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Metabolic profiles of groundnut (*Arachis hypogaea* L.) genotypes differing in *Sclerotium rolfsii* reaction

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Abstract A total of 60 compounds of known structure, comprising sugars, sugar alcohols, fatty acids, amino acids, organic acids, phenols and sterols were identified in stem extracts of groundnut using GC-MS. Sugars and fatty acids were predominant in stem extracts as compared to other metabolites. Distinguished metabolite patterns were observed in control and 96 h after infection (h.a.i.). Succinic acid, pentitol, scopolin, D-glucose and D-turanose, myo-inositol, fructose and mannitol were observed to be higher in control plants, whereas, D-ribopyranoside, thymol, pentadecanoic acid and octadecanoic acid increased at 24 hai than that of control. Interestingly, phenol related compounds such as phenol, hydroquinone, guaicol-.beta.-d-glucopyranoside, scopolin were also found lower in non-infected stems of TG37A. Moreover, tolerant genotypes (CS 319 and CS 19) had higher content of Thymol-.beta.-d-glucopyranoside, pentitol, D-glucose, D-turanose, scopolin and hydroquinone than that of moderately tolerant and susceptible genotypes. Sugar profiles using Ion chromatography revealed that glucose content decreased in moderately susceptible and susceptible genotype after *S. rolfsii* infection. Both constitutive and induced levels of cinnamic acid was observed higher in resistant genotypes than that of susceptible ones which was further supported by phenylalanine ammonia lyase activity. Thus, our study demonstrates the biological role of metabolites specifically sugars, phenolics and fatty acids in plant defense responses.

Keywords Gas chromatography-mass spectrometry · Groundnut · High performance liquid chromatography · Phenol profiles · Stem rot · Sugar profiles

Introduction

Sclerotium rolfsii Sacc., the causal organism of stem rot in many crop plants is also a potential threat to groundnut growers worldwide (Bishi and Vakharia 2015). Up to 30% yield losses were recorded in India by various researchers in farmer's field. The incidence of stem rot has been enhanced year by year due to spread of host range of the pathogen and survival of the sclerotia for several years in soil. Management of the disease in the groundnut field is still challenging due to lack of profitable rotational crops and fresh tillable land, poorly structured farm programmes, undecomposed previous crop residues in the field which act as substrate for the fungal growth, tolerance of the pathogen to the

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fungicide and importantly unavailability of resistant varieties to the groundnut growers (Thirumalaisamy et al. 2015). However, there are few groundnut genotypes reported to be stem rot tolerant (Bera et al. 2014).

Plants have their own defense system which is reflected as metabolic and genetic responses resulting in the production of toxic compounds that either kill the pathogen or deter its growth. These molecules are derived from the secondary metabolism and their production is induced upon detection of a pathogen-associated molecular pattern (PAMP). These highly diverse and specific PAMPs are either preformed metabolites (phytoanticipins) or toxic metabolites (phytoalexins), are perceived by the host cell triggering defense responses. Phytoanticipins are converted into toxic molecules upon pathogen perception while phytoalexins are produced only upon pathogen attack. The pathogen specific toxic phytoalexins are isoflavanoids, which disrupt pathogen metabolism and cellular structure. Plant metabolite profiling techniques have been used for the identification of novel antimicrobial molecules that are induced upon elicitation by pathogens. However, more studies are required to assess the specific function of those unknown metabolites or metabolite blends on plant-microbe interactions (Arbona and Gómez-Cadenas 2016).

Besides being the source of carbon and energy, sugars such as glucose, fructose, and sucrose are recognized as signalling molecules in plants (Koch 2004; Rolland et al. 2006; Bolouri-Moghaddam et al. 2010). The role of soluble sugars in resistance responses to pathogens has been recognized, as they can stimulate isoflavone accumulation in plants as part of defence mechanisms (Morkunas et al. 2005). In plants, phenolics are produced via the shikimic acid and malonic acid pathways which include various defense-related compounds such as flavonoids, anthocyanins, phytoalexins, tannins, lignin, and furanocoumarins (Freeman and Beattie 2008). Phenolics are associated with plant resistance to different fungal pathogens and also their profiling in various crops have already been reported by researchers (Mahatma et al. 2009; Kim et al. 2013; Jadhav et al. 2013a; Scandiani et al. 2015; Hamzehzarghani et al. 2016), however detailed study about stem rot disease in groundnut is missing.

It is essential to identify, characterize and categorize effective sources of resistance to develop disease resistance varieties. Further, early, precise and rapid identification of the various biochemical factors that are conferring the preformed and induced resistance is one of the factors that may aid in breeding disease resistant varieties. To the best of our knowledge, the understanding of the stem rot disease tolerance mechanism in groundnut is very scanty. Therefore, the objective of this study was to study the differential accumulation of preformed and induced metabolites in groundnut genotypes differing in *Sclerotium rolfsii* reaction and further to identify metabolite marker s which will help to differentiate stem rot tolerant and susceptible groundnut genotypes.

Materials and methods

The seeds of groundnut genotypes CS19 and CS 319 (Stem rot tolerant), GG16 (Moderately susceptible) and GG20 and TG 37A (Susceptible) were procured from Plant Breeding Section, ICAR-Directorate of Groundnut Research, Junagadh, Gujarat, India. These genotypes were selected based on previous reports on stem rot incidence (Bera et al. 2014; Thirumalaisamy et al. 2015). All the genotypes were raised in earthen pots under PII glass house to maintain uniform temperature and humidity during *kharif* 2013–14 and 2014–15. A potting mixture (Vertisol, sand and farm yard manure in a 2:1:1ratio) with diammonium phosphate at 1 g kg^{-1} of soil was used. Each genotype was grown in six pots, three pots were kept for control and three for inoculation of stem rot pathogen (i.e. two sets). Four plants were kept in each pot (20 kg soil capacity). The pathogen, *S. rolfsii* was isolated from stem rot infected TG37A genotype grown in field and multiplied on autoclaved sorghum grains for 15 days and used for inoculation (@ 20 g per pot) on 60 days old plants. The inoculum was spread on soil surface and covered with wheat straw (Thirumalaisamy et al. 2015). Stem samples were collected at 24, 48 and 96 h after infection (h.a.i.) for various biochemical analysis at 60 days after sowing (DAS). Stem was divided into three parts and middle stem was taken for all analysis. Three replications for each analysis were taken.

Metabolite profiles using gas chromatography–mass spectrometry (GC-MS)

Stem rot infected and control stems were taken at 24, 48 and 96 hai for metabolite extraction. Metabolites were

extracted and analysed following the method described by Aliferis and Jabaji (2012) with some modifications.

Stem samples were collected from infected and non-infected plants and immediately immersed into liquid N for quenching of metabolism. These samples were kept at −80 °C until further analysis. Groundnut stems (200 mg) were homogenized in pestle and mortar with methanol: ethyl acetate solution (50:50,*v*/v), this was repeated three times with centrifugation at 5000 rpm for 5 min. The supernatant was taken in screw-cap glass tubes and evaporated using a nitrogen stream. Metabolites were derivatized as per the method described by Fiehn et al. (2000). Carbonyl moieties were protected by methoximation, using 100 μL methoxylamine hydrochloride solutions (20 mg ml^{-1} in pyridine) at 30 °C for 120 min. Then, acidic protons were derivatized by adding 100 μLMSTFA (N-methyl-N-trimethylsilyl trifluoroacetamide) at 37 °C for 90 min. One-microliter of derivatized samples were injected into a DB-17 MS capillary column (50%-phenyl)-methylpolysiloxane (30 mt length, 0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies Inc.) equipped with Shimadzu GC-2010 coupled with MS-QP2010 Plus. Electron ionization of 70 eV was used. Full scan mass spectra were acquired at the mass range of 50 to 1000 Da at 1 scan 0.5 s^{-1} with a 10.0-min solvent delay. The temperature for the ion source was set to 230 °C, for the transfer line to 280 °C, and for the injector to 280 °C. The oven temperature was initially kept at 100 °C for 5 min, followed by increasing 5 °C min^{-1} to 290 °C for 1 min. Carrier gas (Helium) was used at a constant flow rate of 1 ml min^{-1}. Instrument was calibrated daily using the default automatic calibration mode as recommended by the manufacturer. Chromatogram acquisition, peak deconvolution, and MS library searches were performed using GCMS Solution version 2.71, Shimadzu Corporation-Japan. Reagent peaks and peaks corresponding to column bleeding were excluded from further analyses. Metabolites were putatively identified by matching their mass spectra to spectra in NIST 14 library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Pre-processing of total ion chromatograms (TIC) such as baseline correction, alignment, peak picking, and integration were performed using the ACD/Spec Manager v.12.00 (Advanced Chemistry Development, Inc., ACD/Labs, Toronto, Canada). CSV comma delimited files were created for data analysis.

Sugar profile of groundnut stems using ion chromatography

Oligosaccharides from stems (500 mg) of groundnut genotypes were extracted in 80% ethanol as described by Swami et al. (2015). Glucose, fructose, myo-inositol, mannitol, trahalose, sucrose, lactose, raffinose, stachyose, and verbascose were used as standards. The internal standard was used as lactose during the analysis. Varied concentrations of these sugars were used in the standard mixture to get distinct peak of each sugar in chromatogram (Supplementary Fig S1). Extracted sugars from groundnut stems were membrane-filtered and 25 μL of aliquot from each samples was injected in the injection port of ion chromatograph (Dionex, ICS 3000) equipped with amino trap column, CarboPac PA10 guard column followed by CarboPac PA10 analytical column. Sugars were eluted using 150 mM NaOH as isocratic mobile phase with a flow rate of 1 ml min^{-1}. Data integration was accomplished by using Chromeleon software supplied with the equipment (Bishi et al. 2015).

Identification of phenolics using high performance liquid chromatography (HPLC)

Extraction of phenolics from stem

Phenolics were extracted from groundnut stems following the method described by Mahatma et al. (2011) with minor modifications. One gram of stem tissues were taken and immersed into 80% HPLC grade methanol in screw cap glass tubes. These tubes were kept in refrigerator at 4 °C for 48 h. Samples were homogenized using pestle and mortar and at 10,000 rpm for 10 min. The extraction procedure was repeated four times on the pellet. The supernatant from every centrifugation was collected in volumetric flask and made up to 25 ml with 80% methanol. The supernatants were evaporated to dryness under vacuum dryer at 50 °C and residue was dissolved in 1 ml mobile phase.

Extract was filtered (PTFE, 25 mm × 0.45 μm) and 10 μL extract was injected by glass syringe in injection port. Separation of the phenolics was achieved using RP-C18 column (4.6 × 250 mm, 5 μm; from Merck) using isocratic mobile phase of methanol and water in the ratio of 25:75 (*v*/v) and 1% of acetic acid (v/v). Phenolics were detected by UV detector at 290 nm. All separations were performed at 25 °C and the flow

rate was 1 ml per min. Total nine standards of phenolics (gallic acid, catechol, chlorogenic acid, caffeic acid, syringic acid, cinnamic acid, coumaric acid, ferulic acid and salicylic acid) were used for identification of the phenolic compounds in groundnut stems (Supplementary Fig S2). Phenolics from stems were identified comparing their retention times with standards and confirmed by adding internal standards into the samples (Czerniewicz et al. 2008). Concentration of the identified phenolics within plant material was expressed as mean values of three replications.

Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) enzyme assay

Three hundred milligrams of stem tissues were homogenized using 3 ml extraction buffer contained 50 mM borate–HCl buffer (pH 8.5) and 0.04% β-mercaptoethanol in pre-chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 15 min and clear supernatant was used as the enzyme source (Jadhav et al. 2013b). For the assay of PAL activity, 3.0 ml of reaction mixture contained of 0.1 M sodium borate buffer (pH 8.8) and 0.5 ml of 0.1 M phenylalanine as substrate (dissolved in 0.1 M sodium borate buffer, pH 8.8) was added in test tube. Enzyme extract (0.1 ml) was added in the each test tube except blank to initiate reaction. The tubes were incubated at 37 °C for 2 h. The optical density (OD) was read at 290 nm after 2 h**.** Protein content of enzyme extract was estimated and standard curve of cinnamic acid was prepared to express enzyme activity as μM cinnamic acid h^{-1} g^{-1}protein.

Data processing and statistical analysis

Statistical and fold Change analysis were undertaken using MetaboAnalyst 3.0 (Xia et al. 2015), an online statistical package. For statistical analyses peak areas were taken into consideration. Data were normalised with respect to the internal standards (adonitol) and pareto scaling. Chromatography peaks were considered significant where the signal to noise (S/N) ratio was >50, the Fold Change (FC) was >2.0, and *p*-values were 0.05. To identify important metabolite and the outliers at each stage, a Partial Least Square-Discriminant Analysis (PLS-DA) was employed (Karpe et al. 2015). PLS-DA is a supervised method used to analyse large datasets and has the ability to assess linear/polynomial correlation between variable matrices by lowering the dimensions of the predictive model, enabling easy discrimination between samples and the metabolite features that cause the discrimination (Wold et al. 2001). A heat map and dendrogram analysis conducted to visualize the relative levels and relationships of metabolites (Xia et al. 2015).

Data of assay of PAL and phenol and sugar profiles were analysed by completely randomized design (CRD) and mean differences were compared by critical differences (CD) at $P < 0.05$ for significance.

Results

To have a better understanding of the biochemical changes induced in groundnut plants during *S.rolfsii* infection, we monitored the activation of plant defense mechanisms in a time-course experiment at 60 DAS with 24, 48 and 96 hai.

Metabolite profiles by GC-MS

A total of 60 compounds of known structure, comprising sugars, sugar alcohols, fatty acids, amino acids, organic acids, phenols and sterols were identified in stem extracts of groundnut using NIST library (Fig 1). Sugars and fatty acids were predominant in stem extracts as compared to other metabolites. Distinguished metabolite pattern was observed in the stem samples taken from control and 96 hai. Highly abundant metabolites at different stages were identified by PLS-DA using variable importance in projection (VIP). Succinic acid, pentitol, scopolin, D-glucose, D-turanose, myo-inositol, D-fructose and D-mannitol were observed higher in control plants, whereas, D-ribopyranoside, thymol, pentadecanoic acid and octadecanoic acid were increased at 24 hai than that of control. Further, these metabolites gradually decreased at later stage of infection. Some induced metabolites i.e. D-ribopyranose, 2,3,4-trihydroxybutyric acid, 2-furancarboxaldehyde, Stigma sterol and quinic acid increased at 96 hai (Supplementary Fig S3).

Heat map of metabolites (Fig. 1) showed that in non-infected stems of TG37A (highly susceptible genotype), sugars content such as 2-deoxy ribose, D-ribofuranose,xylitol, D-glucose, D-turanose, glucofuranoside and mannofuranoside were lower than that of moderately tolerant and tolerant genotypes. Fatty acids such as monopalmitin, beta-hydroxybutyrate,

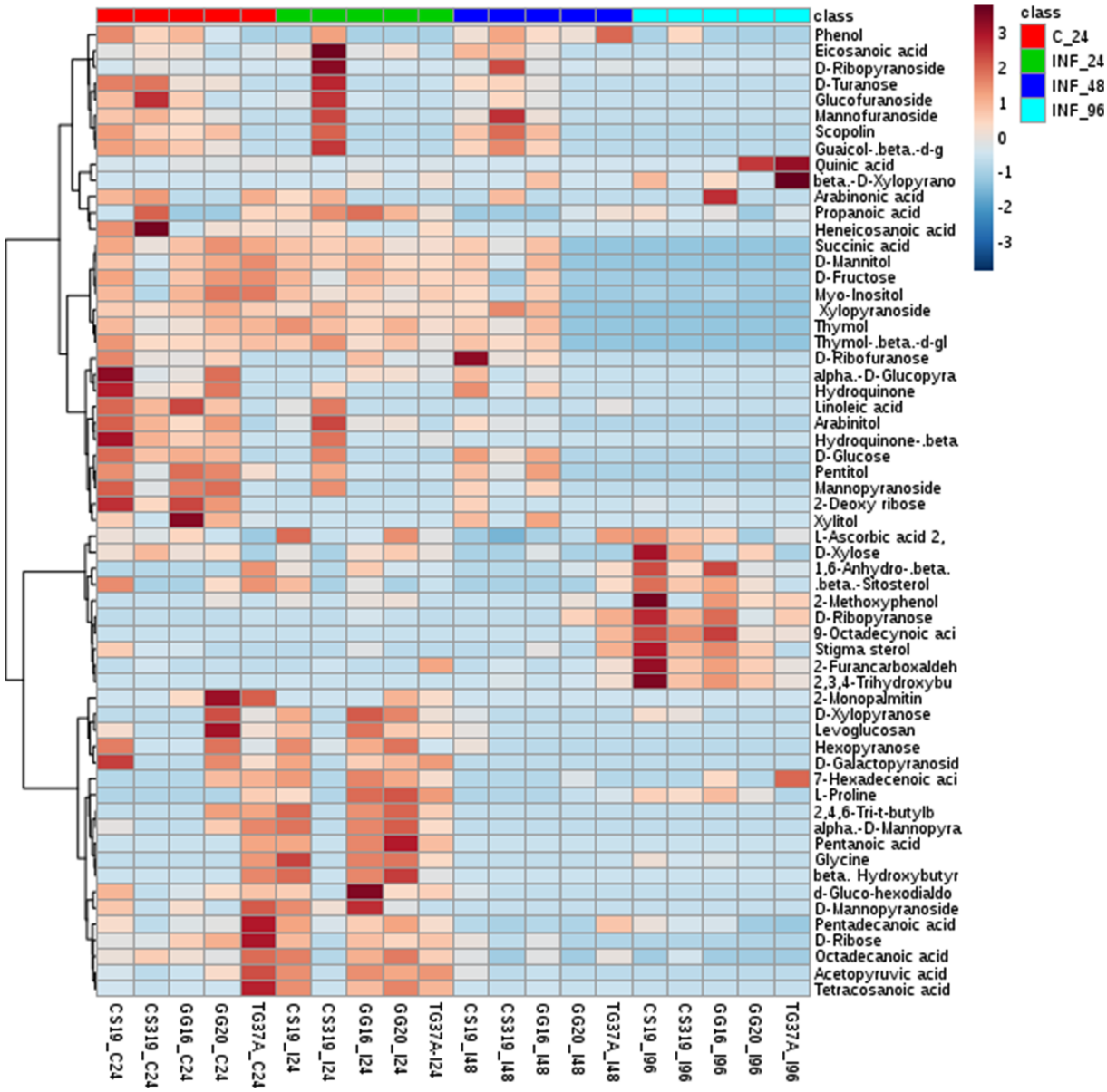

Fig. 1 Heatmap analysis showing abundance of metabolite (log2) in groundnut genotypes (stem rot tolerant: CS19 and CS319; moderately tolerant: GG16; susceptible: GG20 and TG37A) at different stages. Where; C: control and I: infected; 24, 48 and 96 stands for hour after infection. On the log scale bar brick red colour indicates increased metabolite levels, and sky blue represents decreasedlevels

tetracosonoicacid, archidic acid were higher in GG20 and TG37A while linoleic acid was not observed in TG37A. Interestingly, the content of phenol related compounds such as phenol, hydroquinone, Guaicol-.beta.-d-glucopyranoside, scopolin were also found lower in non-infected stems of TG37A. Moreover, tolerant genotypes (CS 319 and CS 19) had higher content of Thymol-.beta.-d-glucopyranoside, pentitol, D-glucose, D-turanose, scopolin and hydroquinone than that of moderately tolerant and susceptible genotypes.

Based on metabolite pattern, 2 clusters were formed in which cluster-II comprised all genotypes at 96 hai along with TG 37A and GG20 at 48 hai (Fig. 2). Whereas, in cluster-I, two sub-clusters were formed under which all genotypes at 24 hai were observed on sub-cluster-I while non-infected (control) susceptible genotype TG37A

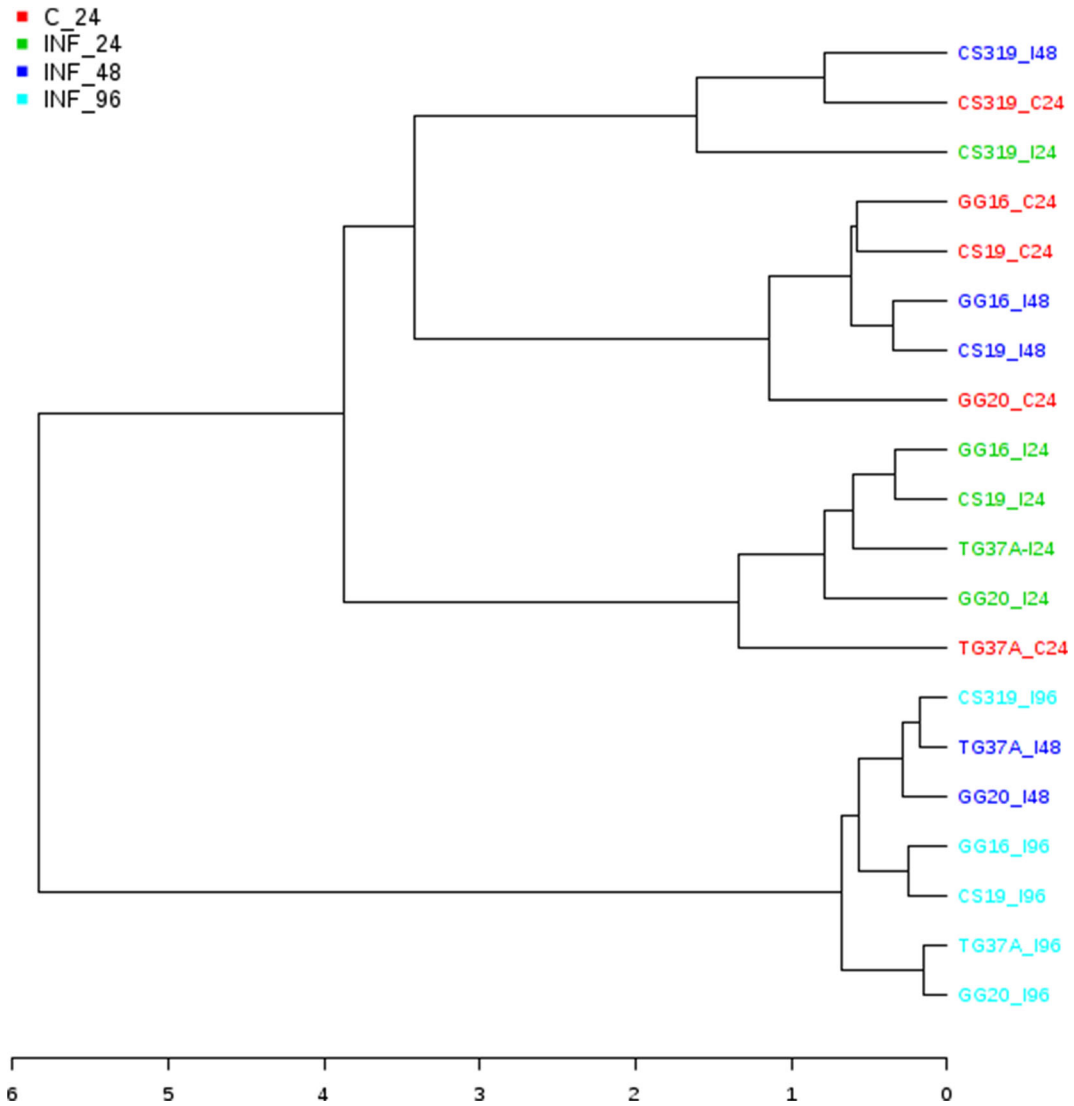

Fig. 2 Clustering pattern shown as dendrogram (distance measure using euclidean, and clustering algorithm using ward) of groundnut genotypes at different stages (stem rot tolerant: CS19 and CS319; moderately tolerant: GG16; susceptible: GG20 and TG37A). Where; C: control and Inf: infected; 24, 48 and 96 stands for hour after infection

accommodated in sub-cluster-II. Tolerant genotype CS 319 observed in different sub-sub-cluster of cluster-I. Metabolite pattern of the tolerant genotypes in control and 24 and 48 hai infection almost remained same which is clearly reflected by dendrogram as these genotypes grouped in same cluster at control, 24 and 48 hai. Thus, these results revealed that induced changes after infection are less in CS 319 and CS 19 till 48 hai. These results of clustering pattern were further supported by PLS-DA 2D scoring plots (Supplementary Fig S4). All the metabolites data evaluated by PLS-DA score plot showed two principal components explaining 74.6% of the overall variance. The first principal component (PC1) alone revealed 55.2% of the total variation and was dominated by metabolites of 96 hai stage and susceptible genotypes at 48 hai. The second principal component (PC2) contributed to 19.4% of the total variation and was dominated by the metabolites of tolerant genotypes at 48 hai and rest of genotypes at 24 control and 24 hai except TG37A.

Sugar profiles in groundnut stems

For further confirmation of sugar results observed by non-targeted metabolites by GC-MS we analysed

sugar profiles using Ion Chromatography. Total four sugars i.e. myo-inositol, fructose, glucose and sucrose detected in all genotypes. Myo-inositol content decreased in the stem of all genotypes after infection but it was more pronounced in resistant (CS 19 and CS 319) and moderately resistant (GG16) genotype (Fig. 3a). On the other hand glucose content decreased markedly in the susceptible genotypes after disease incidence while it was increased in resistant genotypes at 24 hai (Fig. 3b). Sucrose content decreased in all genotypes except CS19 after *S. rolfsii* infection, however resistant genotypes had almost 2 times higher content of sucrose at 96 h.a.i. (Fig. 3c). Fructose content also decreased in all genotypes after infection during but increased in resistant genotypes (Fig. 3d).

Phenol profiles in groundnut stems

Total six phenolic acid were (chlorogeic, caffeic, cinnamic, coumaric, ferulic and salicylic acid) detected in stem tissue of groundnut genotypes. Cinnamic acid was abundantly observed among six phenolics followed by chlorogenic acid. In non-infected stems, higher content of chlorogenic acid was observed in all genotypes as compared to infected stems. However, resistant genotypes had about 2-times higher chlorogenic acid. Caffeic acid and coumaric acid were also higher in non-infected resistant genotypes at 60 DAS. Both constitutive and induced levels of cinnamic acid observed higher in resistant genotypes than that of susceptible ones. Content of cinnamic acid hastened in all genotypes after infection but resistant genotypes had 4.5 to 6.75 times more cinnnamic acid compared to susceptible genotypes at 96-hai. Salicylic acid was not detected in non-infected susceptible genotype (TG 37A) at 96 h. Non-infected stems of resistant genotypes had higher content of salicylic acid which was maintained after infection also. However, salicylic acid was decreased in

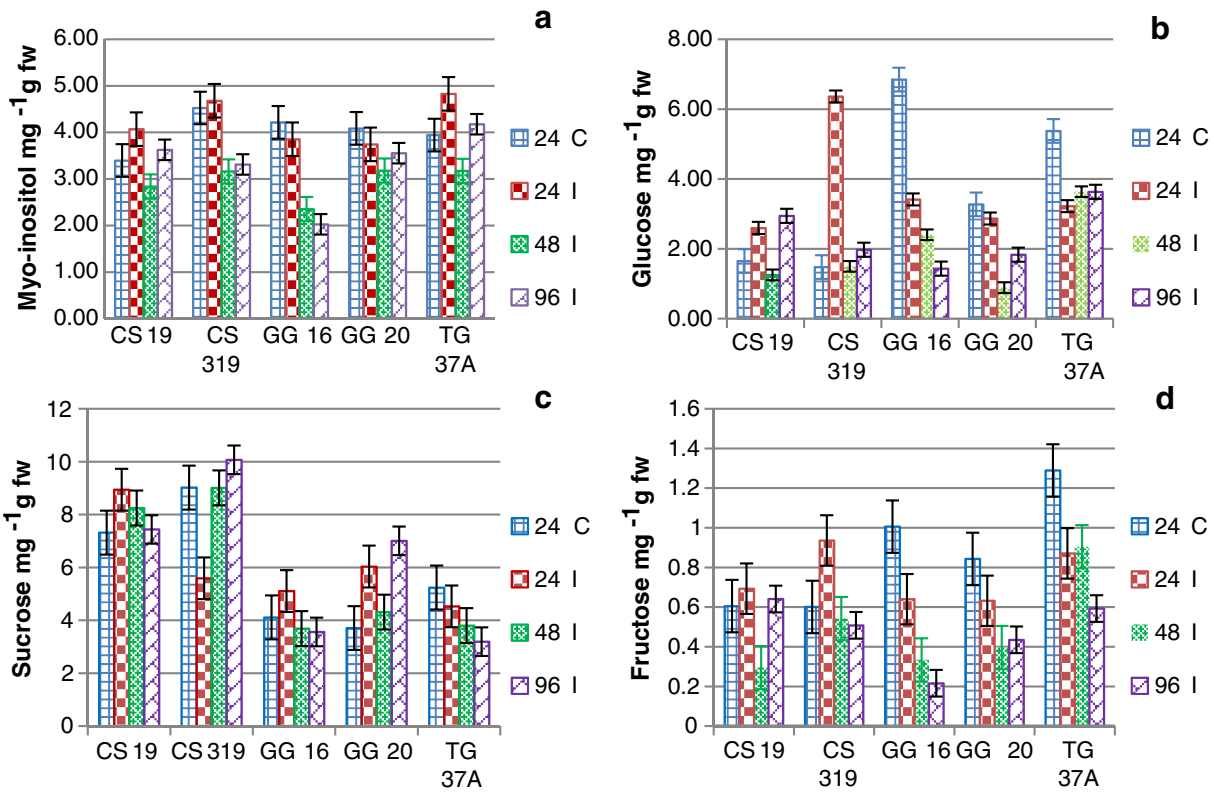

Fig. 3 Sugar (**a**) *myo*-inositol (**b**) glucose (**c**) Sucrose and (**d**) fructose content in groundnut genotypes (stem rot tolerant: CS19 and CS319; moderately tolerant: GG16; susceptible: GG20 and TG37A) at different stages. Where; C: control 24 h and I: infected; 24, 48 and 96 stands for hour after infection. Error bar depicted as CD(0.05) value

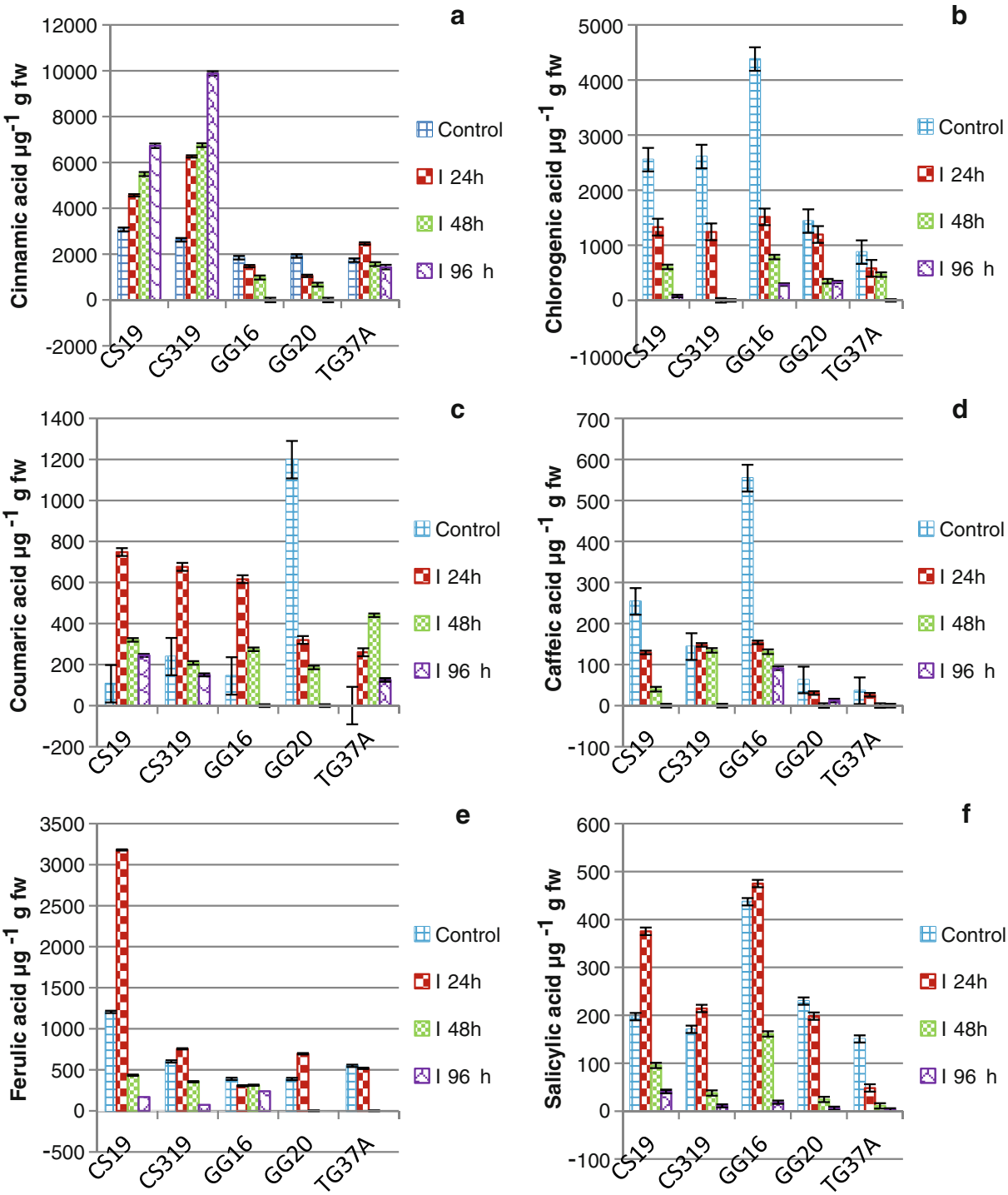

Fig. 4 Phenolic acids (**a**) Cinnamic (**b**) Chlorogenic (**c**) Coumaric (**d**) Caffeic (**e**) Ferulic and (**f**) Salicylic acid content in groundnut genotypes (stem rot tolerant: CS19 and CS319; moderately tolerant: GG16; susceptible: GG20 and TG37A) at different stages after *S.rolfsii* infection. Where; C: control 24 h and I: infected; 24, 48 and 96 stands for hour after infection. Error bar depicted as CD (0.05) value

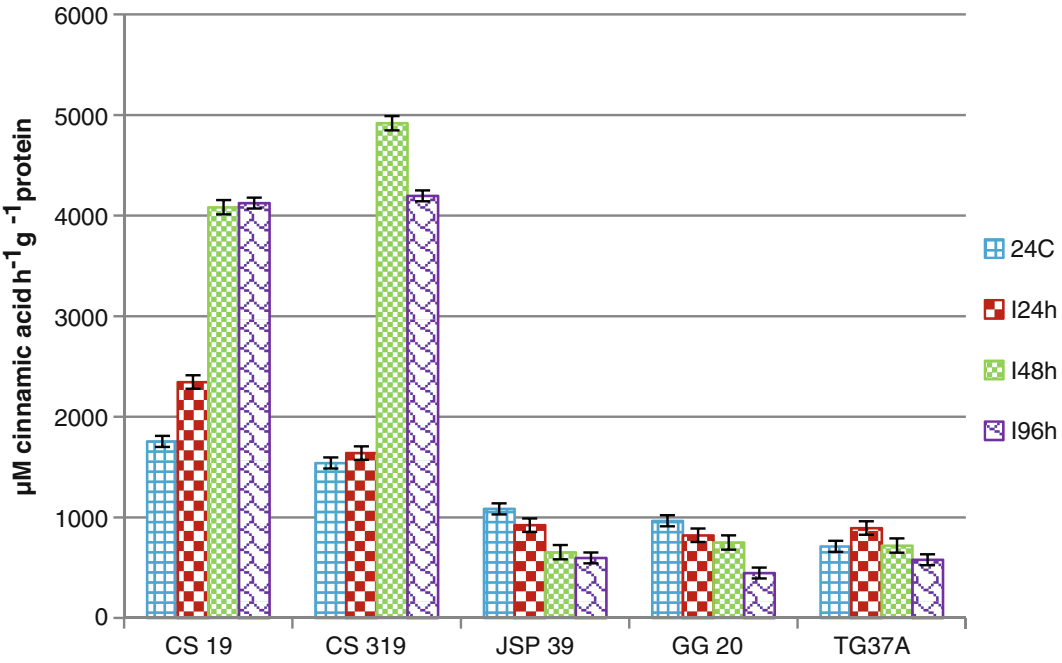

Fig. 5 Phenylalanine ammonia lyase activity in groundnut genotypes (stem rot tolerant: CS19 and CS319; moderately tolerant: GG16; susceptible: GG20 and TG37A) at different stages after *S.rolfsii* infection. Where; C: control 24 h and I: infected; 24, 48 and 96 stands for hour after infection. Error bar depicted as CD (0.05) value

susceptible genotypes after infection at different time interval (Fig. 4).

Phenylalanine ammonia lyase (PAL) activity

Constitutive level of PAL activities were observed higher in resistant genotypes. After infection, it was increased in all genotypes but resistant genotypes had 2–3 fold higher activities than that of susceptible genotypes (Fig. 5).

Discussion

Using GC-MS analysis, distinct metabolites were observed in tolerant and highly susceptible genotypes. Of the various metabolites group, sugar, phenolics and fatty acid were the most altered in groundnut genotypes during infection. Further analysis of targeted metabolites (sugars and phenols) confirmed that stem rot tolerant genotypes had higher content of glucose and sucrose and some phenolics such as cinnamic acid, caffeic acid and salicylic acid.

The earliest responses of plants to the *Sclerotium rolfsii* appeared at 24 h.a.i. (60 DAS) as a decrease of monosaccharides, particularly *myo*-inositol, glucose and fructose suggested an increasing demand on host sugars for biosynthetic intermediates to support fungal growth and sporulation. Investigation on uptake of various C^{14} labelled sugars including asymmetrically labeled sucrose by infected leaf pieces and isolated mycelial suspensions confirmed that glucose is the major carbon and energy source for fungal mycelium (Ayres et al. 1996). Similar alterations in hexoses and sucrose content were noticed in cucumber leaves from 0 to 9 days of infection by *Podosphaera xanthii* (Abood and Lösel 2003). Interestingly, sucrose a transport sugar for entry into glycolysis and the TCA cycle, observed higher in tolerant genotypes. Additionally, higher accumulation of some sugar compounds 2-deoxy ribose, D-ribofuranose, xylitol, D-turanose, glucofuranoside and mannofuranoside in tolerant genotypes also indicating availability of more intermediates or precursor for other metabolic pathways.

Furthermore, increased sugars production has been speculated to provide the phosphate sugars that are used for antioxidant pathway activity and phenolic synthesis (Shetty and Wahlqvist 2004). During infection,

pathogens reallocate the plant sugars for their own needs forcing the plants to modify their sugar content and triggering their defense responses. Sucrose induced phenylpropanoid metabolism (activity of PAL) was observed in lupine during *Fusarium oxysporum* infection (Morkunas et al. 2005, 2011). These results demonstrated that the regulation of the expression of PAL is sugar-related. In present investigation higher PAL activity in resistant genotypes is accomplished with higher glucose and sucrose content.

Higher content of defense-related and signal metabolites (e.g., salicylic acid, Thymol-.beta.-d-glucopyranoside, pentitol, glucose, sucrose, scopolin and hydroquinone), substrate and products of phenylpropanoid (cinnamic acid and caffeic acid) and lipoxygenase (linoleic acid) metabolism were observed in the tolerant genotypes. The shikimate-phenylpropanoid pathway generates numerous antioxidants (e.g.,flavonoids, phenols and lignins) and their precursors (e.g., aromatic amino acids and shikimic acid). Many of the pathogen-induced phenylpropanoids (e.g., coumarins and isoflavonoids) are considered phytoalexins because they exhibit antimicrobial properties in-vitro and accumulate in plant tissues upon infection (Kuc 1995; He and Dixon 2000). Moreover, Phenolic compound, salicyclic acid plays key roles in the signaling network leading to the establishment of both local and systemic resistance (Delaney et al. 1994; Coquoz et al. 1998; Chong et al. 2001).

Role of hydroxycoumarins such as scopoletin and scopolin is well documented in plant disease resistance. Scopoletin can be considered a phytoalexin beacause of its in-vitro antimicrobial activity (Ahl-Goy et al. 1993; Kuc 1995). Phytoalexins generally are believed to exert their antimicrobial activity toward fungi and bacteria. Increased constitutive levels of scopolin and scopoletin were also observed in a *Nicotiana* hybrid that is resistant to viral, bacterial, and fungal diseases (Ahl-Goy et al. 1993). Moreover, transgenic tobacco plants that accumulate tetrapyrrole intermediates were found to accumulate scopolin constitutively in their leaves. Because it is known that the toxicity of tetrapyrroles is exerted via reactive oxygen intermediates (ROIs), scopolin accumulation could represent a means to withstand ROIs in these plants (Mock et al. 1999). Further, Chong et al. (2002) suggested that scopoletin together with other antioxidant systems, plays a role in the regulation of ROI accumulation in living cells surrounding necrotic lesions, either as a substrate of peroxidases or as a direct ROI scavenger. Scavenging of higher accumulation of ROIs is essential for protecting cell proteins, membrane lipids, DNA and other cellular components from serious injury (Zhao et al. 2015).

Enzymatic deamination of L-phenylalanine directs the energy flow to the various branches of the general phenylpropanoid metabolism (Vogt 2010). Caffeic acid is mainly involved in lignification of plant cell walls (Rastogi and Dwivedi 2008) and observed higher in wilt resistant genotypes of castor (Jadhav et al. 2013a). These results suggest that up-regulation of these compounds are likely to re-enforced plant defense responses against invading pathogen.

Elevated level of linoleic acid in avocado elicits resistance to fungal pathogen, *Colletotrichum gloeosporioides* (Madi et al. 2003). Moreover, antifungal activity of linoleic acid has been reported for several plant pathogenic fungi (Liu et al. 2008).

Production of biologically active phyto-oxylipins begins with the oxygenation of either the 9 or the 13 position of the linoleic and linolenic acids by lipoxygenases to form 9- or 13-hydroperoxides. These are substrates for several enzymes involved in the synthesis of final oxylipins, which can act as signal molecules and/or direct antimicrobials in plant defense (Mhaske et al. 2013). Genesis of bioactive lipid mediators such as oxylipins takes place before and concurrent with the onset of pathogen-associated molecular patterns (PAMP)-triggered immunity (Howe and Schilmiller 2002). In conclusion, our study demonstrates the biological role of metabolites specifically sugars, phenolics and fatty acids in plant defense responses.

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Author contributions M.K.M. designed and performed research, collected the data and wrote the paper, L.K.T., K.S.J., P.P.T. and N.K. inoculated disease and performed research, S.K.B. analysed the data and wrote the paper, J.K.J performed the GC-MS analysis and analysed the data, B.A.G. contributed devices and interpretation of metabolites data.

Compliance with ethical standards

Conflict of interest The work published in the research article entitled "Metabolic profiles of groundnut (*Arachis hypogaea* L.) genotypes differing in *Sclerotium rolfsii* reaction" was a part of Institutional Research Project. The article has been scrutinized by the Priority setting, Monitoring and Evaluation (PME) Cell of ICAR-Directorate of Groundnut Research prior to submission and the Number DGR/PME/78 was allotted by the PME cell.

Directorate of Groundnut Research is a research institute of Indian Council of Agricultural Research (ICAR) under Ministry of Agriculture and Farmers Welfare, Government of India. ICAR is the sole financing authority of the research work carried out by the authors and the authors were not paid by any other organizations other than the Government of India.

The article is the authors' original work carried out at the Directorate of Groundnut Research. This manuscript has not been previously published, is not currently submitted for review to any other journal, and will not be submitted elsewhere before a decision is made by this journal.

No potential conflicts exist or would arise in publishing the article and all co-authors have been agreed for submission to European Journal of Plant Pathology.

The authors have not used any material, figures or anything received from any other Persons/organization/agency/anybody to carry out their research mentioned the article.

The results of the article will not have any commercial value.

The authors have no direct or indirect commercial interest in publishing the results.

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