Metabolomics of groundnut (*Arachis hypogaea* L.) genotypes during groundnut- *Sclerotium rolfsii* interaction at different stages of infection

Tatmiya Ritisha N.[1], Ambalam Padma[2] and Tomar Rukam S.[3]*
1. Department of Biosciences, Saurashtra University Rajkot, Gujarat 360005, INDIA
2. Christ Campus, Vidya Niketan, Saurashtra University, Rajkot, Gujarat 360005, INDIA
3. Department of Biotechnology and Biochemistry, Junagadh Agricultural University, Junagadh, Gujarat 362001, INDIA
*rukam@jau.in

Abstract
Groundnut (Arachis hypogaea L.) genotype exhibits different levels of resistance to fungus Sclerotium rolfsii, a causative agent of stem rot disease. The current study was aimed to evaluate the metabolomic and enzymatic response of resistant (CS-319) and susceptible (JAL-42) groundnut genotype against S. rolfsii pathogen. Both the genotypes were assessed at control, 24, 72 and 120 h Post Inoculation (HPI). To discern early metabolic markers that can differentiate amongst resistant and susceptible genotypes, GC-MS analyses and enzyme activity were performed from fresh leave samples. Whole metabolites data were processed and statistically analysed using the online statistical package MetaboAnalyst 4.0.

The result revealed presence of a total of 82 known compounds such as fatty acids, organic acids, sugar acids, sugar alcohols, sugars and other compounds. The principal component analysis showed the metabolic profile of leaves in resistant and susceptible genotype was differentiated. In addition, pathway topology analysis showed that 11 recognized pathways were significantly expressed under stem rot conditions (FDR < 0.05 in both genotypes).

Keywords: GC- MS, Groundnut, Metabolomics, *Sclerotium rolfsii*.

Introduction

Groundnut (*Arachis hypogaea* L.) is consumed as staple diet in many parts of the world including India. It is known for its health benefits with around 48 % oil, 3 % fiber, 26 % protein, high content of thiamine, niacin, calcium and compounds of medicinal importance such as resveratrol, polyphenols (p-coumaric acid, flavonoids, isoflavones), antioxidants, vitamins especially vitamin E, niacin and folic acid.[9] India is second largest producer of groundnut in the world and was grown in 4.94 m ha and yield of 6.69 m t and 1.35 t ha^{-1} respectively.[8]

Fungal infection is the serious threat to groundnut plants. Stem rot disease triggered by the fungal pathogen *S. rolfsii*, is also known for a potential hazard to groundnut producers around the world.[24] Stem rot can cause an average loss of 25-80 % in the yield.[13] The managing of stem rot infection in the groundnut field is difficult due to absence of beneficial rotational crops, undecomposed post harvest deposits in the field area (substrates for the fungal growth), fungicide resistant fungal strains and non-accessibility of resistant genotypes.[31]

Analysis of primary metabolites improves understanding about potential of the plants nutrition precursors for secondary metabolite synthesis. They facilitate biochemical and physiological changes in plants, production of defense enzymes and pathogenesis-related (PR) proteins which in turn prevent the pathogen invasion. The defense mechanism in the host plant is attributed to a specific or a group of metabolites which provides resistance against pathogens by interfering in the development and multiplication of the pathogen. The plant metabolites concerned in stress reactions includes incorporate polyols (mannitol and sorbitol), dimethylsulfonium (dimethylsulfopropionate, glycine) and sugars (glucose, sucrose, trehalose, fructan) that function as osmolytes and osmo-protectants.[30] The major secondary products are phenolics, flavonoids, anthocyanins, fatty acids, tannins and lignin which intensify them during resistance to diverse environmental stresses. The shikimic acid and malonic acid pathways form phenolic substances which are involved in plant protection against different fungal pathogens.[10]

In this study, attempts are made to profile the untargeted (whole) metabolites at different stages of *S. rolfsii* infection in resistant (CS-319) and susceptible (JAL-42) groundnut genotype using gas chromatography-mass spectrometry (GC-MS).

Material and Methods

Plant material and experimental design: Groundnut leave samples of both genotypes (CS-319 and JAL-42) were grown at the Department of Biotechnology and Biochemistry, Junagadh Agricultural University, Junagadh, Gujarat, India (21°30′23.2″N, 70°26′57.2″E). The genotypes were selected on the basis of the resistance level against stem rot disease.[3] Forty-day old plants of resistant (CS-319) as well as susceptible (JAL-42) genotypes of *A. hypogaea* were inoculated by *S. rolfsii* inoculums spread on the surface of the soil. The inoculums were prepared according to the method of Thirumalaisamy et al.[30] Infection was successfully done by *S. rolfsii* inoculums spreading in the morning time.[31]

Fresh leave samples of resistant and susceptible genotypes were collected in three biological replicates at four different stages i.e. control stage, 24, 72 and 120 h post inoculation. Simultaneously, a microscopic study was carried out to study pathogen morphology and to investigate the mycelium network around first and second collar region of stem tissue which is necessary for confirmation of pathogenic activity.[22] The summary workflow of the experiment is shown in fig. 1.

Extraction and derivatization of samples for untargeted metabolomic analysis: The metabolites were extracted from the fresh leave samples of resistant and susceptible groundnut genotype at control, 24, 72 and 120 HPI with minor modifications as described by Lisec et al.[17] The derivatization and metabolomic analysis by GC-Ms were performed according to the method of Sanimah et al[27] and Mahatma et al[19] respectively.

Data processing and statistical analysis: Untargeted metabolites data were processed and statistically analysed using the online statistical package MetaboAnalyst 4.0.[38] Data were normalized with respect to the internal standards (ribitol) and pareto scaling to put all variables on equal footing, adjust for measurement errors and minimize variable redundancy. Pareto scaling helps to increase amplification of scarce ions suppressing amplification of raw data noise. The MetaboAnalyst4.0 software was also used to carry out the multivariate statistical analyses (PCA, PLS-DA). A Partial Least Square-Discriminant Analysis was used to describe significant metabolites at each point.[15] PLS-DA is a process used to analyse large datasets and seems to have the capability to verify the linear/polynomial association among variable matrices by reducing the predictive model dimensions, allowing for simple discrimination among samples and the discrimination-causing metabolite attributes.[37] > 1 value of VIP score was considered as comparison of metabolites between control and infection stages of resistant and susceptible genotype using VIP-PLSDA.[20] A heat map analysis was performed to illustrate the relative levels and relationships of metabolites.[38]

Pathway analysis: Pathway analysis was conducted using MetPA (Metabolomic Pathway Analysis), a web-based tool integrated into the MetaboAnalyst 4.0 platform. The data of identified and detected metabolites in all samples were submitted to MetPA with annotation based on common chemical names. At the same time several pathways were tested, so the statistical p-values from the enrichment analysis were adjusted by estimating the False Discovery Rate. For pathway analysis, the *Oryza sativa* pathway library was used.

Results and Discussion

SEM analysis: SEM images of control stage, 24, 72 and 120 HPI in the resistant and susceptible genotypes were taken. There was no visible difference was observed in the transverse sections of stem observed in control, 24 and 72 HPI of both CS-319 and JAL-42 genotype. The penetration of fungal mycelium was not observed in the transverse section of first (fig. 2a) as well as second (fig. 2b) collar region of stem in CS-319 genotype at 120 HPI.

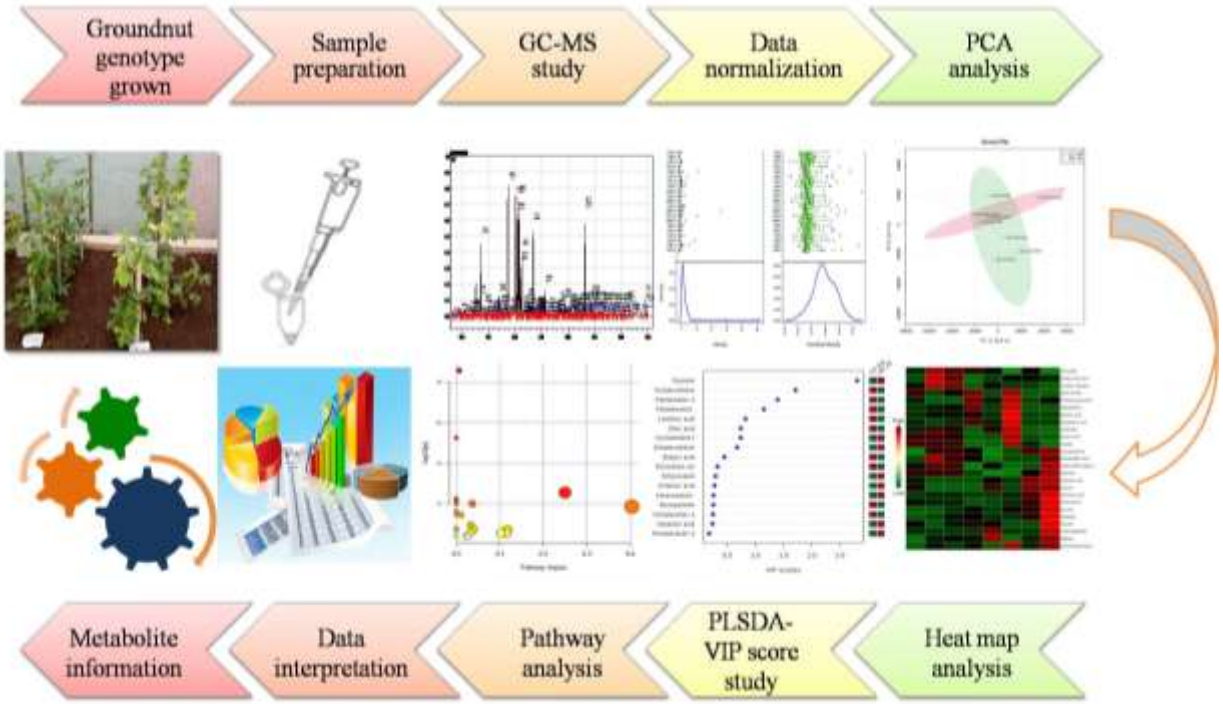

Figure 1: A detailed graphical workflow performed for the identification of metabolites in different stages of resistant and susceptible genotypes of *Arachis hypogaea* against *Sclerotium rolfsii*

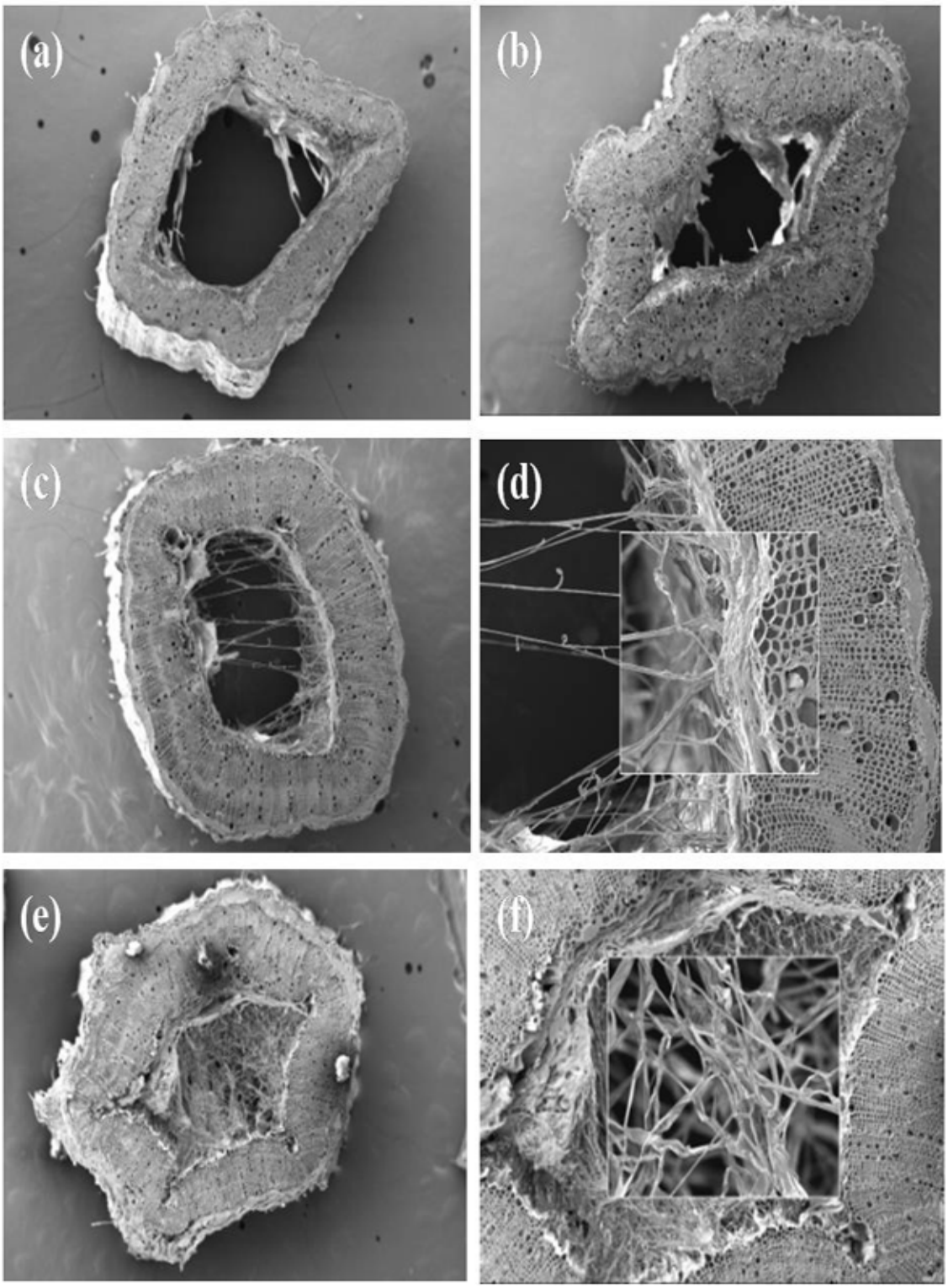

Figure 2: Scanning electron microscopy of groundnut genotype where (a) First collar region of CS-319 at 120 HPI (b) Second collar region of CS-319 at 120 HPI (c and d) First collar region of JAL-42 at 120 HPI (e and f) Second collar region of JAL-42 at 120 HPI

However, the transverse sections taken from first collar region at 120 HPI of JAL-42 showed maximum infection in the stem portion (fig. 2c and 2d) in the form of rich mycelium network connected to the surrounding tissues whereas the stem transverse section taken from second collar region (fig. 2e and 2f) showed less number of mycelia without any network.

VIP-PLSDA and PCA analysis: Metabolites were extracted from leaf samples of three biological replicates for each of the experimental classes i.e. control stage, 24, 72 and 120 HPI of CS-319 and JAL-42 plants and were analyzed by GC-MS. A measure of the variable importance in the PLS-DA is the VIP (Variable importance in projection) score. A total of 17 metabolites were found in resistant and

susceptible genotype based on >1 VIP score[33] (fig. 3). The VIP values for component 1 and log2 fold change ≥1[25] of these metabolites were shown in table 1.

In order to visualize sample grouping and reduce the dimension of the data, we conducted an unsupervised multivariate data analysis on the GC-MS generated data. According to the PCA (Principal Component Analysis) models, 5 principal components were gained from the comparison between resistant and susceptible genotype. The PCA scoring plots further supported these clustering pattern outcomes. All PCA score plot evaluated metabolites information showed two principal components that explained 50.2 % of the overall variance. Alone the first principal component (PC 1) had 28.8 % of the total variation and 21.4 % of the total variance was contributed by the second principal component (PC 2)[18] (fig. 4).

Heat map analysis: Heat map results revealed a total of 82 known structure compounds from leaf samples at various stages of SRI such as control stage, 24, 72 and 120 HPI in CS-319 and JAL-42 genotype (fig. 5). Among these, the expressions of sugars (44 %) and fatty acids (21 %) were abundant in comparison to all metabolites in both CS-319 and JAL-42 genotype (fig. 6).

Plants exhibits resistance against pathogens by giving rise to several defense responses like cell wall deposition of lignin and suberin, accumulation of specific metabolites and enzyme synthesis. A suberin component was significantly expressed in the cutin, suberin and wax biosynthesis pathway. The metabolites involved in defense systems are chiefly accomplished with plant primary and secondary metabolites of plant defense mechanisms. During pathogen infection in plants, increase of primary and secondary metabolites suggests the activation of induced systemic resistance (ISR) as described by Jahangir and co-workers in infected *Brassica leaves* by different bacterial infection.[14]

The sugars such as mannose, D-xylose, beta-L-galactopyranose, deoxy-galactopyranose, deoxy-galactopyranose, D-Gluco-hexodialdose, deoxy-ribose, turanose, deoxy-galactose and D-glycero-D-glu-heptose were found higher in control stage of CS-319 than that of control of JAL-42.

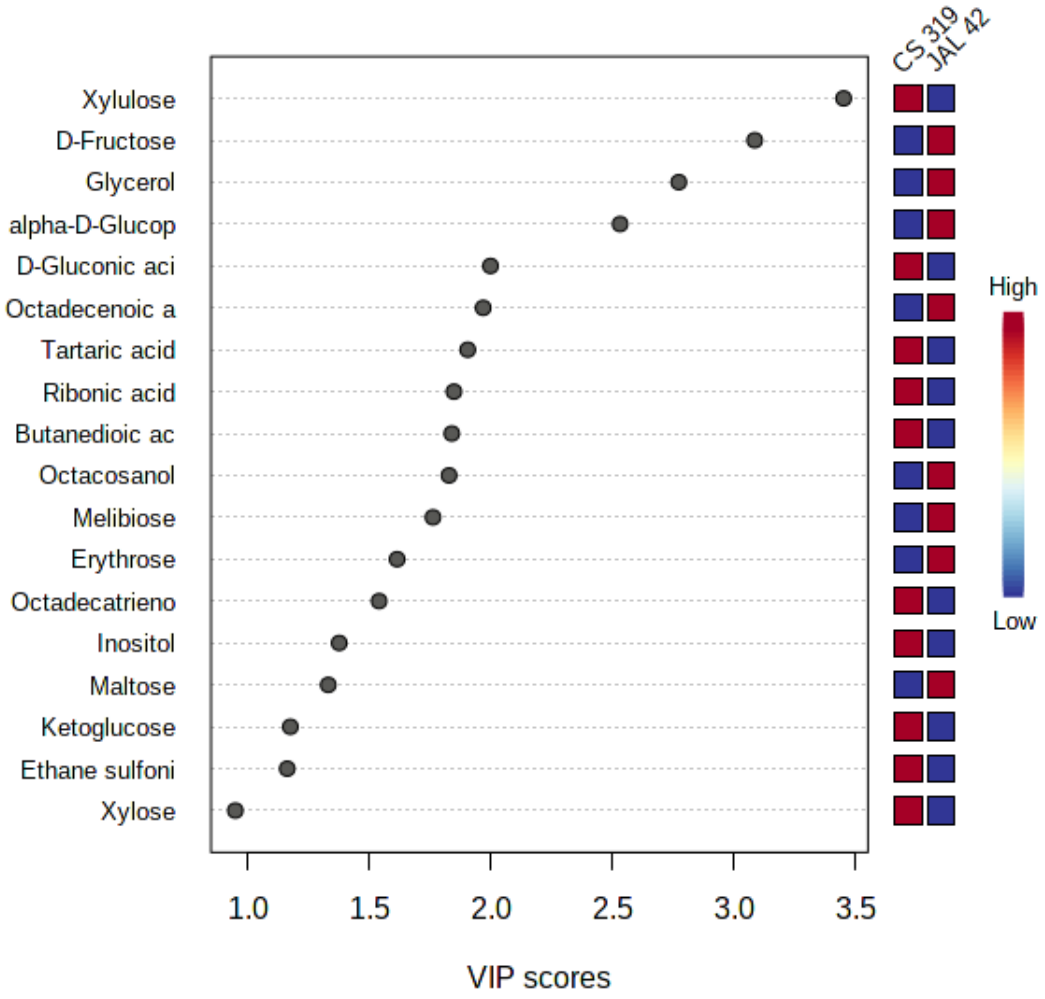

Figure 3: Important metabolites identified by PLS-DA using variable importance in projection (VIP) score through MetaboAnalyst web-based platform. Colored boxes indicate the relative concentrations of the corresponding metabolite in CS-319 and JAL-42 genotype (red, up-regulation; blue, down-regulation)

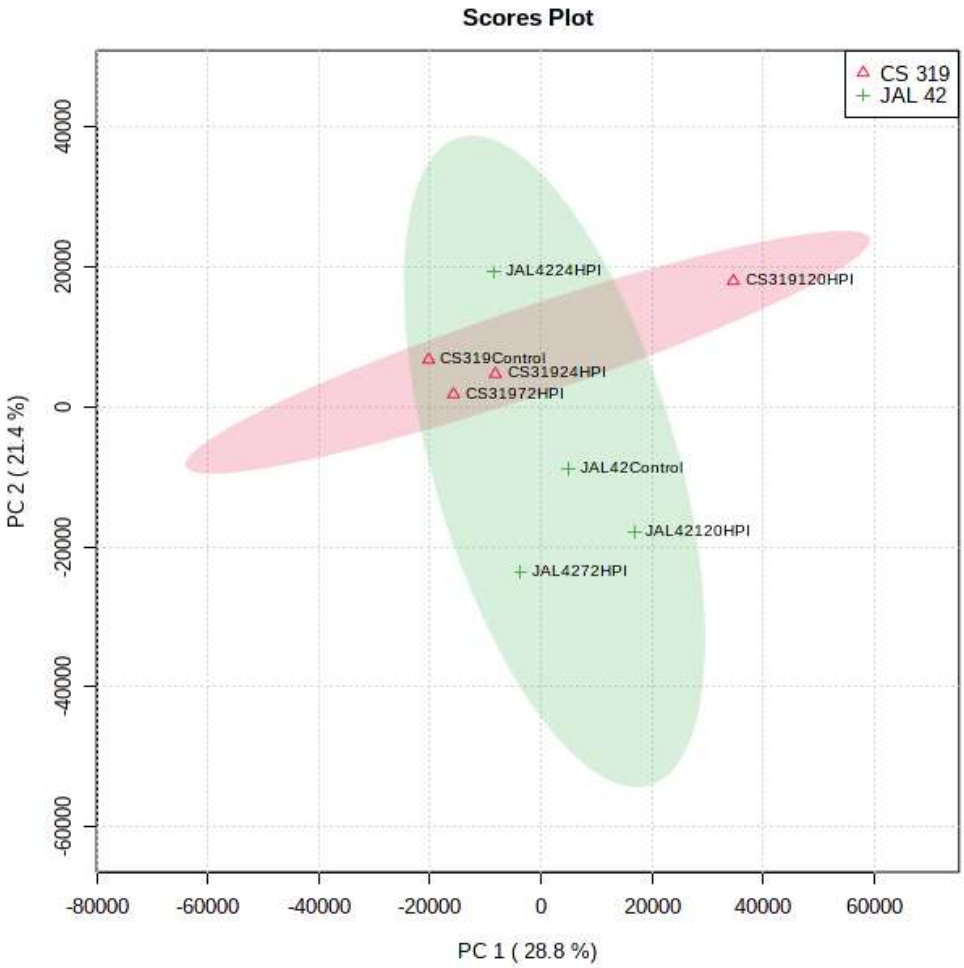

Figure 4: Multivariate statistical analysis of metabolomics data from control and post inoculation of CS 319 and JAL 42 groundnut leaves. Principal Component Analysis (PCA) score plots are shown in the upper and lower panels respectively

Table 1
VIP (Variable Importance in the Projection) values for Component 1 metabolites and fold change in comparison between CS-319 and JAL-42 genotype

S.N.	Name of metabolite	VIP value	Log2 FC
1.	Xylulose	3.4513	-0.67288
2.	D-Fructose	3.0855	1.3295
3.	Glycerol	2.7732	5.2586
4.	alpha-D-Glucopyranoside	2.5315	3.416
5.	D-Gluconic acid	1.9984	-1.2798
6.	Octadecenoic acid	1.9681	0.55947
7.	Tartaric acid	1.9046	-0.89872
8.	Ribonic acid	1.8479	-1.6364
9.	Butanedioic acid	1.839	-1.3574
10.	Octacosanol	1.8277	1.2787
11.	Melibiose	1.7615	1.2194
12.	Erythrose	1.614	0.63102
13.	Octadecatrienoic acid	1.54	-2.62
14.	Inositol	1.3757	-0.42955
15.	Maltose	1.3306	1.0717
16.	Ketoglucose	1.1749	-1.5459
17.	Ethane sulfonic acid	1.1618	-0.96083

Figure 5: Heatmap analysis showing abundance of metabolites in groundnut genotypes (stem rot resistant: CS-319 and susceptible: JAL-42) at different stages. From control, 24, 72 and 120 HPI stand for h post inoculation. On the log scale brown color indicates increased metabolites levels and dark blue represents decreased levels

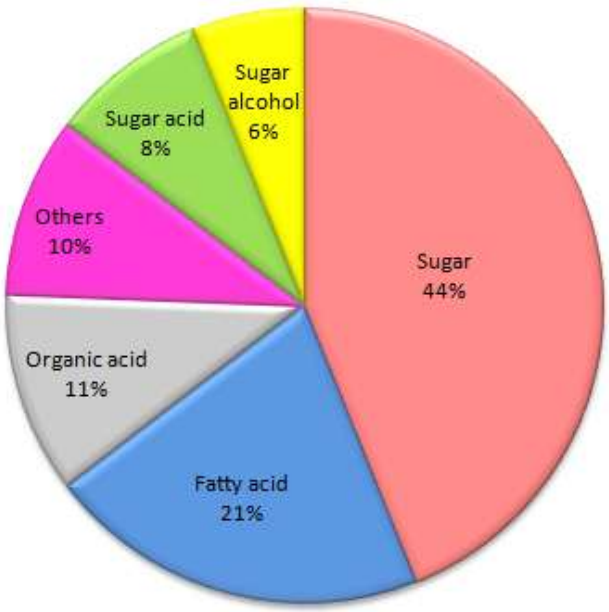

Figure 6: Whole metabolites identified by GC-MS analysis was classified into six groups as represented in the pie chart

However, in resistant genotype, distinct sugars were observed higher than susceptible genotype at different infection stages. At 24 HPI, D- galactose, xylulose, glycoside, glucopyranose and alpha-D-Mannopyranoside were found higher, whereas arabinohexosulose and galactose were found higher at 72 HPI. Sugars such as D-galactose, xylose, erythropentose, ketoglucose, lyxose, alpha-D-galactoside and N-acetyl glucosamine were observed higher at 120 HPI in CS-319. Higher content of salicylic acid and D-turanose was observed in resistant genotype than that of susceptible genotype. Also, these metabolites slowly reduced at later stage of infection. Mahatma et al[19] also reported increased level of D-turanose and salicylic acid during SRI in groundnut.

The level of glucose and D-fructose increased in the susceptible genotype at 24 HPI compared with the control. A higher concentration of glucose could suggest a role in host pathogen interactions. The sugars act as energy and carbon sources and play a signaling role in coordination with hormonal signaling pathways.[7] It was suggested that an increased demand for biosynthetic intermediates for the host sugars supported fungal development and sporulation. Examination of various ^{14}C named sugars including asymmetrically labeled sucrose from contaminated parts of leaf and isolated mycelial suspensions revealed that glucose is the important source of energy and carbon for fungal mycelium.[25]

In addition, greater accumulation of some deoxy ribose and turanose sugar in resistant genotype also indicates accessibility for other metabolic pathways of more intermediates or antecedent. The sugar compounds such as D- glucose, glycerol and alpha-D- galactose were expressed in the galactose metabolism pathway. The expanded production of sugars was theorized to supply the phosphate sugars used for phenolics acid synthesis and antioxidant pathway activity.[29] The decreased sugars level is indication of the alteration of carbohydrates metabolism such as sugar involvement in energy generation, biosynthesis of secondary metabolite as a precursor as shown by the increased level of flavonoids and phenyl propanoids known for defensive role during pathogen attack.[32]

The butyric and octadecatrienoic acid were higher in CS-319 (resistant genotype) compared to JAL-42 (susceptible genotype) in control stage. Pentanedioic acid was observed higher during 24 and 72 HPI with higher 9, 12-octadecadienoic acid was found at 72 HPI in resistant genotype. The 9, 12-octadecadienoic acid is a precursor of plant signal metabolite jasmonic acid and this underlines that up regulation of jasmonate signaling pathways was involved in groundnut defense responses against *S. rolfsii.* In response to pathogen stress, fatty acid desaturases are regulated both at transcriptional and post-translational levels.[34]

Moreover, icosanoic acid, linolenic acid, tetradecanoic acid, oleic acid, octadecenoic acid, hexadecanoic acid and monopalmitin were observed higher during 120 HPI in CS-319. The linolenic acid triggers membrane expansion fluidity resulting in intracellular segment spillage and cell death during different stress degrees.[4]

The avocado linoleic acid provides protection from *Colletotrichum gloeosporioides*, a fungal pathogen.[18] Higher content of linolenic acid (substrate of lipoxygenase), resistance related and signal metabolites e.g. glucopyranoside, pentitol and glucose metabolism were observed in the resistant genotype.[11]

These metabolites are particularly produced under stress conditions to cope with oxidative damage. The release of

18:3 by stress activated lipases from plant membrane lipids is thought to provide the lipoxygenase substrate and subsequent octadecanoid (oxylipin) pathway synthesis of JA and methyl jasmonate. In the signal regulation of a number of plant processes including wound and pathogen defence responses, the JA and methyl jasmonate was involved.[36] The expression of organic acid such as ethyl tartrate, malic acid, ethanedioic acid and propanoic acid was high in resistant genotype compared to the susceptible genotype during control stage.

It is assumed that these organic acids play important function in various metabolic processes along with exchange of electrons and protons connected to the oxidation-reduction of significant redox pairs in plant cells like NAD, NADP, ascorbate and glutathione.[12] Propanoic acid is an intermediate in the biosynthesis of benzoic and salicylic acids, both antifungal and active in plant defense signaling mechanisms.[20] Butanoic acid and propanoic acids were observed to be high in later stages of susceptible genotype.

Similarly, Hamzehzarghani et al[10] also found higher amount of butanoic and propanoic acids in *Phytophthora infestans* infected potato tubers. The ethansulfonic acid was higher at 24 HPI while phosphoric acid, salicylic acid and tartaric acid were found higher in 120 HPI of CS-319. The other compounds comprise 10 % of total classes of metabolites which include butenoic acid, pyridine carboxylic acid and uridine more in 24 HPI while succinic acid was found higher at 120 HPI in CS-319. In addition, salicyclic acid, a phenolic compound, assumes main function in the signaling system promoting the foundation of local and systemic strength.[5]

In later stages of infection in JAL-42 genotype, increased biosynthesis of a-tocotrienol was observed, in addition to the regulation of phenolics pathway. Phenolics and tocopherol level was increased in potato sprout during *Rhizoctonia solani* infection.[1] When a pathogen manages to beat constitutive resistance obstacles, it may recognize on plant cells plasma membrane.

The activating inducible plant defense reactions is probably accomplished by recognizing invariant pathogen-associated molecular patterns (PAMPs), which are normal for whole groups of microbial organisms. PAMP discernment systems trigger signaling cascades whose recognition in prevalent plant pathogen encounters is subject to responses in all ways.[23]

The sugar acid and sugar alcohol comprise 8 % and 6 % of total metabolite classes respectively. At 120 HPI, glucaric acid, ribonic acid and D-gluconic acid increased in resistant genotype. The sugar alcohol inositol was higher during control and later stages of infection in both the genotype while arabinitol was found higher in the infected genotype than in the pre infected plants. Scandiani et al[28] reported higher accumulation of inositol and arabinitol in soybean during root infection caused by *Fusarium tucumaniae*. The inositol was expressed in inositol phosphate metabolism pathway.

Moreover, accumulation of sugar alcohols like mannitol or sorbitol has been also connected to stress tolerance.[2] Metabolites such as carboxaldehyde, alpha-D-Glucopyranoside, methyl alpha-D-galactopyranoside, cyclopentane tridecanoic acid, pyridine carboxylic acid, valeric acid, pentitol were observed in susceptible genotype but their specific function was not observed.

Pathway analysis: A more comprehensive study of the related pathways and networks influenced by stem rot was carried out using MetPA (Metabolic Pathway Study), a web-based method that concatenates findings from topology analysis and efficient pathways enrichment. The statistical test conducted was hypergeometric distribution and raw p-values < 0.05 indicated substantial enhancement of some metabolites in a pathway. In addition, pathway topology analysis showed that 11 recognized pathways were significantly expressed under stem rot conditions (FDR < 0.05 in both genotypes).

This includes metabolic pathways such as galactose metabolism, fructose and mannose metabolism, starch and sucrose metabolism, biosynthesis of unsaturated fatty acids, alpha-linolenic acid metabolism, cutin, suberine and wax biosynthesis, fatty acid biosynthesis, sulfur metabolism, citrate cycle (TCA cycle), glycerolipid metabolism and inositol phosphate metabolism (fig. 7, table 2). Galactose metabolism, fructose and mannose metabolism, starch and sucrose metabolism and citrate cycle (TCA cycle) pathways were reported in plant pathogen interaction in plant of rosaceae family.[16]

Sugars play a significant role in signaling molecule and serve as metabolic precursors in plants such as galactose metabolism, GABA, fructose and mannose metabolism.[6] The precursor molecule for phyto-oxylipin biosynthesis was alpha-linolenic acid released from the membrane lipid through regulated lipase activity. The chloroplast modulation of oleic acid (18:1) level is central to the normal expression of defence responses to pathogens in *Arabidopsis.*[32]

Conclusion

A detailed metabolic profile of resistant and susceptible groundnut genotype against *S. rolfsii* revealed that arabinitol, inositol, octadecadienoic acid, propanoic acid, pentadecanoic acid, butanoic acid, salicylic acid, glucose, D-turanose and D-fructose showed important function in CS-319 against SRI. Metabolites such as carboxaldehyde, alpha-D-Glucopyranoside, methyl alpha-D-Galactopyranoside, cyclopentane tridecanoic acid, pyridine carboxylic acid, valeric acid, pentitol were observed in susceptible genotype. Thus metabolomics might prove to be appropriate approach for the rapid characterization of the cultivar response to stem rot.

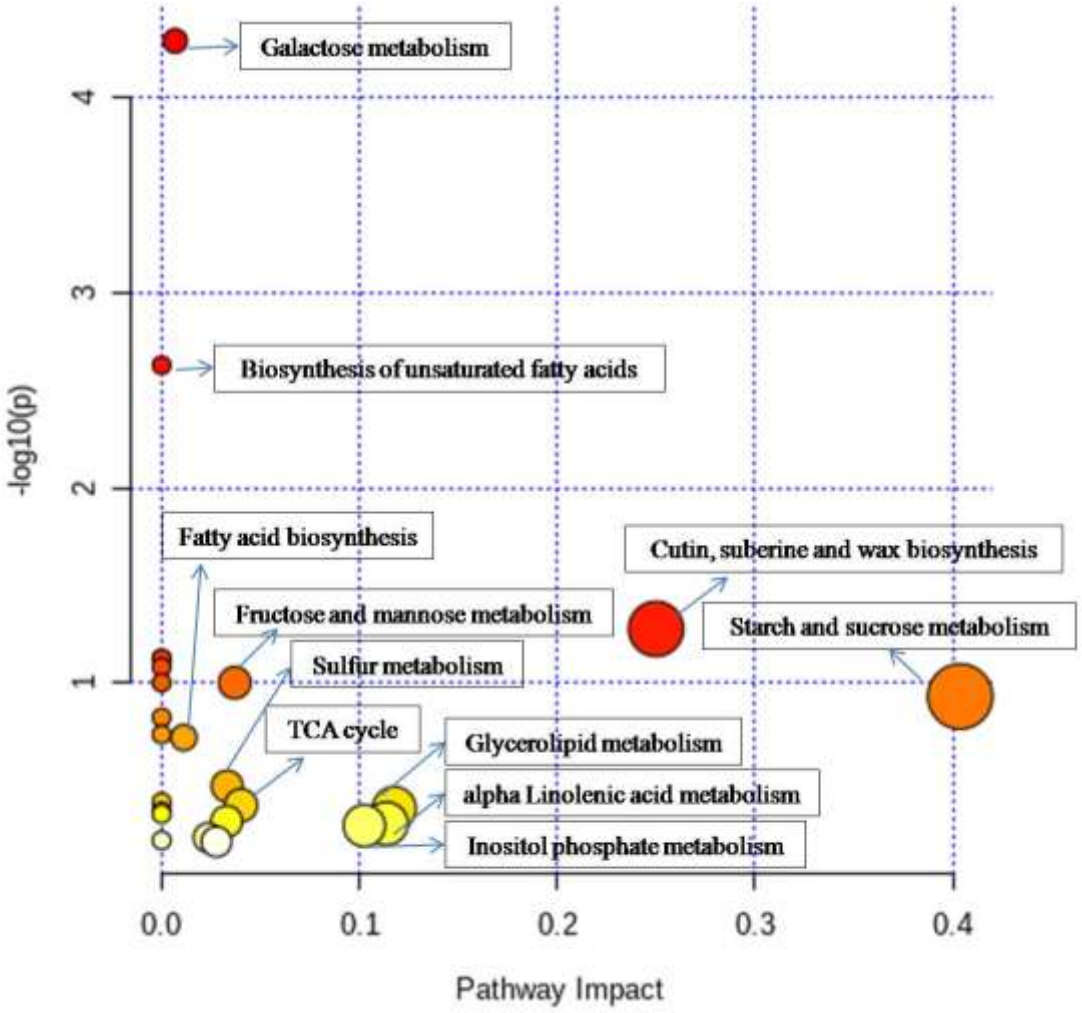

Figure 7: Metabolomic Pathway Analysis (MetPA) as generated by MetaboAnalyst software package. All the matched pathways are displayed as circles. The color of each circle is based on p-values (darker colors indicate more significant changes of metabolites in the corresponding pathway), whereas the size of the circle corresponds to the pathway impact score. The most impacted pathways having high statistical significance scores are annotated

Table 2
Detailed results from the Metabolomic Pathway Analysis (MetPA). Only pathways with FDR < 0.05 are shown

S.N.	Pathway	Total compound	Hits	Raw p F	-log(p)	FDR
1.	Galactose metabolism	27	6	5.14E-05	4.29E+00	4.88E-03
2.	Biosynthesis of unsaturated fatty acids	22	4	2.36E-03	2.63E+00	1.12E-02
3.	Cutin, suberine and wax biosynthesis	14	2	5.30E-02	1.28E+00	2.30E-02
4.	Fructose and mannose metabolism	20	2	1.00E-02	1.00E+00	1.20E-02
5.	Starch and sucrose metabolism	22	2	4.18E-03	9.30E-01	6.50E-03
6.	Fatty acid biosynthesis	56	3	1.91E-02	7.19E-01	5.50E-03
7.	Sulfur metabolism	15	1	3.39E-02	4.70E-01	4.90E-03
8.	Citrate cycle (TCA cycle)	20	1	4.96E-02	3.72E-01	2.56E-02
9.	Glycerolipid metabolism	21	1	4.41E-02	3.56E-01	3.40E-03
10.	alpha-Linolenic acid metabolism	27	1	4.87E-02	2.78E-01	3.90E-03
11.	Inositol phosphate metabolism	28	1	4.73E-02	2.68E-01	5.10E-03

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References

1. Aliferis K.A. and Jabaji S., FT-ICR/MS and GC-EI/MS metabolomics networking unravels global potato sprout's responses to *Rhizoctonia solani* infection, *PLoS One*, **7**, 8 **(2012)**

2. Arbona V., Manzi M., Ollas C.D. and Gómez-Cadenas A., Metabolomics as a tool to investigate abiotic stress tolerance in plants, *International Journal of Molecular Sciences*, **14(3),** 4885-4911 **(2013)**

3. Bera S., Kasundra S., Kamdar J., Ajay B., Lal C., Thirumalasmy P., Dash P. and Maurya A., Variable response of interspecific breeding lines of groundnut to *Sclerotium rolfsii* infection under field and laboratory conditions, *Electronic Journal of Plant Breeding*, **5(1)**, 22-29 **(2014)**

4. Buchanan B., Gruissem W. and Jones R., Biochemistry and molecular biology of plants, Rockville MD USA, American Society of Plant Biologists **(2000)**

5. Chong J., Pierrel M.A., Atanassova R., Werck-Reichhart D., Fritig B. and Saindrenan P., Free and conjugated benzoic acid in tobacco plants and cell cultures, Induced accumulation upon elicitation of defense responses and role as salicylic acid precursors, *Plant Physiology*, **125(1)**, 318-328 **(2001)**

6. Darko E., Végh B., Khalil R., Marček T., Szalai G., Pál M. and Janda T., Metabolic responses of wheat seedlings to osmotic stress induced by various osmolytes under iso-osmotic conditions, *PloS One*, **14**, 2 **(2019)**

7. Desai H.V., Mandavia M.K., Nidhi R.J. and Golakiya B.A., Metabolite profiling of mango cv. Kesar leaf using gas chromatography-mass spectrometry, *IJCS*, **6(4)**, 2731-2735 **(2018)**

8. FAOSTAT, Food and Agriculture Organization, http:// faostat.fao.org/2018 **(2018)**

9. Francisco M.L.D. and Resurreccion A., Functional components in peanuts, *Critical Reviews in Food Science and Nutrition*, **48(8)**, 715-746 **(2008)**

10. Hamzehzarghani H., Vikram A., Abu-Nada Y. and Kushalappa A.C., Tuber metabolic profiling of resistant and susceptible potato varieties challenged with *Phytophthora infestans*, *European Journal of Plant Pathology*, **145(2)**, 277-287 **(2016)**

11. He X.Z. And Dixon R.A., Genetic manipulation of isoflavone 7-O-methyltransferase enhances biosynthesis of 4′-O-methylated isoflavonoid phytoalexins and disease resistance in alfalfa, *The Plant Cell*, **12(9)**, 1689-1702 **(2000)**

12. Igamberdiev A.U. and Bykova N.V., Role of organic acids in the integration of cellular redox metabolism and mediation of redox signalling in photosynthetic tissues of higher plants, *Free Radical Biology and Medicine*, **122**, 74-85 **(2018)**

13. Iquebal M.A., Tomar R.S., Parakhia M.V., Singla D., Jaiswal S., Rathod V.M. And Kumar D., Draft whole genome sequence of groundnut stem rot fungus *Athelia rolfsii* revealing genetic architect of its pathogenicity and virulence, *Scientific Reports*, **7(1)**, 1-10 **(2017)**

14. Jahangir M., Abdel-Farid I.B., Choi Y.H. and Verpoorte R., Metal ion-inducing metabolite accumulation in *Brassica rapa*, *Journal of Plant Physiology*, **165(14)**, 1429-1437 **(2008)**

15. Karpe A.V., Beale D.J., Morrison P.D., Harding I.H., Palombo E.A. and Boden R., Untargeted metabolic profiling of *Vitis vinifera* during fungal degradation, *FEMS Microbiology Letters*, **362**, 10 **(2015)**

16. Lee D.K., Ahn S., Cho H.Y., Yun H.Y., Park J.H., Lim J. and Kwon S.W., Metabolic response induced by parasitic plant-fungus interactions hinder amino sugar and nucleotide sugar metabolism in the host, *Scientific Reports*, **6(1)**, 1-11 **(2016)**

17. Lisec J., Schauer N., Kopka J., Willmitzer L. and Fernie A.R., Gas chromatography mass spectrometry–based metabolite profiling in plants, *Nature Protocols*, **1(1),** 387 **(2006)**

18. Madi L., Wang X., Kobiler I., Lichter A. and Prusky D., Stress on avocado fruits regulates Δ9-stearoyl ACP desaturase expression, fatty acid composition, antifungal diene level and resistance to *Colletotrichum gloeosporioides* attack, *Physiological and molecular plant pathology*, **62(5)**, 277-283 **(2003)**

19. Mahatma M.K., Thawait L.K., Jadon K.S., Thirumalaisamy P.P., Bishi S.K., Jadav J.K., Khatediya N. and Golakiya B.A., Metabolic profiles of groundnut (*Arachis hypogaea* L.) genotypes differing in *Sclerotium rolfsii* reaction, *European Journal of Plant Pathology*, **151(2)**, 463-474 **(2018)**

20. Michaletti A., Naghavi M.R., Toorchi M., Zolla L. and Rinalducci S., Metabolomics and proteomics reveal drought-stress responses of leaf tissues from spring-wheat, *Scientific Reports*, **8(1)**, 1-18 **(2018)**

21. Mustafa N.R. and Verpoorte R., Chorismate derived C6C1 compounds in plants, *Planta*, **222(1)**, 1-5 **(2005)**

22. Nandi S., Dutta S., Mondal A., Adhikari A., Nath R., Chattopadhaya A. and Chaudhuri S., Biochemical responses during the pathogenesis of *Sclerotium rolfsii* on cowpea, *African Journal of Biotechnology*, **12**, 25 **(2013)**

23. Nuernberger T. and Lipka V., Non-host resistance in plants: new insights into an old phenomenon, *Molecular Plant Pathology*, **6(3)**, 335-345 **(2005)**

24. Pasupuleti J., Nigam S., Pandey M.K., Nagesh P. and Varshney R.K., Groundnut improvement: use of genetic and genomic tools, *Frontiers in Plant Science*, **4**, 23 **(2013)**

25. Peter G.A., Malcolm C.P. and Spencer-Phillips P.T., Effects of Pathogens and Parasitic Plants on Source-Sink Relationships, In Photoassimilate Distribution Plants and Crops Source-Sink Relationships, Routledge, 479-500 **(2017)**

26. Rathod V., Hamid R., Tomar R.S., Patel R., Padhiyar S., Kheni J. and Munshi N.S., Comparative RNA-Seq profiling of a resistant and susceptible peanut (*Arachis hypogaea*) genotypes in response to leaf rust infection caused by *Puccinia arachidis*, *3 Biotech*, **10**, 1-15 **(2020)**

27. Sanimah S., Maheswary V., Sarip J., Qistina O.N. and Vasanthi S., Identification of phytochemicals and the associated genes in

Eksotika papaya at ripening index 5 using functional genomics, *J Trop Agric and Fd Sc.*, **41(2)**, 283-308 **(2013)**

28. Scandiani M.M., Luque A.G., Razori M.V., CiancioCasalini L., Aoki T., O'Donnell K., Cervigni G.D. and Spampinato C.P., Metabolic profiles of soybean roots during early stages of *Fusarium tucumaniae* infection, *Journal of Experimental Botany*, **66(1)**, 391-402 **(2015)**

29. Shetty K. and Wahlqvist M., A model for the role of the proline-linked pentose-phosphate pathway in phenolic phytochemical bio-synthesis and mechanism of action for human health and environmental applications, *Asia Pacific Journal of Clinical Nutrition*, **13(1)**, 1-24 **(2004)**

30. Shulaev V., Cortes D., Miller G. and Mittler R., Metabolomics for plant stress response, *Physiologia Plantarum*, **132(2)**, 199-208 **(2008)**

31. Thirumalaisamy P., Kumar N., Radhakrishnan T., Rathnakumar A., Bera S., Jadon K., Mishra G., Rajyaguru R. and Joshi B., Phenotyping of groundnut genotypes for resistance to sclerotium stem rot, *Journal of Mycology and Plant Pathology*, **44**, 459-462 **(2015)**

32. Tomar R.S., Gajera H.P., Viradiya R.R., Patel S.V. and Golakiya B.A., Phylogenetic relationship among Mango (*Mangifera indica* L.) Landraces of Saurashtra based on DNA fingerprinting, *Journal of Horticulture and Forestry*, **13**, 379–385 **(2011)**

33. Treutter D., Significance of flavonoids in plant resistance: a review, *Environmental Chemistry Letters*, **4(3)**, 147 **(2006)**

34. Upchurch R.G., Fatty acid unsaturation, mobilization and regulation in the response of plants to stress, *Biotechnology Letters*, **30(6)**, 967-977 **(2008)**

35. Wang Y., Xu L., Shen H., Wang J., Liu W., Zhu X. and Liu L., Metabolomic analysis with GC-MS to reveal potential metabolites and biological pathways involved in Pb & Cd stress response of radish roots, *Scientific Reports*, **5,** 18296 **(2015)**

36. Wasternack C., Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development, *Annals of Botany*, **100(4)**, 681-697 **(2007)**

37. Wold S., Sjöström M. and Eriksson L., PLS-regression: a basic tool of chemometrics, Chemometrics and intelligent laboratory systems, *Chemometrics and Intelligent Laboratory Systems*, **58(2)**, 109-130 **(2001)**

38. Xia J., Sinelnikov I.V., Han B. and Wishart D.S., MetaboAnalyst 3.0-making metabolomics more meaningful, *Nucleic Acids Research*, **43(W1)**, W251-W257 **(2015)**.

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