

Original Research Article

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Study of Plant Pathogen Interaction in Groundnut Challenged with *Sclerotium rolfsii* by Scanning Electron Microscopy

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ABSTRACT

The present investigation was aimed to understand early infection process and plant pathogen interactions involved in tolerance and susceptibility in groundnut challenged with *Sclerotium rolfsii* by Scanning Electron Microscopy (SEM). The histo-pathological changes were recorded at different time intervals in two groundnut genotypes with differential reaction to stem rot disease viz., Cv: "ICGV 86590" (resistant) and Cv: "Narayani" (susceptible). These genotypes were grown in glass house and challenged with stem rot pathogen. The infection process and host-pathogen interactions were examined at cellular level in both resistant and susceptible cultivars at 24, 48 and 72 hours after inoculation (HAI). The SEM observation showed the direct penetration of fungal hyphae through the cuticle was observed within 24 HAI of inoculation in Narayani whereas no sign of mycelial growth was found in resistant genotype ICGV 86590. In the *S. rolfsii* challenged tissues, fungal hyphae were developed in both inter and intra-cellular layers within 48 HAI in Narayani and completely colonization with fungal mycelium was observed within 72 HAI and thereby lead to tissue collapse in susceptible genotype. In contrast, the resistant genotype has no mycelial growth in xylem vessels even at 72 HAI. In Cv. Narayani, after invasion of the fungus, rapid degradation of cell wall occurred in the stem followed by intercellular and intracellular spread of the fungal mycelium was observed. Finally, tissues of the stem lost their integrity and seemed as rotten mass covering with dense mycelium. The SEM study in groundnut clearly demonstrated the difference in histo-pathological responses in resistant and susceptible cultivars while the infection process of *S. rolfsii*.

Keywords

Groundnut,
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Introduction

Stem and pod rot is one of the major constraints in groundnut production as it severely affect the yield and quality of the

produce (Mehan and McDonald, 1990). In India, it occurs in all groundnut growing states and most severe in Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Orissa and Tamil Nadu (Kumar *et*

al., 2013). Yield losses range from 10 to 25% annually. The disease incidence will be more severe reach upto 80% during stem rot epidemics coincides with wet climatic conditions prevailed at pod filling (Akgul *et al.*, 2011).

Stem rot is caused by *Sclerotium rolfsii* Sacc., is a ubiquitous, soil-borne, necrotrophic pathogen with a wide host range of agricultural and horticultural crops belonging to 100 families (Punja *et al.*, 1985). It attacks at any stage of crop growth and affects both above and underground plant parts ranging from roots to shoots whereas stem infection at the collar region is the most common and devastating (Ganesan *et al.*, 2007). The pathogen also attacks pods and diseased pods show characteristics bluish-gray discoloration known as “blue damage” (Madhan *et al.*, 2013) and severely reduce the quality of the seeds and recovery of the produce and thereby reduces yield and fetches poor price. Fungal attack in groundnut triggers a variety of host defense mechanisms including production of phytoalexins and antifungal proteins that degrade fungal cell walls or cause other deleterious effects on the invading pathogen (Zinnat and Robert, 2012) which in turn helps in restraining the pathogen from establishment and further multiplication and thereby results in resistance. In a susceptible disease reaction, once the pathogen comes in contact with the groundnut plant surface, the spores germinates and hyphae spreads both intra and inter cellular growth results in a sponging interaction between the host and the pathogen (David and Brown, 1997). *S. rolfsii* can penetrate into the non-wounded host seedlings directly by the formation of appressoria. It may also gain entry through natural openings such as lenticels and stomata and the disease progresses in both the directions from the sponging point. Smith *et al.*, (1986) reported that the hyphae from germinating sclerotia

ramify over various host tissues within 24-48 hrs following inoculation. The persistence of the pathogen in the soil and wide range of hosts often limits the effectiveness of management of the stem rot disease (Buensanteai *et al.*, 2012). Development of resistant cultivars could be an effective and economical management strategy especially for soil-borne polyphagous pathogens like *S. rolfsii*. Resistance breeding in groundnut for stem rot disease management requires a better understanding of the plant pathogen interactions (Ma *et al.*, 2009) and key factors resulted in resistance reaction. Presently, research on plant pathogen interaction studies of stem rot pathogen and groundnut genotypes are scanty. The present investigation was undertaken to understand the host-pathogen interaction (*sclerotium rolfsii* & groundnut cultivars) during infection processes and thereby formulate effective disease management strategies.

Materials and Methods

Source of plant material

Two contrasting groundnut genotypes with respect to stem rot viz., Cv: “ICGV 86590” and Cv: “Naraynai” were obtained from RARS, Tirupati, India. Cv: “ICGV 86590” is also a Spanish bunch groundnut genotype with medium duration of 120 days with tolerance to biotic stresses like rust, late leaf spot, stem and pod rot where as Cv: “Naraynai” is a Spanish bunch groundnut genotype with short duration (100 days) and good plant architecture but susceptible to most of the biotic stresses including stem rot.

Source of stem rot pathogen

Pure culture of *S. rolfsii* isolate most prevalent in Tirupati was obtained from Dept. of Plant pathology, IFT, RARS, Tirupati to carry out studies described here.

Multiplication of *Sclerotium rolfii* inoculum

The stem rot fungus was multiplied on potato dextrose agar (PDA) media. One matured sclerotial body from pure culture was aseptically transferred to the center of PDA media and the plates were incubated at $27\pm 3^{\circ}\text{C}$. Proper mycelial growth was obtained within 5-7 days and mature sclerotial bodies were formed after 15-20 days.

Challenging groundnut plants with *Sclerotium rolfii*

Contrasting genotypes to stem rot viz., Cv: "Narayani" and Cv: "ICGV 86590" were sown in pots under glass house conditions. The 45 days old groundnut plants were challenged with the 2 cm diameter mycelium disc of *S. rolfii* along with one germinated sclerotial body near collar region (hereafter mentioned as sample). The congenial conditions for *S. rolfii* were maintained at challenged portion by placing absorbent cotton at the site of inoculation. Challenged samples were collected at 24 hrs interval up to three days after inoculation and further used for Scanning Electron Microscopy (SEM) studies.

Sample preparation for Scanning Electron Microscopy (SEM)

Groundnut stem samples at collar region were collected at 24 hrs interval after inoculation up to four days as described by Nandi *et al.*, (2010) with slight modifications and another set of samples after 30 days after inoculation. The samples were sectioned with a thickness of 0.2 to 0.5 mm with a fine edged razor and dried in hot air oven at 50°C for four days. The dried samples were mounted on a SEM aluminum stubs using double-sided adhesive tape and sputter-coated with gold particles. The gold particles were ionized through Ion

coater prior to SEM. The mycelial growth was recorded in challenged and respective control samples of groundnut Cv: "Narayani" and Cv: "ICGV 86590". The photographs were taken under a scanning electron microscope (ZEISS-EVO-18 Special edition).

Results and Discussion

After challenging the groundnut genotypes of both Cv: "Narayani" and Cv: "ICGV 86590" collar region with *S. rolfii* at 0 HAI (hours after inoculation) showed compactness of xylem vessels with no mycelial network (Figure 1A and 2A). In challenged "Cv: Narayani" at 24 HAI, the pathogen reached the xylem vessel of the stem and clear mycelial structures were initiated to form in xylem vessels at 48 HAI (Fig. 1B & C) and in contrast no signs of mycelial growth was found in resistant genotype viz., "Cv: ICGV 86590" (Fig. 2B & C).

Plants possess inducible defense system to withstand the attack of the pathogens. A susceptible disease reaction requires the establishment of a parasitic relationship between the pathogen and the host, once the pathogen has gained entry to the plant (David and Brown, 1997). *S. rolfii* penetrates into the non-wounded host seedlings directly by the formation of appressoria. It may also gain entry through natural openings such as lenticels and stomata. Smith *et al.*, (1986) reported that the hyphae from germinating sclerotia ramify over various host tissues within 24-48 hrs following the inoculation.

Early recognition of the pathogen and activation of resistance responses is often responsible for determining the compatibility or incompatibility of host-pathogen interaction. *S. rolfii* in groundnut is a necrotrophic pathogen showing typical symptoms of vascular wilt pathogens by the growth of mycelium in the xylem vessels and

interfere with translocation of water which in turn leads to wilting of the affected branches (Yadeta and Thomma, 2013).

As the infection progressed, the hyphae of *S. rolf sii* were developed rapidly by inter and intra-cellular colonization of xylem vessels in the stem tissues of Cv: "Narayani". Host cells were disorganized and eventually collapsed. The growth of mycelium in the xylem vessel was more prominent at 72 HAI in Cv: "Narayani" (Fig. 1D).

As xylem vessels were occupied by mycelium in Cv: "Narayani" which hampers solute transport causing wilting in susceptible genotype. In Cv: "ICGV 86590" (resistant genotype) even at 72 HAI also no hyphae were observed in xylem vessels and the stem sections were very clear (Fig. 2D). This genotype effectively curtailed the pathogen entry in initial stages itself.

The hyphal growth in "Cv: Narayani" at 30 days after challenging with *S. rolf sii* displayed complete distorted xylem vessels occupied by fungal hyphae and complete rotting and death of the stem at collar region when compared to 0 HAI (Figure 3A & B). In resistant genotype, "Cv: ICGV 86590" samples doesn't show any hyphal growth in xylem vessels even after 30 days after challenging with *S. rolf sii* (Figure 4A & B). This clearly showed that the genotype "Cv: ICGV 86590" effectively controlled the entry of the pathogen in the initial stages itself and there by resulted in resistant reaction to stem rot.

Garg *et al.*, (2010) reported hypersensitive response associated with resistant genotype against *Sclerotium sclerotiorum* in *Brassica napus* whereas hyphae continued to grow in intercellular and intracellular spaces in susceptible genotype. Similar kind of growth response was found in *Ascochyta rabiei* which produce appressoria that penetrated

both cuticle and stomata in chickpea 3 DAI (Ilarslan and Dolar, 2002). Sunflower challenged with *Sclerotinia sclerotiorum*, the susceptible host cells are completely colonized by mycelium within 48 hours (Davar *et al.*, 2012) which in turn led to tissue collapse. Nandi *et al.*, (2013) also reported mycelial growth of *S. rolf sii* in cowpea xylem vessels at 3 DAI.

Compact xylem vessels with no mycelium hyphae were observed in "Cv: ICGV 86590" even after 72 HAI. Similarly histopathological differences between cucumber cultivars with differential reaction to fusarium wilt showed slower growth of hypha in the vessels of the resistant cultivar when compared to that of susceptible cultivar and hyphae were not observed in the parenchyma cell spaces of the resistant cultivar (Chen *et al.*, 2003).

Sujit kumar (2015) reported the formation of tyloses as a resistance mechanism in groundnut genotype CS19 against *S. rolf sii* at 5 DAI whereas no such cellular responses were seen in susceptible genotype. This kind of structures was not noticed in Cv: "ICGV 86590" tolerant genotype.

The tolerant genotypes halt or restrain the pathogen entry as the initial response and further spread in the xylem vessels will be restricted by the formation of tyloses (Sujit kumar, 2015). In addition to this, chemical defense responses which include biochemical components like cell wall degrading enzymes, production and accumulation of pathogenesis related (PR) proteins also prevents invasion of the pathogen and inhibit its growth.

The present study has detailed the infection processes and pathogen development was both inter and intra cellular in the susceptible host plant ("Cv: Narayani") when compared to resistant genotype.

Fig.1 Scanning electron photomicrographs of Cv: “Narayani” healthy and infected stem tissues stem anatomy showing features of cellular responses at 24 hrs interval up to three days after inoculation (A-D). A. Healthy stem tissue showing the compactness of xylem vessels with no traces of *S. rolfsii* mycelium. B, C. Infected stem anatomy showing distorted xylem vessel at 24, 48 HAI. D. A closer view of xylem tissue occupied by *S. rolfsii* hyphae (white arrow) at 72 HAI

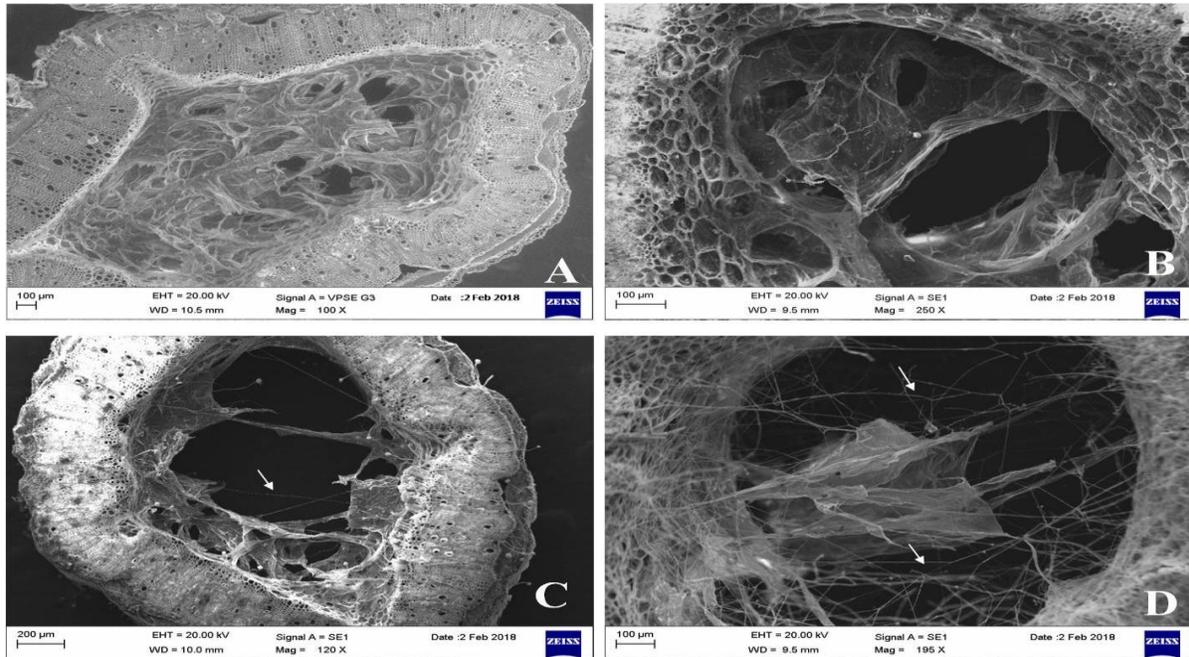


Fig.2 Scanning electron photomicrographs of Cv: “ICGV 86590” healthy and infected stem anatomy showing features of cellular responses at 24 hrs interval up to three days after inoculation (A-D). (A). Healthy stem anatomy showing the compactness of xylem vessels at 0 HAI. Infected stem anatomy showing the compactness of xylem vessels at 24, 48, 72 HAI without hyphal growth (B), (C), (D)

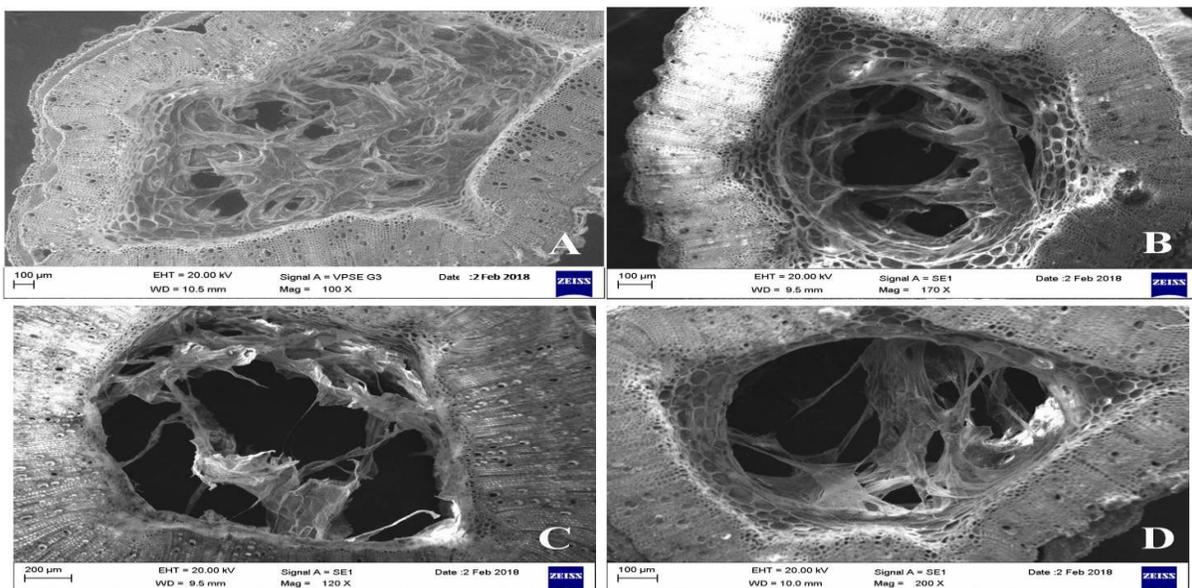


Fig.3 Scanning electron photomicrographs of Cv: “Narayani” healthy and infected stem tissues with *Sclerotium rolfsii* at 0 HAI and 30 days after inoculation. (A). Healthy stem tissue showing no traces of mycelial growth with compact xylem vessels. (B). Infected stem anatomy showing distorted and collapsed xylem vessel at 30 days after inoculation

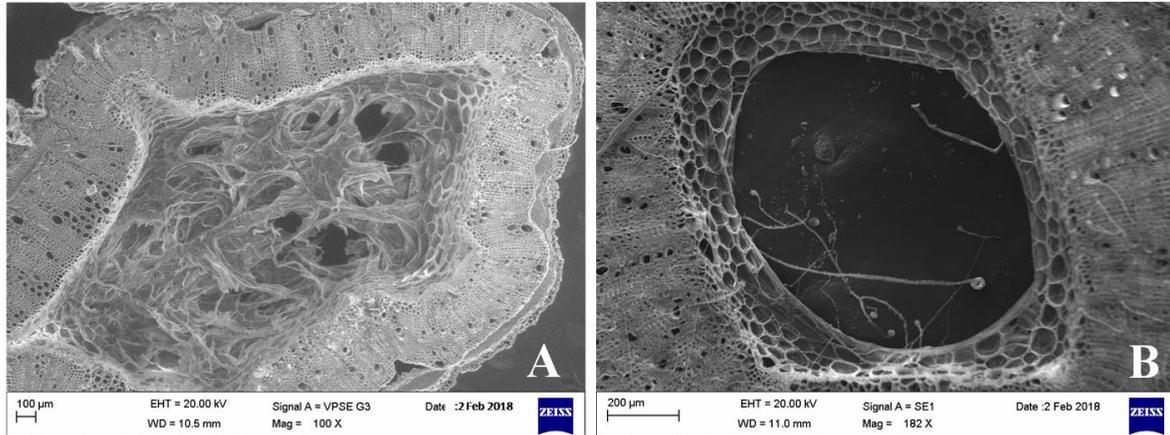
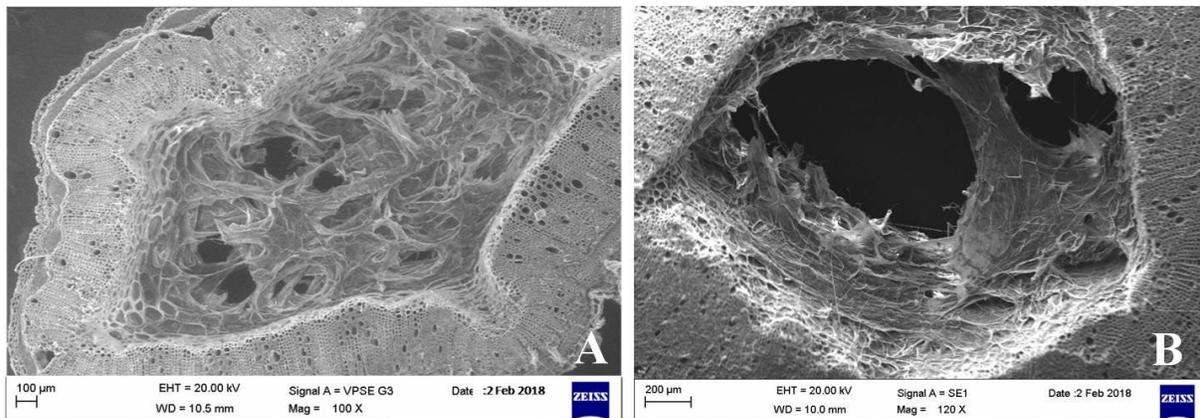


Fig.4 Scanning electron photomicrographs of healthy and infected stem tissues of Cv: “ICGV 86590” at 0 hrs and 30 days after inoculation. (A). Healthy stem anatomy showing the compactness of xylem vessels with no mycelial growth at 0 HAI. (B) Infected stem anatomy without mycelial growth even at 30 DAI (days after inoculation)



Also our studies have depicted the presence of distorted xylem vessels occupied by the fungal hyphae which hampers solute transport causing wilting in susceptible genotype after 72 HAI whereas resistant genotype (“Cv: ICGV 86590”) did not show any traces of mycelial growth. Overall, our studies have demonstrated the difference in histopathological responses both in resistant and susceptible cultivars during infection process to stem rot.

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