lib.) de Bary is a ubiquitous necrotrophic fungal pathogen capable of infecting at least 408 plant species of 75 families. No highly resistant variety or germplasm is found in hosts including Cruciferae plants. In *Brassica napus*, the disease results in severe yield losses. But in non-host plants such as some Gramineae species, the pathogen is avirulent or weakly virulent developing no lesions or very small ones before dying or forming sclerotia. To understand how these non-hosts resist the pathogen, we inoculated *S. sclerotiorium* mycelium onto the leaves of bamboo, wheat, maize and oilseed rape. Different responses were observed in these plants after 24 hpi (hours post inoculation). No lesion was found on bamboo and maize leaves. Small lesions were observed on wheat, but the lesion size varied among different cultivars. Larger lesions were observed on oilseed rape leaves than any other Gramineaes at earlier time after inoculation. Scanning electron microscope (SEM) clearly showed a layer of membrane glutinous to the bamboo surface, and transverse sections of infected leaves under optical microscope unveiled hyphae penetrating the epidermal cell. The modes that hyphae grew into the leaves were also different between bamboo and oilseed rape. They grew mainly under the oilseed rape epidermal and in the intercellular space, while limitedly in epidermis and mesophyll cells in bamboo leaves. We assumed that surface substance and cell wall composition were obstacles to *S. sclerotiorium* hyphae in non-host Gramineae plants. Further work needs to be done to analyze these compounds in comparison with oilseed rape and interaction between the pathogen and bamboo leaf.

Key words: *Sclerotinia sclerotiorium*, non-host, oilseed rape, interaction, resistance

Introduction

Sclerotinia scleotiorium is a ubiquitous necrotrophic fungal pathogen infecting at least 408 plant species among 75 families (Boland and Hall, 1994), including important crops such as rapeseed, soybean and sunflower. No highly resistant resource was found yet in host plants. However, it is not clear whether some non-host monocotyledonous plants can be infected.

S. sclerotiorium infecting of healthy tissue depends on the formation of an appressorium, which may be simple or complex in structure depending on the host surface (Tariq and Jeffries, 1984). In most cases, penetration is directly through the cuticle and not through stomata. Appressoria develop from terminal dichotomous branching of hyphae growing on the host surface and consist of a pad of broad, multi-septate, short hyphae that are attached by mucilage (Hegedus and Rimmer, 2005). There is no report describing appressoria formation on non-host surface.

The objects of our study are to observing the interaction between non-hosts, Gramineae species (such as bamboo, barley and wheat), and *S. scleotiorium*, and to understanding the resistant mechanism of non-host.

Materials and methods

Sclerotia were collected from infected stem of rapeseed in Wuhan and cultured on PDA. Agar plugs of mycelia were inoculated on detached leaves. The inoculated adult leaves were collected from *Brassica napus cv* zhongshuang No. 9 (reserved by breeders in OCRI); *Rorripa indica* (weed in rape fields); bamboo（*Fargesia dracocephala*）; barley (*Hordeum vulgare*) and wheat line Ye er yan (*Triticum aestivum*, offered by Dr. Qingqin Zhang, Guizhou University); *Avena fatua*; *Beckmannia syzigachne* and *Polypogon monspeliensis* (grass besides the rape fields). Lesion samples were fixed and stained with typan blue (TB) or Toluidine blue (TOB), or prepared for SEM. The transverse sections of infected leaves were stained with TOB before observing under optical microscope. Bamboo and Gramineae grasses were rubbed by carborundum on the upper side of leaves.

Results

Different resistance to infection among species tested

Different resistance to infection were observed among species tested. The tested species can be divided into 3 groups by their resistance: susceptible, which including *Brassica napus cv* zhongshuang No. 9 and *Rorripa indica*; tolerant, which including wheat line ye er yan (data not shown), barley, *Avena fatua* (data not shown), *Beckmannia syzigachne* (data no shown) and *Polypogon monspeliensis*; resistant, which including bamboo (Fig. 1), corn and rice (data not shown).

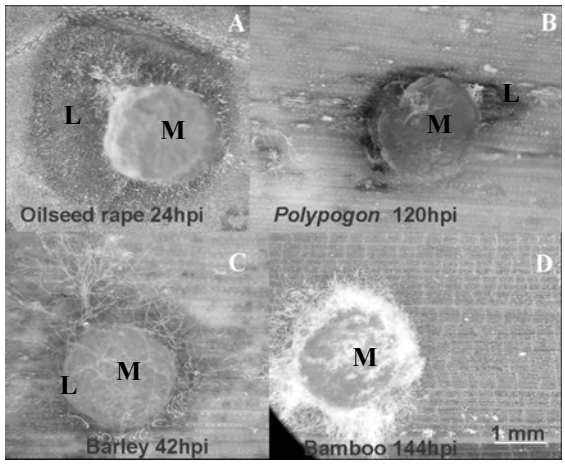

Fig 1 Differences of resistance to infection among species tested. There were big (A) or small (B) lesions developed on inoculated detached leaves. A: *Brassica napus cv* zhongshuang No. 9, 24 hours post inoculation (hpi); B: *Polypogon monspeliensis*, 120 hpi; C: barley, 42 hpi; D: bamboo, 144 hpi with no lesion. M: mycelia plug; L: lesion.

Appressoria on healthy and carborundum rubbed surface

Different size of appressoria was observed on inoculated leaves of different species. Small appressoria were seen on the rapeseed leaf, and bigger ones were found on bamboo and other Gramineae species. The rubbing treatment led to infection of bamboo leaves. The hypha developed more dichotomous branches on rubbed bamboo.

Mucilage material was formed on rubbed bamboo leaves

The SEM showed a layer of mucilage compound formed on rubbed bamboo after inoculation. This kind of material was also found on hypea surface developing on healthy bamboo without white dots. No mucilage was found on inoculated rapeseed leaf before penetration.

Different behaviors after penetration in rapeseed and rubbed bamboo

The pathogen could penetrate into bamboo leaf after carborundum rubbing. But even wounded by rubbing, the haphal growth was different between rapeseed and bamboo. In 24 hpi rapeseed leaves, the pathogen went directly under the cuticle and emerged on the non-inoculated epidermal. While in 24 hpi bamboo leaves, its growth was limited in epidermis and mesophyll cells.

Discussion

In this study, we investigated the interaction between *S. sclerotiorium* and it's Gramineae non-host species. Different resistance are observed among non-host plants. Some are immune to *S. sclerotiorium,* others are tolerant to the pathogen, which suggests that there is diversity of resistance mechanism among non-hosts. Different infection process was also observed among different inoculated species in this study. Bigger and more complex appressoria were found on inoculated bamboo, which supported that there are different disease resistance in non-host. It is interesting to unpuzzle the mechanism of non-host resistance.

Caborundum rubbing of bamboo leaves led to shift from resistance to susceptibility, which suggests that the surface structure is important protection of bamboo and other Gramineae species. Cell wall is the first obstacle to defense the pathogen. Bamboo leaves with intact cell wall and secondary metabolites on the surface can prevent the penetration and infection. More detailed work should be done to discover the biochemical change after inoculation and rubbing treatment.