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BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN CHICKPEA (CICER ARIETINUM L.) DURING PATHOGENESIS OF COLLAR ROT FUNGI (SCLEROTIUM ROLFSII SACC)

Agriculture Science

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ABSTRACT

Sclerotium rolfsii (Sacc.), causing collar rot (CR) of chickpea is one of the most perilous soil-borne pathogens that parasitize chickpea at a very early stage, therefore, leading to huge yield loss. The present experiment aimed to study the relative changes in defence-related enzymes and phenolics which take place in chickpea (both in resistant and susceptible genotypes) upon CR infection. Emphasis was given to study the changes in total soluble proteins, peroxidase and total phenol content at 7, 14, and 21-days post inoculation (dpi). The experiment was set up by taking moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) genotypes and the experiment was conducted under both inoculated and non-inoculated conditions. The results indicated that total phenol content was significantly higher (3 folds) at 7dpi and then declined gradually upto 21dpi. MR cultivars had higher phenol content than the (MS) and the susceptible non-inoculated control. Total soluble protein content decreased with the days after post-inoculation and this reduction is greater in the susceptible cultivar as compared to the MR cultivars. Total soluble protein content was 3 times higher in the inoculated cultivars than in the in-inoculated control. Peroxidase (POD) activity was also decreased from 7dpi to 21dpi and maximum POD activity was recorded at 7dpi in MR cultivars (12.61 g-1 fresh wt.) than the susceptible one (1.31 g-1 fresh wt.). Scanning electron microscopy (SEM) was also done to compare the extent of the mycelial network within the xylem vessels of inoculated MR, MS and susceptible cultivars.

KEYWORDS

Chickpea, collar rot, total soluble proteins, peroxidase, total phenol and SEM

Chickpea (*Cicer arietinum* L.) is the third most important food legume and belongs to the Fabaceae family grown almost all over the world. India ranks first in terms of chickpea production with 10.21 million tons which constitute 46% of the total pulses production in India (FAOSTAT, 2021). In India chickpea is mainly grown in the winter rain-fed crop and is mostly famous for its high nutritive value (20-23% protein) along with its content of vitamins, minerals, and fibres (17.4%). It could be a good alternative source for animal proteins due to the presence of easily digestible proteins (Hossain et al., 2010), unsaturated fatty acids and β-carotene (Jukanti et al., 2012).

Despite the immense potential, the yield of chickpeas is challenged by several biotic and abiotic factors (Pande et al., 2011). Among biotic stresses, collar rot (CR) pathogen *Sclerotium rolfsii* parasitizes the crop at a very young stage near the root and collar region thus producing CR symptoms accompanied by wilting and yellowing, rendering 55-95% mortality in Indian conditions (Sharma and Ghosh, 2017) and crop loss is reported within 30 days after sowing under conducive environment (Tamang et al., 2021). Management of CR is quite challenging because of the wide host range of the pathogen, and its survival potential as a facultative parasite. Therefore, the best way to manage the disease could be the use of disease-resistant varieties. But, till now no varieties showed truly resistance to this disease.

The first line of defense exhibited by the host against invading pathogens is the hypersensitive response (HR). It is associated with a highly coordinated and integrated set of metabolic activities which are instrumental in impeding further ingression of the pathogen and a variety of novel proteins and secondary metabolites are produced (Nandi et al., 2013). This wide array of proteins and antifungal secondary metabolites act as a determinant factor for the host to become either resistant or susceptible.

Considering the above facts in mind, an experiment was designed to unveil biochemical changes that took place within chickpea upon inoculation of *S. rolfsii.* A few important defence-related biochemical compounds like total phenol, total soluble proteins and peroxidase (POD) were considered and quantified to understand differential responses produced by resistant and susceptible cultivars of chickpea upon CR infection. The experiment was further reinforced through scanning electron microscopy (SEM) to determine the ingression of the pathogen inside the host of resistant and susceptible cultivars. Hence, the present study would provide a holistic insight into understanding the defense mechanism in chickpea against CR which will eventually help to develop sustainable disease management strategies.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the pots at the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia in the year 2018-19 and 2019-20.

The host

Seeds of 13 different chickpea cultivars showing differential reactions against CR infection under field conditions were chosen for study namely, Anuradha (MS), FLIP09-194C (MR), XIIth77-S2 (MR), FLIP10-81C (MR), FLIP09-436C (MR), FLIP10-6C (MR), FLIP10-70C (MR), FLIP07-314C-S7 (HS), FLIP09-112C (S), XIIth86-S5 (HS), FLIP10-19C (S), FLIP09-250C (S), and FLIP07-314C-S6 (HS).

The seeds were collected from AICRP on Chickpea, BCKV. Among the chosen cultivars, *Anuradha* is the most popularly cultivated variety under West Bengal conditions.

Isolation of the Pathogen

The pathogen *Sclerotium rolfsii* was isolated from the diseased chickpea plants showing typical CR symptoms. Pure culture of *S. rolfsii* was maintained aseptically in the petri plates containing sterilized PDA medium and was incubated at 25±2°C.

Inoculation of chickpea cultivars by the pathogen

The seeds of the cultivars were grown in six inches pot in sterilized soil and 10 days old seedlings were inoculated at the collar region by the fungal mycelium.

An active growing culture of *S. rolfsii* (fourth day old) was cut into a 2 mm disc and wrapped lightly with moist absorbent cotton taped around the pricked collar region of the chickpea. Un-inoculated plants were used as control. Both the inoculated and un-inoculated plants were kept at temperature 28^{0}C and 95% RH under controlled conditions with 12/12-day night cycle.

Experimental design
The experiment was laid out in Randomised complete block design RCBD with three replications and 13 genotypes. The trial was performed under laboratory conditions following artificial inoculation and in each set of experiments un-inoculated plants act as a control. The data was collected at 7, 14, and 21 days after inoculation. Infected tissues (surrounding collar region) along with roots were considered under study.

Biochemical assay
Infected tissues (surrounding collar region) along with roots were collected separately from randomly the chosen plants at 7, 14 and 21days post inoculation (dpi) and frozen immediately in liquid nitrogen and stored at - 20°C. These tissue samples were used to isolate the major biomolecules for assessing their activity.

Extraction of Antioxidant Enzymes
The root tissues (500 mg fresh weight) were ground in 0.1 M phosphate buffer (pH 7.0, containing 0.5M EDTA) in a pre-chilled mortar and pestle, and the homogenate was filtered through four layers of cheesecloth. The filtrate was centrifuged at 15000 X g at 4°C for 30 min and the supernatant was used as the source of enzyme.

Activity Assay of Peroxidase (EC 1.11.1.7)
Peroxidase activity was determined according to the procedure reported earlier by Kar and Mishra (1976) with few modifications described herewith. The peroxidase assay mixture was made with 2 ml 0.1 M of phosphate buffer, pH 7.0, 1 ml 0.01 M of pyrogallol ($C_6H_6O_3$), 1 ml of 0.005 M hydrogen peroxide (H_2O_2), and 1 ml of the 2 times diluted enzyme extract (1:2 ratio dilution). The reference cuvette contained an equal volume of boiling inactivated enzyme and an assay mixture of peroxidase containing pyrogallol. Incubation was done for 5 min. at 25°C after stopping the reaction by adding 0.5 ml of 5% (v/V) H_2SO_4. Absorbance due to purpurogallin formed was recorded at 420 nm. One unit of peroxidase activity was calculated as the amount of the enzyme that caused a change of 0.01 absorbance unit per minute under the present assay condition and it was expressed as a Unit per gram fresh weight (unit g-1 fresh wt).

Isolation and quantification of total phenols
The total phenol content in the fresh root tissue was extracted using the method described by Vinson et al. (1998). 0.1g fresh root samples were ground and extracted with 15 ml of 1.2 N HCl in 50% aqueous methanol by shaking in a water bath at 90° C for 2 hours. After cooling, the extracted material was centrifuged at 10,000 rpm for 30 minutes. The supernatant was decanted off in a beaker and evaporated to dryness. The dried content was diluted to a suitable volume, which was analysed using 0.5 ml Folin Ciocalteau Reagent (FCR). Total (conjugated plus un-conjugated) phenol content in triplicated samples was determined by taking absorbance at 650 nm against the reagent blank and a standard curve made from gallic acid. The amount of total phenol was expressed as mg gallic acid equivalent per gram fresh weight (mg g-^{1} fresh wt).

Isolation and quantification of total soluble proteins
500 mg of root material of each cultivar was extracted and centrifuged at 10,000 rpm for 15 min with 1 ml of 0.1 M Tris buffer. The supernatant was used to precipitate the protein by following TCA-Acetone Precipitation Method described by Damerval et al. (1986) and Chatterjee et al. (2012) with some modifications. Supernatant was kept overnight with freshly prepared 2 mL of 20% TCA, in cold acetone. Following precipitation, the set was again centrifuged at 10,000 rpm for 15– 20 min at 4^{0}C and the supernatant was discarded. The pellet thus was obtained and washed twice in ice-cold acetone and the washed pellet was dissolved in 5 ml of 0.1 N NaOH, stirred well and centrifuged again for 15 min. The supernatant was saved as total soluble and was then estimated by the method of Lowry et al. (1951) using Folin Ciocalteau Reagent (FCR). The amount of protein was calculated by taking absorbance at 660 nm by using a spectrophotometer (Shimadzu UV-1800) against the blank. The protein content in triplicated samples was calculated concerning the standard curve for bovine serum albumin and expressed in mg g^{-1} fresh wt.

Sample preparation for scanning electron microscopy (SEM)
For SEM study from each group of genotypes showing MR (cv. XIIth77-S2), MS (cv. Anuradha) and S (cv. FLIP10-19C) reactions against CR were selected and grown under greenhouse conditions 7 days old seedlings were selected for inoculation and the infected region were taken for study 7, 14, and 21days post inoculation (dpi). The infected region was transversely cross-sectioned at 0.2 to 0.5 mm thickness with a sharp razor and used as the sample for SEM.

Sectioned samples were dehydrated at acetone for 3 minutes. These dehydrated samples were transferred to Quorum K850 CPD (Critical point drying) machine with CO_2 gas at a critical point temperature 35^{0}C. Fully dried samples were taken at aluminium stubs with carbon tape. Gold coating was done for 135 seconds at Quorum SC7620 sputter coater. Gold coated samples were analysed in Carl Zeiss Gemini 450 (Gemini 2) FESEM.

Data analysis
The data were analysed through MS Excel (level of significance, interaction effects and ANOVA) and graphs were prepared by data visualization software name- GraphPad Prism, Version 9.3 of Prism 9.

RESULTS
In all the cases total phenol, total protein and peroxidase production was found to be higher in the inoculated conditions than in its non-inoculated counterparts. Moreover, there was a non-significant change in the content of these products upto 21 days when considered the healthy non-infected condition. Therefore, a comparative study was undertaken between the cultivars of chickpea showing differential reactions upon inoculation with collar rot pathogen (only inoculated condition) and discussed hereby.

Total Phenol
The greater changes in the accumulation of phenol were recorded in the inoculated cultivars rather than the uninoculated healthy ones. The changes in phenol content at different stages of disease development in different cultivars are presented in (Table 1). Diverse cultivars showed a differential pattern in the amount of phenol present at different dpi expressed in mg g-1 fresh wt. Mean phenol content at different dpi varied significantly among the cultivars differential reaction. At 7dpi highest phenol content was measured in FLIP09-436C (2.34) followed by XIIth77-S2 (2.30) and FLIP10-81C (2.21) though their differences were statistically non-significant except for the FLIP09-436C while susceptible cultivar FLIP09-112-C contained the lowest value of total phenol (0.54 mg.g-1.fr.wt) that was statistically at par with XIIth86-S5 (0.61) and FLIP07-314C-S6 (0.57). Phenol content was statistically significant in FLIP314C-S7 (1.71) followed by FLIP10-19C (0.69), FLIP09-250C (0.76) and Anuradha (0.68) but later three genotypes did not show any significant differences. 3-fold increases in total phenol content were observed in moderately resistant cultivars over the highly susceptible cultivars (Table 1).

Among the different infection stages, phenol content significantly varied between 0.20 and 2.34 mg.g^{-1}.fr.wt. The general trend reflected a steady increase in phenolics accumulation in respect to Anuradha at 7dpi and at the subsequent dpi phenol content decline in all the cultivars and ultimate phenol content (at 21dpi) remained higher in MR cultivars in comparison to susceptible cultivars (Fig. 1).

The total phenol content decreased at 14dpi in comparison to 7dpi and in most of the cultivars, their difference was statistically at par. At 14dpi significantly highest and lowest phenol content was recorded in XIIth77-S2 (1.93 mg.g^{-1}.fr.wt) and FLIP10-19C (0.44 mg.g-1.fr.wt) respectively. Among the MR cultivar significantly lowest phenol content was documented in FLIP09-194C (0.96 mg.g^{-1}.fr.wt).

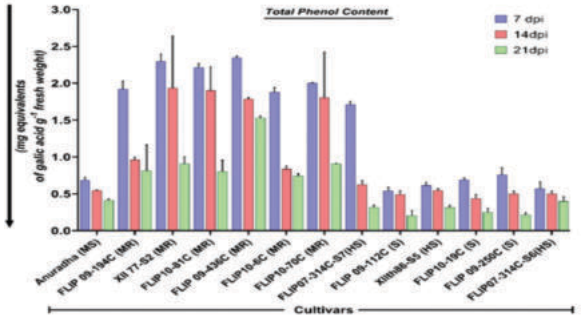

Figure 1. Comparative changes in total phenol content among different cultivars of chickpea at different days post inoculation (dpi) with CR pathogen

Table1. Comparative changes in total phenol, peroxidase and total soluble protein content among moderately resistant and moderately susceptible, and susceptible cultivar of chickpea at different days post inoculation (dpi) with collar rot pathogen dpi = days post inoculation. Same English letter followed by mean are not significantly differ from each other

Cultivars	Total Phenol mg g-1 fresh wt				Peroxidase unit g-1 fresh wt				Total soluble protein mg g-1 fresh wt			
	7dpi	14dpi	21dpi	Mean	7dpi	14dpi	21dpi	Mean	7dpi	14dpi	21dpi	Mean
FLIP09-194C (MR)	1.92 cd	0.96e	0.81 bcd	1.23 b	11.75 a	7.14 ac	6.58 bcde	8.49 ab	2.83 c	2.46 a	2.31a	2.53 a
XIIth77-S2 (MR)	2.30 ab	1.93 a	0.91 bc	1.72 bc	12.46 a	7.99 a	7.53 a	9.33 a	2.98 a	2.38 a	1.98 a	2.45 a
FLIP10-81C (MR)	2.21 b	1.90 ab	0.80 cde	1.64 bcd	9.82 c	7.43 a	6.34 acd	7.86 ab	2.47 a	2.11 b	1.48 b	2.02 b
FLIP09-436C (MR)	2.34 a	1.78 abcd	1.53 a	1.89 cde	10.35 b	7.94 a	6.89 ghi	8.39 ab	2.28 b	2.01 b	1.78 b	2.03 b
FLIP10-6C (MR)	1.88 d	0.83ef	0.74 cde	1.15 bdf	12.11a	7.85 a	7.10 efgh	9.02 ab	2.45 a	2.13b	1.57 b	2.05 b
FLIP10-70C (MR)	2.00 c	1.80abcd	0.90 b	1.57 bcdefg	12.61a	7.46 a	7.33 def	9.13 a	2.15 b	1.88 c	1.59 b	1.87 b
Anuradha (MS)	0.68e	0.54 ef	0.41 f	0.54 a	1.41 d	1.31 d	1.26 j	4.47 c	0.98 c	0.96 e	0.94 c	0.95 d
FLIP09-112C (S)	0.54 f	0.49 ef	0.20 h	0.41 ah	8.00 c	7.28 a	5.38 i	6.89 b	1.46 c	1.29 d	0.99 c	1.25 cd
FLIP10-19C (S)	0.69 e	0.44 f	0.25 fgh	0.46 ah	8.63 c	6.29 c	4.66 fghi	6.53 b	1.76 b	1.49 d	1.02 c	1.43 c
FLIP09-250C (S)	0.76 e	0.50 ef	0.21 h	0.49 ah	8.03 c	6.92 ac	5.45 hi	6.8 b	1.07 c	0.99 e	0.78 c	0.98d
FLIP07-314C-S7(HS)	1.71 g	0.62ef	0.32 fgh	0.89 abfh	8.74 c	6.75 a	5.14 i	6.88 b	1.98 b	1.36 d	1.12 c	1.49 c
XIIth86-S5 (HS)	0.61 f	0.54 ef	0.31 fgh	0.49 ah	8.77 c	6.55 b	5.81ac	7.04 b	1.38 c	1.15 e	0.89 c	1.14 d
FLIP07-314C-S6(HS)	0.57 f	0.50 ef	0.40 fg	0.49 ah	7.98 c	6.24 c	4.59 acd	6.27 b	1.25 c	1.01e	0.78 d	1.01d
LSD (p=0.05)	0.11	0.48	0.17	0.58	1.85	1.37	0.92	1.82	0.58	0.23	0.33	0.26
CV%	4.71	8.66	17.66	13.26	11.19	11.73	9.40	14.72	17.75	8.36	14.55	9.46

At 21dpi, MR variety FLIP09-436C (1.53 mg.g^{-1}.fr.wt) showed the highest concentration of phenol followed by FLIP10-70C (0.90 mg.g-1.fr.wt) and XIIth77-S2 (0.91 mg.g-1.fr.wt) and their differences were statistically significant except the latter two. Significantly lowest phenol content was observed in FLIP09-112C (0.20 mg.g-1.fr.wt) followed by FLIP09-250C (0.21 mg.g^{-1}.fr.wt) and their differences were statistically insignificant. All the other genotypes under susceptible reaction showed statistically insignificant variation in their phenol content in comparison to the moderately susceptible Anuradha. Rather, moderately resistant genotypes recorded significant variation.

Peroxidase (POD) assay

Peroxidase is another important component involved in the production and release of active oxygen species which play various roles in reducing pathogen activity directly or indirectly Passardi et al. (2005).

The activities of peroxidase increased in root tissues of all the varieties at 7dpi and declined thereafter in subsequent stages (Table 1) irrespective of whether the plants were susceptible or tolerant. Significantly highest POD activity was observed in moderately resistant genotypes as compared to the susceptible genotypes. Results showed a decrease in peroxidase activity from 7dpi to 21dpi and maximum POD activity at 7dpi was recorded in FLIP10-70-C (12.61 unit g^{-1} fresh wt) followed by XIIth 77-S2 (12.46 unit g^{-1} fresh wt and FLIP10-6C (12.11 unit g^{-1} fresh wt) and their differences were not statistically significant. The trend showed a maximum upsurge of POD in moderately resistant genotypes rather in susceptible genotypes in all stages. Upon infection, peroxidase activity increased by 108.72 to 75.84% in the roots of MR varieties, whereas, in the susceptible cultivars it ranges from 57.49 to 40.26%, respectively. In susceptible to highly susceptible genotypes the range of POD vary from 6.27 to 7.04 unit g^{-1} fresh wt and their differences were statistically at par. In moderately susceptible cultivar Anuradha significantly lowest increase of POD concentration was observed i.e 4.47 unit g^{-1} fresh wt (Fig. 2).

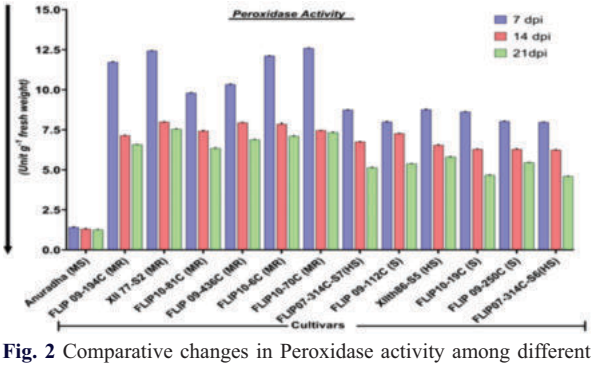

Fig. 2 Comparative changes in Peroxidase activity among different cultivars of chickpea at different days post inoculation (dpi) with CR pathogen

Total soluble protein

Total soluble protein content was found to vary considerably among the cultivars under inoculated conditions on different days post inoculation as presented in Table 1. Among the cultivars, XIIth77-S2 hold a significantly higher amount of total soluble proteins content (2.98 mg.g^{-1}.fr.wt.) and it was at par with cultivar FLIP10-81C (2.47 mg.g^{-1}.fr.wt and FLIP10-6C (2.47 mg.g^{-1}.fr.wt). Moderately susceptible cultivars contained the lowest level of total soluble proteins ranging from 1.07 to 1.98 mg g^{-1} fr wt. FLIP09-250C contained the lowest level of total soluble proteins which were at par with FLIP07-314C-S6 and FLIP09-112C. At different infection stages, the total protein content decreased significantly from 2.98 to 1.98 mg.g^{-1}.fr.wt.in the moderately resistant cultivar whereas in susceptible cultivars it reduced from 1.07 to 0.78 mg.g-1.fr.wt. Total soluble protein contents drastically reduced in XIIth77-S2 from 7 to 21dpi i.e 2.98 to 2.38 to 1.98 mg.g^{-1}.fr.wt respectively rather decrease gradually in FLIP09-194C i.e 2.83 to 2.46 to 2.31 mg.g^{-1}.fr.wt.

The total soluble protein content significantly declined with the advancement of pathogen infection. Moderately resistant and moderately susceptible cultivars had less reduction of total soluble proteins, while susceptible to highly susceptible cultivars had a greater reduction in total soluble proteins.

Inoculated chickpea cultivars showed significantly higher (2.5 fold increase) total soluble proteins content (1.01 to 2.53 mg.g^{-1}.fr.wt) when compared among highly susceptible to moderately resistant one while moderately susceptible variety Anuradha showed the static presence of total soluble protein at different days after post inoculation (Fig. 3).

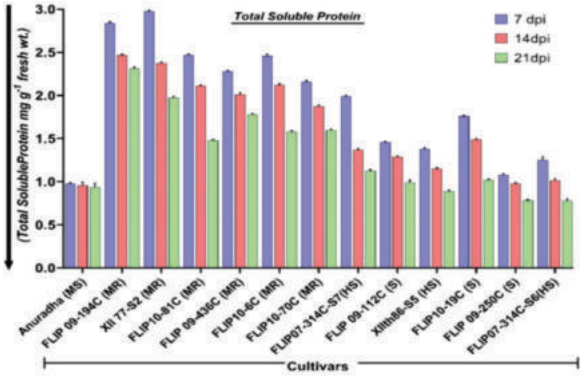

Fig. 3 Comparative changes in Total soluble protein content among different cultivars of chickpea at different days post inoculation (dpi) with CR pathogen

Scanning electron microscopy

The histo-pathological changes took place during host pathogen interactions at different time interval were recorded and photograph captured through SEM (Figs. 4, 5 and 6). The phenomena were observed at cellular level in both moderately resistant and susceptible genotypes at 7, 14, 21 and 30dpi though at 30dpi complete degradation of tissues happened in susceptible genotypes therefore no SEM could be performed.

Scanning electron microscopy revealed both intra and inter-cellular growth of fungal mycelium inside the host and the disease spread both direction from the point of infection. At 7dpi infection reached upto xylem vessels but, in case of moderately resistant genotypes the mycelia present at the periphery of the cell wall whereas, in susceptible genotypes fungal mycelium invaded the internal tissue and occupy the cell to a greater extend (Fig. 4a and 5a).

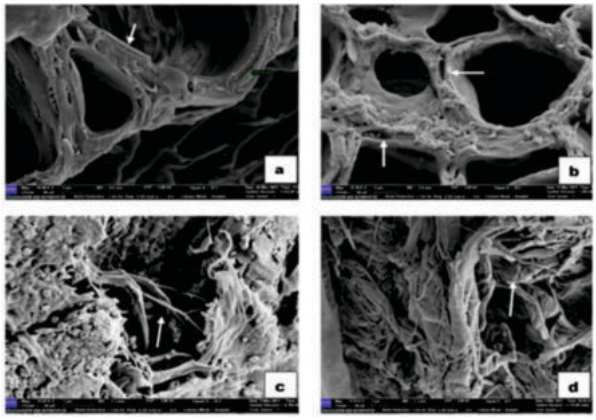

Fig. 4 SEM photograph shown compatible (cv. FLIP10-19C) interaction between Sclerotium rolfsii and chickpea taken at 7dpi (a), 14dpi (b) and 21dpi (c) and 30dpi (d)

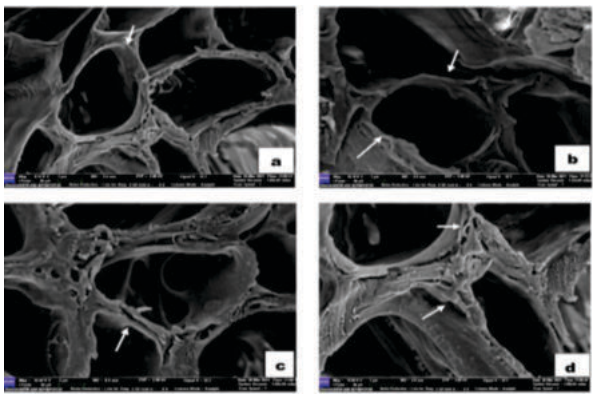

Fig. 5 SEM photograph shown incompatible (cv. XIIth77-S2) interaction between Sclerotium rolfsii and chickpea taken at 7dpi (a), 14dpi (b) and 21dpi (c) and 30dpi (d)

At 14dpi, Fig. 4 showed rapid progression of the hyphae of *S. rolfsii* into the xylem vessels referred inter and intra-cellular colonization and clogging of almost 50% (Fig. 4b). In the moderately resistant genotype progression of hyphae also observed but rate of advancement was less prominent than the susceptible one (Fig. 5b and 6c).

As a consequence of clogging up of xylem vessels in susceptible cultivar hampered solute transport causing wilting in susceptible genotype (Fig. 4d). However, the moderately resistant genotype managed to withstand the infection expressed by its phenotypic appearance and supported by SEM (Fig. 5c and 5d) which showed less occupied tissues inside vessels.

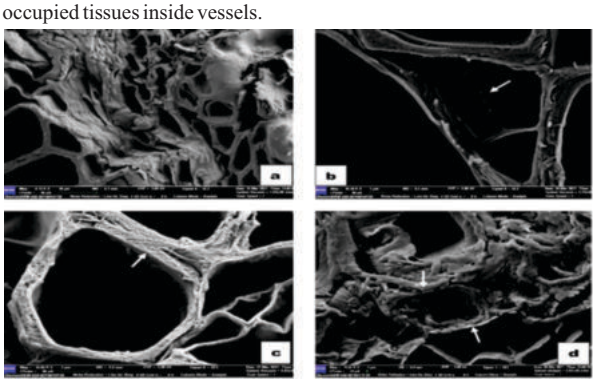

Fig. 6. SEM photograph shown moderately susceptible (cv. Anuradha) interaction between *Sclerotium rolfsii* and chickpea taken at healthy (a), mycelia passing from one xylem vessel to neighbouring at 7dpi (b) and 14dpi (c) and 30dpi (d)

Complete distortion of xylem vessels observed in susceptible genotype at 21dpi of *S. rolfsii* invasion exposed entire cells occupied by fungal hyphae leads to complete rotting and death of the cells (Fig. 4d) as compared to the MR genotype (Fig. 5c and 5d) which showed restricted growth of the fungus inside the xylem. It showed in MR and MS genotypes effectively delayed the pathogen invasion initiated upon the entry of the pathogen and colonize the host tissue at a slower rate as showed up in Fig. (6c and 6d) than the susceptible cultivar.

In the susceptible genotype, tissues of the infected region lost their integrity, became disorganized and eventually collapsed and appeared as rotten mass covering with dense mycelium. The SEM study in chickpea clearly demonstrated the difference in histo-pathological responses in moderately resistant and susceptible cultivars while challenged with *S. rolfsii*.

DISCUSSION

CR is one of the most alarming diseases of chickpeas. During infection, the pathogen brings about several metabolic or biochemical changes inside the host that are highly instrumental in impeding further pathogen ingression of the pathogen inside the host. It includes a variety of novel proteins and secondary metabolites, phenolic compounds etc. This research emphasised to determine the relative changes of total phenol, total protein and peroxidase enzymes in chickpea (both in MR, MS and S genotypes) upon *S. rolfsii* infection at 7, 14, and 21 days post-inoculation and its correspondence SEM study was performed to identify the histopathological changes that took place inside the host varied in disease reaction.

Overall observation established the fact that total phenol content was higher (3-fold) in moderately resistant cultivars than in the susceptible cultivars of chickpea infected with *Sclerotium rolfsii* but decrease gradually with the days after inoculation. The concentration of total phenol was recorded highest at 7dpi then decrease gradually upto 21dpi. This decline of total phenols with the progression of the disease in both moderately resistant and susceptible plants may lead to the termination of hypersensitive reactions (Sarma et al., 2003). Observation in the current study indicated that the rate of phenol accumulation was lower in susceptible than in moderately resistant cultivars with respect to moderately susceptible (Anuradha). This result is in corroboration the report by Datta and Lal, 2018. Additionally, these research results are in agreement with the findings suggested by Rathod and Vakharia (2011); Khan et al. (2005); Singh et al. (2003) who reported that there was an increase in phenolic content of susceptible and moderately resistant cultivars of chickpea upon inoculation with the *F. oxysporum, f.sp. ciceris*. Aravind and Brambhatt (2018) reported higher content of total phenol in the resistant genotypes than the moderately resistant cultivars of okra when infected by root and collar rot caused by *Macrophomina phaseolina*.

The study revealed POD activity was greater in the moderately resistant inoculated cultivars than the susceptible cultivars but decrease gradually with the days after infection that has been already validated earlier by Datta and Lal (2018). A similar response was recorded by Nandi et al. (2013) in *S. rolfsii* infected cowpea, *Fusarium* infected chickpea by Rathod and Vakharia (2011). Peroxidases mainly catalyses the final polymerization step of lignin synthesis and therefore may be directly associated with conferring resistance. It participates in phenyl propanoid metabolism induced by pathogen infection and plays an important role in reinforcing cell wall during biotic stress by polymerising the lignin precursors and by cross-linking structural cell wall proteins to polysaccharides and polyphenols to form a dense network around the vicinity of the infection sites Van de Rhee et al. (1994).

Ferreira et al. (2006) reported proteins are major weapons in a plant–pathogen open warfare produced by both organisms. Similarly, chickpea plants when exposed to *S. rolfsii* showed highest total protein content in moderately resistant cultivars while susceptible cultivars showed the lowest. Generally, total protein content is reduced as the infection proceeds both in susceptible and moderately resistant cultivars. This may happen because of amino acids being utilized by the fungus for their development within the disease plants.

These results are in corroboration the findings reported by Kumari et al. (2020) in chickpea-*S.rolfsii* pathosystem; by Awan and Sohain (2018) in tomato – *Alternaria solani* pathosystem. A different class of proteins e.g. PR proteins involved in the host defense mechanism (Hameed et al., 2017) and due to the upregulation of both i.e. the activation of the host defense and the pathogen attack - the infected plants may show a high protein percentage. In the present study same trend has been observed. It has also been reported that protein contents were significantly decreased in symptomatic plants of chickpea genotypes infected by a phytoplasma (Nasir et al., 2017).

SEM study has been done by several scientists to decode host-pathogen interaction at the cellular level. Rajasekhar et al. (2019) disclosed SEM of groundnut differential host response while challenged with *Sclerotium rolfsii.* Garg et al. (2010) reported hypersensitive response of resistant genotype in *Brassica napus* against *Sclerotium sclerotiorum* whereas continuous growth of hyphae inter and intracellularly within the susceptible genotype. Davar et al. (2012) performed SEM in sunflower - *Sclerotinia sclerotiorum* interaction and revealed that the susceptible host to be completely colonized by mycelium within 48 hours leads to tissue collapse. Nandi et al. (2013) also published SEM of mycelial growth of *S. rolfsii* in cowpea xylem vessels at 3dpi.

CONCLUSION

This study envisioned a variety of complex mechanisms involved in the induction of plants defense induced by pathogen infection thereby resulting in a hypersensitive reaction that confers resistance to plants. It includes biosynthesis and accumulation of different metabolites and constitutive proteins that are directly or indirectly related to the plants defense response. In this study, the interaction between *S. rolfsii* and chickpea cultivars (susceptible and moderately resistant) were studied and the differential defense responses among the cultivars were justified with SEM study.

Root traits play a major role in pathogen tolerance in natural environments. In compatible interaction between *Sclerotium rolfsii* and chickpea, the pathogen grew intercellularly in the root cortex, reached the xylem, and progressed upwards in the stem xylem ultimately interfering in water uptake (as shown in SEM Fig 4). However, incompatible interaction only occupies the intercellular spaces of the root cortex failing to reach the xylem (Fig. 5). When moderately resistant genotypes under study was challenged by the pathogen it suffered both way due to pathogen infection like thickening of the xylem vessel and gradual clogging up of the vessel (Fig. 6).

This study disclosed the changes in the behaviour that took place inside the host upon pathogen infection along with the comparative changes that demarcate resistant cultivars from the susceptible ones. Here, evidence of histopathological deviations had also been documented through scanning electron microscopy that took place due to pathogen infection inside the moderately resistant and susceptible cultivars. Thus this study provides an overall comprehensive idea that contributes a step towards the understanding of host-pathogen interaction.

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