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SDS-PAGE-BASED DIFFERENTIAL PROTEIN EXPRESSIONS OF SOYBEAN GENOTYPES IN RESPONSE TO *Sclerotium rolfsii* INFECTION

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ABSTRACT

Sclerotium rolfsii is an important soil-borne pathogen and acting as causative agent of collar rot disease in soybean. The present study was undertaken to study the differential expressions of protein profiles of soybean during infection of *Sclerotium rolfsii*. Two soybean cultivars, MAUS 162(collar rot tolerant) and MAUS 731 (collar rot susceptible), were analyzed at different stages of infection. Disease development was monitored during 0^{th} to 7^{th} days after inoculation, The root, collar and stem tissues were collected from control and infected tissue seedlings at 0^{th}, 3^{rd}, 5^{th} and 7^{th} days after inoculation. White mycelial growth appeared from 1^{st} day after inoculation, sclerotia formation was started at 5^{th} days after inoculation, and severe plant damage followed by death was observed at 7^{th} days after inoculation. Protein profiles during differential expressions were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and band intensities were quantified through densitometric analysis. Changes in the number and relative intensity of protein bands were observed among soybean cultivars, tissues and infection stages. SDS-PAGE analysis showed clear differences in protein banding patterns among the two soybean cultivars, different tissues, and stages of *Sclerotium rolfsii* infection. A total of 18 aligned protein bands were detected from the 48 analyzed samples, and all the bands were polymorphic. Densitometric analysis further showed variation in the relative intensity of protein bands between control and infected samples and across different stages of infection. The approximately 70 kDa region showed changes in band prominence during infection in both MAUS 162 and MAUS 731, particularly at later sampling stages. Band-frequency analysis showed that Band_06 was the most frequently detected band (52.08%), followed by Band_09 (43.75%), whereas Band_17 had the lowest frequency (4.17%). Overall, the observed variation in band number, relative intensity and band frequency indicated changes in the detectable protein profiles of soybean tissues during *S. rolfsii* infection. These observations suggest that some protein regions may be associated with the plant response to pathogen-associated stress; however, their specific biological functions cannot be confirmed from SDS-PAGE analysis alone.

Key words : Soybean, *Sclerotium rolfsii*, Collar rot, Protein profiling, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most important legume and oilseed crops grown worldwide. It is valued for its high-quality protein and edible oil and is widely used as food, animal feed and an industrial raw material. Soybean also contributes substantially to the global supply of vegetable protein and oil. Recent FAOSTAT data showed that global soybean production increased substantially between 2010 and 2024 (FAO, 2025), reflecting its growing importance in the agricultural and food sectors. In India, soybean is being cultivated as an important commercial oilseed crop, particularly in the

central and southern regions and its productivity is affected by several biotic stresses.

Among the soil-borne diseases of soybean, collar rot caused by *Sclerotium rolfsii* is an important constraint (Bhamra and Borah, 2022). The pathogen is a necrotrophic fungus that survives in soil mainly through sclerotia and can infect plants under favourable environmental conditions. Infected plants commonly show white mycelial growth near the collar region, followed by formation of sclerotia and progressive tissue damage. Recent studies have confirmed the occurrence and variability of *S. rolfsii* associated with soybean collar rot in India. Hiremath *et al*. (2024), reported biochemical variation among *S. rolfsii* isolates collected from soybean-growing regions. The disease is therefore important not only because of its effect on plant survival and yield, but also because soybean genotypes can differ considerably in their response to infection.

Plant infection by fungal pathogens involves changes in the physiological and biochemical processes of the host plant (Kong and Yang, 2023). Proteins play an important role in metabolism, signalling, stress response and plant defence mechanism. Consequently, changes in protein abundance or accumulation can occur during pathogen infection and may differ according to the response of genotypes of crops, type of tissue and stages of disease development (Balotf *et al*., 2022). Protein profiling therefore provides a primary and useful approach for comparing changes in the host protein pattern during plant–pathogen interactions.

Protein profiling using SDS-PAGE is relatively simple approach for studying changes in the protein composition of plant tissues following pathogen infection. Pathogen infection can induce the synthesis, accumulation, suppression or modification of specific proteins associated with stress responses, pathogen recognition, defense signaling, and structural protection. Resistance or tolerance to *S. rolfsii* is a complex plant response involving morphological, biochemical and molecular mechanisms. Understanding the biochemical changes occurring in resistant/tolerant and susceptible soybean genotypes during pathogen infection can provide valuable information about the mechanisms associated with disease tolerance. Therefore, comparison of protein profiles between selected tolerant (MAUS-162) and susceptible cultivar (MAUS-731) at different stages of infection can be helped to identify proteins or protein bands that are potentially associated with the host response to *S. rolfsii*. The utilization of collar rot-tolerent soybean cultivars MAUS-162 will be worked as one of the most effective and environmentally sustainable approaches to develop sustainable disease management strategies.

Plant–pathogen interactions involve a dynamic series of biochemical and molecular responses in which both the pathogen and host modify their physiological processes (Kaur *et al*., 2022). When soybean encounters *S. rolfsii*, pathogen-associated signals can activate host defense mechanisms, resulting in changes in the expression and accumulation of numerous proteins. These changes may occur in the root, collar and stem tissues through which the disease progresses. Protein expression profiling provides an opportunity to monitor these changes and compare the responses of soybean genotypes differing in their reaction to collar rot. SDS-PAGE (Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis) separates proteins primarily according to their molecular weight and allows comparative visualization of protein banding patterns between healthy and infected tissues. Appearance or disappearance of bands, as well as changes in their relative intensity, may indicate alterations in protein accumulation associated with pathogen infection.

Therefore, in the present study, cultivars, MAUS 162 (collar rot tolerant) and MAUS 731 (collar rot susceptible) were selected to examine changes in soybean protein profiles during *S. rolfsii* infection. Root, collar and stem tissues were analysed at 0^{th}, 3^{rd}, 5^{th} and 7^{th} days after inoculation under control and infected conditions which will provide a useful approach for understanding the biochemical basis of host response. SDS-PAGE gel separation technique was used to compare the detectable protein banding patterns. Monitoring protein profiles across different tissues and stages of infection can reveal differential protein accumulation associated with disease progression and host tolerance. Differentially expressed protein bands can be served as candidate markers for subsequent proteomic characterization. Further, identification of such proteins through advanced techniques such as LC-MS/MS or MALDI-TOF/MS may provide deeper insights into the mechanisms underlying soybean tolerance to collar rot and support the development of reliable biochemical or molecular markers for disease resistance breeding.

Materials and Methods

Isolation of *Sclerotium rolfsii* and infection to seedlings

Diseased soybean plants showing typical collar rot symptoms were collected from Latur, Maharashtra and brought to the laboratory for isolation of *Sclerotium rolfsii*. The pathogen was isolated on potato dextrose agar (PDA) as per protocol described by Hiremath *et*

al. (2024), Matale *et al*. (2025) and pure culture was maintained PDA slants. The characteristic white cottony mycelium and brown sclerotia were used for cultural identification. Fifteen-day-old soybean seedlings of tolerant cultivar MAUS 162 and susceptible cultivar MAUS 731 were inoculated with *S. rolfsii* using the sick-soil method (Matale *et al.*, 2025). The fungal inoculum was mixed with sterilized top soil at a 1:1 (w/w) ratio and applied around the collar region at 200 g pot^{-1}. Uninoculated plants were maintained as controls for the further experiment.

Collection of plant material and protein extraction

Root, collar and stem tissues were collected separately from control and infected seedlings at 0^{th}, 3^{rd}, 5^{th} and 7^{th} DAI. The procedure for extraction of plant protein described by Carmo-Silva *et al*. (2024) was utilized with modifications. Approximately 100 mg of each tissue was finely ground in a pre-chilled mortar and pestle and homogenized in 1× phosphate buffer. The homogenate was incubated at –20°C for approximately 1.5 h, thawed for 10 min and centrifuged at 10,000 rpm for 10 min at 4°C. The clear supernatant was collected and used for protein analysis.

Preparation of protein samples

Protein extract (80 µL) was mixed with 20 µL of sample loading buffer containing Tris-HCl, β-mercaptoethanol, SDS, bromophenol blue and glycerol. The samples were incubated at 95°C for 5 min to denature the proteins before electrophoresis. Protein samples with SDS-containing loading buffer were heated before SDS-PAGE separation which is a standard procedure for obtaining denatured protein profiles (Laemmli, 1970; Sigma-Aldrich, 2026).

SDS-PAGE and electrophoresis

Protein profiling was performed by SDS-PAGE following protocol described by Laemmli (1970). A 10% resolving gel and 4% stacking gel were prepared using a Bio-Rad Mini-PROTEAN system. Approximately 25 µL of each denatured sample was loaded along with a pre-stained molecular-weight marker. Electrophoresis was initially performed at 80 V for 20 min and subsequently at 100 V until adequate separation of protein bands was obtained. SDS-PAGE separates denatured proteins primarily according to their molecular size and remains a standard approach for comparative protein profiling (Laemmli, 1970).

Staining and visualization of protein bands

Coomassie Brilliant Blue staining method is used for visualization of proteins separated by SDS-PAGE (Wang *et al*., 2024). After electrophoresis, the gels were stained overnight with Coomassie Brilliant Blue R-250 dye 25 mg/L with methanol and glacial acetic acid in 4:1 proportion with gentle shaking and subsequently destained with methanol and glacial acetic acid in 4:1 proportion for 5 hours for clear protein bands. The gels were photographed under transilluminator and the apparent molecular weights of the protein bands were estimated using the molecular-weight marker.

Data analysis

The stained gel images were analysed using ImageJ/Fiji software for densitometric measurement of protein bands. Fiji is an established open-source platform used for quantitative biological image analysis (Schindelin *et al*., 2012). The area of individual bands was measured from the densitometric profiles and expressed as relative band intensity based on the total detected band area of the respective sample. The detected protein bands were aligned and coded as Band_01 to Band_18 and band frequency was calculated from the number of samples in which each band was detected. Relative band-intensity data were further analyzed in software R to generate a heatmap for visual comparison of protein profiles among cultivars, tissues, treatments and infection stages. The combined banding and densitometric analyses were used to assess changes in the detectable soybean protein profile associated with *S. rolfsii* infection.

Results and Discussion

SDS-PAGE protein expression profiles

The protein was extracted from infected and control samples of soybean cv. MAUS 162 (Tolerant) and MAUS 731 (Susceptible) and separated by SDS-PAGE (Figs. 1 and 2). SDS-PAGE revealed clear changes in the detectable protein banding patterns of MAUS 162 (Tolerant) and MAUS 731 (Susceptible) during *Sclerotium rolfsii* infection at 0^{th}, 3^{rd}, 5^{th} and 7^{th} DAI within root, collar and stem tissues. The changes were observed in differences in the protein band number, distribution and apparent intensity were observed between treatments and sampling stages. Such changes are expected during plant–pathogen interactions because infection can alter proteins involved in metabolism, signaling, stress response and defence (Rózewicz *et al*., 2021; Mierziak and Wojtasik, 2024).

During analysis of tissues of collar rot tolerant cultivar MAUS 162, at 0^{th} DAI, the control and infected samples showed comparable initial protein bands in root, collar and stem tissues. A protein band at approximately 70 kDa was visible in the analyzed tissues, but its intensity was comparatively low. As 0^{th} DAI represented the initial

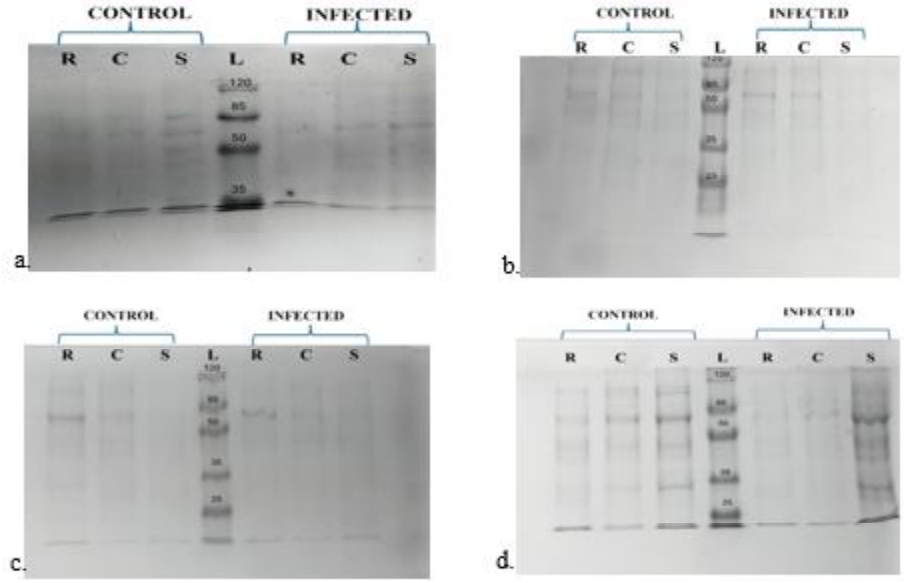


Fig. 1 : Protein profile of soybean cv. MAUS 162 under control and SR-infected condition a) 0 DAI b) 3 DAI c) 5 DAI d) 7 DAI, R- Root tissue, C-Collar tissue, S- Stem tissue, L-Ladder (120 kDa).

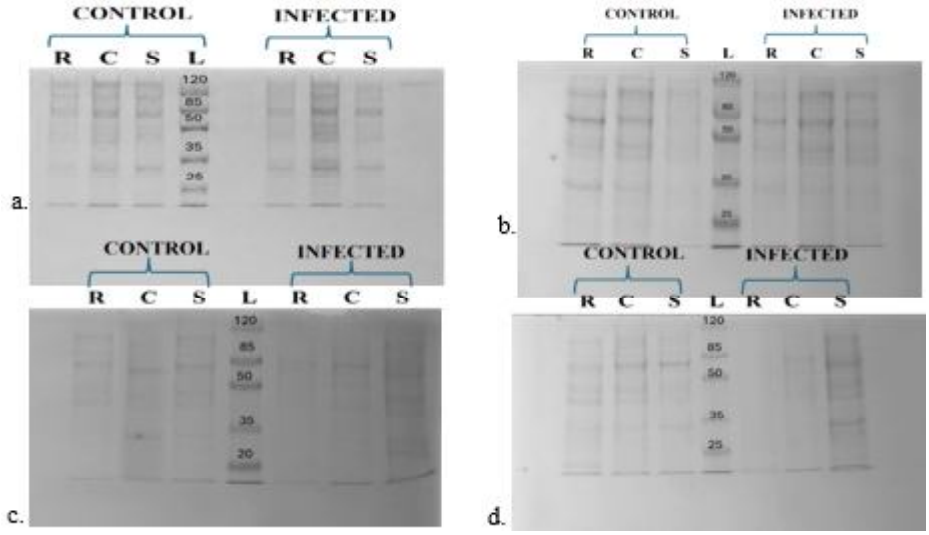


Fig. 2 : Protein profile of soybean cv. MAUS 731 under control and SR-infected condition a) 0 DAI b) 3 DAI c) 5 DAI d) 7 DAI, R- Root tissue, C-Collar tissue, S- Stem tissue, L-Ladder (120 kDa).

stage, this band may represent a protein that was present under the normal physiological condition of the seedlings. During 3rd DAI, visible white mycelial growth of *S. rolfsii* was observed. Changes in the protein banding pattern were noticed in the infected tissues compared with the initial samples. The approximately 70 kDa region appeared more prominent in the infected root tissue. This increase in expression may indicate an early response of the plant to pathogen-associated stress. Since MAUS 162 is considered as tolerant cultivar, the increased prominence of this band at this stage may possibly be associated with an early host response; however, the protein cannot be recognized as a defence protein from SDS-PAGE experiment alone. At 5th DAI, when initial sclerotia development was observed, further changes in the protein pattern were detected. The approximately 70 kDa region appeared more prominent in the infected collar tissue. The change from the earlier root-associated observation to the collar region may indicate that the response associated with this protein region varied with the stage and tissue affected during infection. This observation may also reflect increasing pathogen-associated stress around the collar region. During 7th DAI, disease development was progressed in the infected seedlings. Further changes in the protein banding pattern were visible, and the 70 kDa region appeared more prominent in the infected stem tissue. The appearance of this region at different tissues at successive sampling stages may suggest a temporal and tissue-associated response during disease development. However, this pattern may prove the movement of the pathogen from root to stem and tissue assisted activation of defense protein and blocking the entry of pathogen and its subsequent spread. The approximately 70 kDa band may therefore be considered a putative infection-associated protein band, possibly related to stress or defense response, which requires further protein identification for confirmation.

The same comparison was made for susceptible cultivar MAUS 731, the susceptible variety. At 0th DAI, the approximately 70 kDa band was present in both control and infected root, collar and stem tissues. Its presence at this stage may represent its basal occurrence before clear disease development. During 3rd DAI, when white mycelial growth was visible, the approximately 70 kDa band remained detectable in both control and infected tissues. The continued presence of this band in the infected tissues may indicate that, the corresponding protein was already present during the early stage of

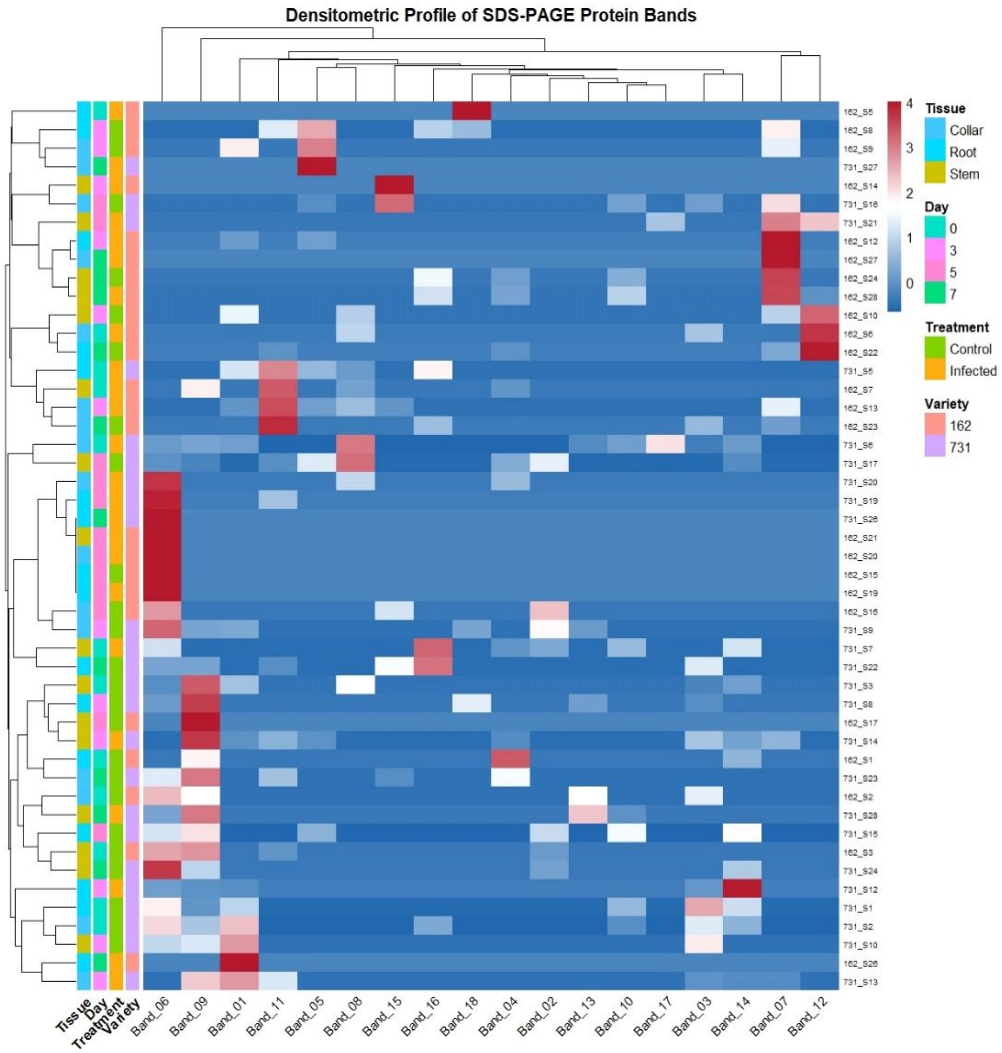


Fig. 3 : Densitometric heatmap showing relative intensity of SDS-PAGE protein bands in root, collar and stem tissues of soybean varieties MAUS 162 and MAUS 731 under control and *Sclerotium rolfsii*-infected conditions at 0th, 3rd, 5th and 7th days after infection.

pathogen challenge. However, during 5th DAI, the ~70 kDa protein showed increased prominence in the infected collar tissue compared with the earlier stage. This change may reflect an alteration in the host protein response with increasing infection-associated stress. The observation was broadly comparable with the pattern observed in MAUS 162 at this stage. At 7th DAI, the ~70 kDa region appeared more prominent in the infected stem tissue. Thus, in MAUS 731 also, the band showed a visible change in prominence from the earlier stages towards the collar and stem regions as infection progressed. Since MAUS 731 is susceptible, the increased prominence of this band may represent a general stress- or infection-associated response occurring during disease progression.

Overall, both soybean varieties showed changes in their differential protein banding patterns following *S. rolfsii* infection. The ~70 kDa band was particularly noticeable because, it was present at the initial stage and showed changes in prominence during later stages in infected tissues. A predominance of ~70 kDa protein fragment was found in tolerant cultivar MAUS 162 rather than susceptible cultivar MAUS 731 and exhibited differential expression pattern during pathogen interaction. Therefore, the ~ 70 kDa region may represent a protein response associated with pathogen challenge or plant stress, rather than a confirmed defense protein. Changes in protein abundance are commonly associated with plant responses to pathogen infection, including stress, signaling, and defence-related processes (Figueiredo *et al.*, 2024; Soni and Gurjar, 2024). The exact function of the overexpressed protein *i.e.* ~70 kDa protein, cannot be determined from the present SDS-PAGE analysis and would require further identification through mass spectrometry or immunological/biochemical characterization.

Densitometric analysis using ImageJ/Fiji provided

Table 1 : Sample identification used for analysis.

Variety	Days after infection	Control Root	Control Collar	Control Stem	Ladder	Infected Root	Infected Collar	Infected Stem
MAUS 162	**0th**	162_S1	162_S2	162_S3	**162_S4**	162_S5	162_S6	162_S7
MAUS 162	**3rd**	162_S8	162_S9	162_S10	**162_S11**	162_S12	162_S13	162_S14
MAUS 162	**5th**	162_S15	162_S16	162_S17	**162_S18**	162_S19	162_S20	162_S21
MAUS 162	**7th**	162_S22	162_S23	162_S24	**162_S25**	162_S26	162_S27	162_S28
MAUS 731	**0th**	731_S1	731_S2	731_S3	**731_S4**	731_S5	731_S6	731_S7
MAUS 731	**3rd**	731_S8	731_S9	731_S10	**731_S11**	731_S12	731_S13	731_S14
MAUS 731	**5th**	731_S15	731_S16	731_S17	**731_S18**	731_S19	731_S20	731_S21
MAUS 731	**7th**	731_S22	731_S23	731_S24	**731_S25**	731_S26	731_S27	731_S28

Ladder lanes were used only for molecular-weight reference and were not included in the densitometric analysis.

Table 2 : Frequency and polymorphism of aligned protein bands detected by SDS-PAGE.

Band	Present samples (n)	Absent samples (n)	Frequency (%)	Band type
Band_01	15	33	31.25	Polymorphic
Band_02	7	41	14.58	Polymorphic
Band_03	14	34	29.17	Polymorphic
Band_04	10	38	20.83	Polymorphic
Band_05	10	38	20.83	Polymorphic
Band_06	25	23	52.08	Polymorphic
Band_07	14	34	29.17	Polymorphic
Band_08	9	39	18.75	Polymorphic
Band_09	21	27	43.75	Polymorphic
Band_10	9	39	18.75	Polymorphic
Band_11	13	35	27.08	Polymorphic
Band_12	5	43	10.42	Polymorphic
Band_13	5	43	10.42	Polymorphic
Band_14	12	36	25.00	Polymorphic
Band_15	6	42	12.50	Polymorphic
Band_16	8	40	16.67	Polymorphic
Band_17	2	46	4.17	Polymorphic
Band_18	4	44	8.33	Polymorphic

quantitative support to the visible differences observed in the SDS-PAGE profiles. The relative intensity of protein bands varied among control and infected samples of soybean varieties. In MAUS 162, infected tissues showed changes in the relative contribution of individual bands at 3rd, 5th and 7th DAI compared with the corresponding control samples. Similar changes were also observed in MAUS 731.

The densitometric heatmap (Fig. 3) further showed differences in overall band-intensity patterns among the samples. Samples with similar intensity profiles were grouped closer together, whereas samples with greater differences were separated in the hierarchical clustering. Thus, the heatmap supports the general observation of protein expression patterns changing with tissue, variety, treatment and progression of infection.

The densitometric results also support the observation of changes around ~70 kDa protein. Since the values were calculated relative to the total detected band area of each sample, a higher relative intensity indicates greater contribution of that band to the detected protein profile and does not by itself establish absolute protein abundance.

Such changes in protein profiles are due to plant–pathogen interactions because pathogen challenge can alter proteins involved in metabolism, signalling, stress responses and defence. Recent proteomic studies have similarly reported stage- and genotype-dependent changes in plant protein expression following pathogen infection (Peng *et al*., 2023 and Lopes *et al*., 2023). Therefore, the present densitometric results provide supporting evidence for changes in soybean protein profiles during *S. rolfsii* infection, while identification of the proteins responsible for the observed bands would require further analysis.

Protein band frequency and polymorphism

Across all 48 samples (Table 1), 18 aligned protein bands (Band_01–Band_18) were detected. All 18 bands were polymorphic because their presence varied among the samples. The frequency of individual bands ranged from 4.17 to 52.08% (Table 2). Band_06 was the most frequently detected band, occurring in 25 of 48 samples (52.08%), followed by Band_09 in 21 samples (43.75%). Band_01 occurred in 15 samples (31.25%), whereas Band_17 showed the lowest frequency, occurring in only 2 samples (4.17%) as described in Table 2.

Conclusion

The present study demonstrated that *Sclerotium rolfsii* infection was associated with clear changes in the detectable protein profiles of soybean. SDS-PAGE analysis of MAUS 162 (tolerant) and MAUS 731 (susceptible) showed variation in protein banding patterns among root, collar and stem tissues and at different stages of infection.

Densitometric analysis confirmed changes in the relative intensity of protein bands between control and infected samples during disease progression. The ~70 kDa protein region showed changes in prominence during infection in both cultivars, suggesting a possible association with the plant response to pathogen-associated stress. However, its specific function as a defence protein cannot be confirmed from SDS-PAGE alone. Overall, the combined banding, densitometric and frequency analyses stated stage- and tissue-associated changes in soybean protein profiles following *S. rolfsii* infection. The results provide a useful basis for identifying protein regions that may be associated with the soybean response to *S. rolfsii* and can be further investigated through protein identification techniques

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Conflict of interest : The authors declare that they have no conflict of interest.

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