Indian Journal of Experimental Biology
Vol. 58, July 2021, pp. 476-483

Changes in expression of polyamines and ethylene biosynthesis genes in groundnut (*Arachis hypogaea* L.) genotypes during *Sclerotium rolfsii* infection

Rakesh Nogiya[1], MK Mahatma[2]*, Avinash Gowda H[1], SS Raval[1], V Rathod[1], HP Gajera[1], RS Tomar[1] & Narendra Kumar[2]

[1]Department of Biotechnology and Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh-362 001, Gujarat, India

[2]ICAR-Directorate of Groundnut Research, Post Box No. 5, Junagadh-362 001, Gujarat, India

Received 05 March 2020; *revised 29 October 2020*

Stem rot disease caused by fungal pathogen, *Sclerotium rolfsii* Sacc., is potential threat to groundnut production in warm and humid condition. After host-pathogen interaction, a multitude of plant resistance associated reactions are initiated. In the present investigation we studied the role of polyamines and ethylene during host-pathogen interaction in stem rot tolerant (CS319, GG17 and GG31) and susceptible (TG37A) groundnut genotypes at 24, 48 and 72 h after infection. Stem rot tolerant genotypes showed higher expression of polyamine biosynthesis genes ornithine decarboxylase (*Ordec*), spermine synthase (*Sms*) and lipoxygenase1 (*LOX1*) gene at 72 h after infection than that of susceptible genotype TG37A. The expression analysis of ethylene biosynthesis genes (1-aminocyclopropane-1-carboxylate oxidase: *ACCO* and (*ACCS*) showed up regulation in stem rot susceptible genotype TG37A than that of tolerant genotypes after infection at all stages (24, 48 and 72 h after infection). The expression of amine oxidase (*AMO*) gene was observed highest in stem rot susceptible genotype TG37A while minimum in GJG31. Expression of this gene was remarkably induced in TG37A which may leads to higher accumulation of H_2O_2. Higher content of a polyamine, putrescine was found in the leaves of stem rot tolerant genotypes at 48 and 72 h after infection. These results implied that tolerant genotypes induced higher polyamine biosynthesis which may involve in plant defense and impart tolerance/ resistance. While, susceptible genotype (TG37A), utilized higher flux of S-Adenosyl methionine (SAM) for ethylene biosynthesis which may leads to necrosis of plants. Thus, stem rot resistant genotypes may be developed through genetic manipulation of polyamine biosynthesis pathway.

Keywords: Fungal, host-pathogen interaction, Stem rot disease

Groundnut (*Arachis hypogaea* L.) is an important oilseed legume, mostly grown in arid and semi-arid regions of the world. India is one among the leading producers of groundnut in the world. However, productivity of the crop is very low (1422 kg/ha) as compared to the leading producer China (3893 kg /ha) and USA (4426 kg/ha)[1] mainly because of various biotic and abiotic stresses. Among the biotic stress, stem rot diseases, caused by facultative parasitic fungi, *Sclerotium rolfsii* Sacc., is potential threat to groundnut production in warm and humid condition. The disease is prevailed at 30 to 45 days after germination and at the time of harvest under rain-fed situations due to low and erratic distribution of rainfall. Generally, fungus attacks stem of groundnut plants near the soil surface however, in the light soil, it also damage pegs and pods[2]. Due to wide host range, soil borne nature, late diagnosis of disease[3], and rapid multiplication of the pathogen development, use of the resistant variety is only feasible solution of the problem[4].

Diverse regulatory processes mediate host resistance response to pathogen infection, of which, plant hormone functions have been studied extensively[5]. Upon the perception of the *S. rolfsii* in groundnut, the jasmonic acid-mediated defense pathway gets activated, thereby inducing systemic resistance in groundnut[6]. In tomato, ethylene was imparting resistance against necrotrophic fungi *Botrytis cinerea*[7]. An early and active response of plants to perception of pathogen attack is production of ethylene which is associated with the induction of defense reactions. However, contradictory results have been obtained in different conditions and the plant-pathogen combination[8]. In response to many virulent pathogen infections in plants, ethylene evolution occurs concomitantly with the progression of disease symptoms[9]. Putrescine, spermidine, and spermine are the three most important polyamines in

*Correspondence:
E-Mail: maheshmahatma@gmail.com

plants. In both compatible and incompatible plant-microbial interactions, appreciable changes in polyamines metabolism have been observed[10]. Putrescine and spermidine content increased in maize (*Zea mays*) plants after infection with the fungal biotroph *Ustilago maydis*[11]. Similarly, six-fold increase in spermidine content observed in barley (*Hordeum vulgare*) leaves infected with the brown rust fungal biotroph *Puccinia hordei* compared to control healthy leaves and resulted in the formation of green islands surrounding the site of infection due to chlorophyll retention[12]. In contrary, a decrease in content of polyamine has been observed in tobacco leaves inoculated with fungal, bacterial and viral pathogens[13]. Biosynthesis of putrescine leads by decarboxylation of ornithine by ornithine decarboxylase or arginine by arginine decarboxylase through agmatine, subsequently putrescine is converted to spermidine, and spermine by spermidine synthase and spermine synthase enzyme respectively[14]. S-adenosyl-methionine (SAM) is a common precursor for both polyamine and ethylene[15]. In tomato fruits, ethylene induced by *Rhizopus stolonifer* infection was reported to be responsible for the decreased content of polyamine[16]. Thus, the rate of biosynthesis of both molecules regulates expression of each other, which is a decisive factor in determining the predominance of either of these two pathways during different conditions[17].

Polyamine oxidase and lipoxygenase enzymes are key players for hypersensitive reaction (HR) during host-pathogen interaction[18]. As a signal molecule, H_2O_2 derived from polyamine oxidation mediates cell death, the hypersensitive response and the expression of defense genes[19]. Lipoxygenase is able to generate different functional molecules that include (a) hydroperoxides and free radicals that might be involved in the localized cell death observed during the hypersensitive response; (b) jasmonic acid and its methyl ester that can trigger defense gene expression; and (c) 2-trans-hexenal acts as antimicrobial compounds for direct defense against pathogen attack[20].

In this context, we studied the expression of five different genes *viz*., ornithine decarboxylase, spermine synthase, ethylene biosynthesis, lipoxygenase1, amine oxidase along with polyamine profiling to elucidate the role of polyamines and ethylene in the reaction of different groundnut genotypes to the infection of facultative parasitic fungi, *Sclerotium rolfsii*.

Materials and Methods

Plant material and growth conditions

Seeds of one stem rot susceptible groundnut genotype TG37A and three Stem rot tolerant genotypes CS319, GJG17 and GJG31 obtained from ICAR- Directorate of Groundnut, Junagadh, India. These genotypes were selected based on previous reports on stem rot incidence[4,21]. Seeds were sown in earthen pots (20 kg soil capacity) in PII glass house under controlled atmospheric conditions. A potting mixture consisting Vertisol, sand and farm yard manure in a 2:1:1 ratio with diammonium phosphate at 1.0 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.

Inoculation of *S. rolfsii* in groundnut and sample collection for analysis

S. rolfsii was isolated from TG-37A genotype and cultured in 90 mm Petri dishes containing standard Potato Dextrose Agar (PDA) medium. The fungal pathogen *S. rolfsii* was further mass multiplied on sorghum grains. The fungus multiplied in sorghum grain was inoculated to about 45 days old groundnut plant by placing infested sorghum grain (about 2 g) on soil surface near the main stem. For ensuring the developing of mycelia, the experiment was carried out in controlled condition with natural photoperiod (temperature 30±2°C relative humidity above 70% and soil moisture content near field capacity) in glass house[23]. The pathogen was re-isolated to prove Koch's postulates immediately after the expression of symptoms. Disease symptoms were visually appeared after 48 h (Fig. 1). Leaf samples were collected from infected and non-infected genotypes at 24, 48 and 72 h after infection because hyphae from germinating sclerotia of *S. rolfsii* ramified over host tissue within 24-48 h following inoculation[22]. Second upper leaves were collected from 3 replications of plants at each time interval. Leaf samples used for polyamines analyses were stored at –20°C.

RNA extraction, quantification quality check and cDNA Synthesis

Fresh leaves (0.1 g) were collected at 24, 48 and 72 h after infection from infected and non-infected plants of each genotype. Total RNA was extracted from the leaves by a TRIZOL (Invitrogen) reagent as per standard protocol. The RNA samples with the ratio of 1.7-2.0 at OD 260/280 were used for cDNA synthesis. Agarose gel (0.8% w/v) was prepared in 1.0 X formaldehyde agarose gel buffer and used to check

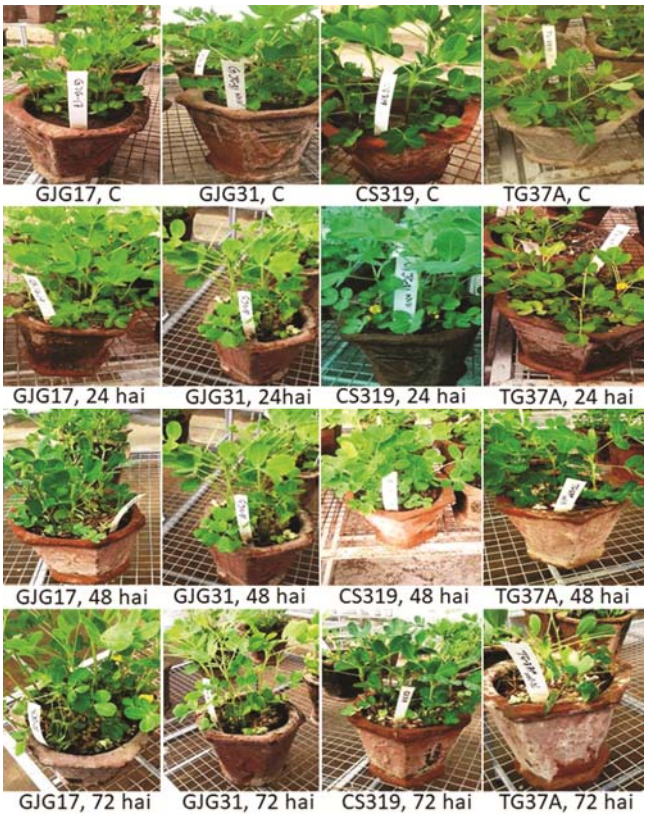

Fig. 1 — Stem rot tolerant (GJG17, GJG31 and CS319) and susceptible (TG37A) groundnut genotypes at 0 (Control), 24, 48 and 72 h after infection. Visual symptoms of disease appeared after 48 h of infection

RNA quality. The cDNA was synthesized using RevertAid™ First Strand cDNA Synthesis Kit (The Thermo Scientific™) following manufacturer's instructions.

Primers for Real Time PCR

Gene sequences for polyamine and ethylene biosynthesis genes along with amine oxidase and lipoxygenese gene of groundnut were searched on NCBI. The primers were designed by using NCBI-primer 3 software (Table 1), while for endogenous control actin primer sequences reported by Morgante *et al*.[23] were used.

Quantitative RT-PCR analysis

PCR was performed using 1.0 µL (≤100 ng/reaction) aliquot of the first strand cDNA in a final volume of 10 µL containing 10 pM of specific primers (forward and reverse). As a endogenous control, the groundnut actin primer[23] was used to normalize each sample for variations in the amounts of RNA used. PCR was carried out using 5 µL 2x QuantiFast SYBR Green PCR Master Mix (Genetix, USA), both reverse and forward primer were 0.5 µL, Template cDNA 1.0 µL and RNase free water 3 µL to final volume 10 µL in a thermal cycler (ABI-7300) programmed as follows. An initial denaturation for 5 min at 95°C, 35 amplification cycles [5 min 95°C (initial denaturation), 10 s at 95°C (denaturation), 30 s at 58°C/60°C (annealing)] for primers. Each sample was analysed in triplicate for all primers. To ensure amplification of a single product with the expected melting temperature and the absence of primer-dimers a melting curve analysis was performed on all samples. The products of each primer set were observed using agarose gel (0.7%) electrophoresis. To estimate relative gene expression, Ct value of both reference and target genes were calculated based on the mean value of three replications. Relative RNA quantities were determined with delta-delta (ΔΔ) Ct, using the formula given by Livak and Schmittgen[24].

Table 1 — List of genes and their primer sequence used for expression analysis

Name of gene	Accession no.	Primer (R- Reverse, F-Forward)
Ornithine decarboxylase (*OrDec*)	GT 735346.1	CATGGAGAATAATCAAAGGATCTTC R AAGGCATGTACTGTCAAGCCAC F
ACC oxidase 2 (*ACCO2*)	EE 125763.1	CCTCAAATGTCCAAACCCAAAG R GCCTTCCAACTTTGTCATCTTG F
ACC synthase (*ACCS*)	EE 125903.1	TGAATTCACTAGCCCATCAGG R TCCTCCCTATGTTCTTCTAGGT F
Spermine synthase (*Sms*)	GT 735283.1	GTCTAACTTTCGGGCTTGCT R CCCTCACACCCTTCTCCTAA F
Amine oxidase (*Amo*)	EE 123718.1	GTTTCAGCCATTTCGGATCTTG R AACAATGCCCAGGAGTTCTAC F
Lipoxygenase 1 (*LOX1*)	AF 231454.1	CAGTATCCTTATGGAGGGCTTATC R GGTGACTCTTCACCATCTCTTC F
Actin	Morgante *et al*.[24]	GAGCTGAAAGATTCCGATGC R GCAATGCCTGGGAACATAGT F

HPLC analysis for polyamines

Polyamines were analyzed through HPLC as described by Flores and Galston[26] with minor modifications. Polyamines were extracted from leaves in 5 % cold perchloric acid ($HClO_4$) at a ratio of about 200 mg/2mL $HClO_4$. Extracted samples were incubated for 1.0 h in an ice bath and centrifuged at 15000 rpm for 20 min. Supernatant containing the free polyamine fraction was collected in Eppendorf tube (1.5 mL) and stored at –20°C till further analysis.

Sample preparation (Benzoylation)

Plant extracts and standards (500 µL) were benzoylated using 1.0 mL of 2 N NaOH and 10 µL benzoyal chloride as per method described by Redmond andTseng[26]. Benzoyalated polyamines were extracted in 2.0 mL diethyl ether by centrifugation at

3000 rpm for 5 min. Ether phase containing polyamines was collected in 1.5 mL eppendorf tube and dried in a vacuum concentrator at 40ºC. These dried polyamines were redissolved in 100 µL methanol (HPLC grade). Standards were treated in a similar way, with up to 100 ppm of each polyamine in the reaction mixture. The benzoylated samples were stored at –20ºC till further analysis. The isocratic solvent system consisted methanol: water run at 60% at the flow rate of 1.0 mL/min. The benzoylated extracts were eluted at room temperature (25±1°C) using a C18 reverse- phase column (4.6×250 mm, 5 µm particle size) and detected at 254 nm. The quantity of individual polyamines was calculated based on area and concentration of standards.

Statistical analysis

Quantitative qPCR data were statistically compared between non-infected and infected samples at each time using two factor (genotype × time) factorial analysis and values were compared P <0.05 for significance. Data processing and time series analysis of polyamines was carried out using MetaboAnalyst 5.0, an online statistical package developed by Pang *et al.*[28].

Results and Discussion

Expression of ornithine decarboxylase and spermine synthase gene

Significant differences in the expression of polyamine and ethylene biosynthesis genes were observed at 72 h afer infection. While at 24 and 48 h afer infection no distinguish expression pattern of polyamine and ethylene biosynthesis genes were observed in susceptible and tolerant genotype. The expression of ornithine decarboxylase (*Ordec*) gene was higher in stem rot tolerant groundnut genotypes (GJG17, GJG31 and CS319) than that of susceptible genotype TG37A at 72 h afer infection (Fig. 2A). Among the different tolerant genotypes, highest expression of *Ordec* gene was observed in GJG31 followed by CS319 and GJG17 at 72 h afer infection. Expression of spermine synthase (*Sms*) gene was increased with progression of disease in all genotypes but CS319 had appreciably higher expression than other genotypes at 72 h afer infection (Fig. 2B). The expression of *Sms* gene in stem rot tolerant genotype CS319 (18.79 fold) was more than 2 fold as compared to susceptible genotype TG37A (8.10 fold) at 72 h afer infection. Higher expression of *Ordec* was further supported by higher putrescine content as shown in polyamine profiling data. Ornithine decarboxylase synthesizes diamine putrescine that is the precursor for the more complex triamine spermidine and tetraamine spermine. The enzymes spermidine synthase and spermine synthas have a key role in polyamine biosynthesis[28]. In tolerant genotype *Ordec* is activated and synthesized putrescine. The results indicate that the activation of *Ordec* is critical while activation of *Sms* is situational and time dependent.

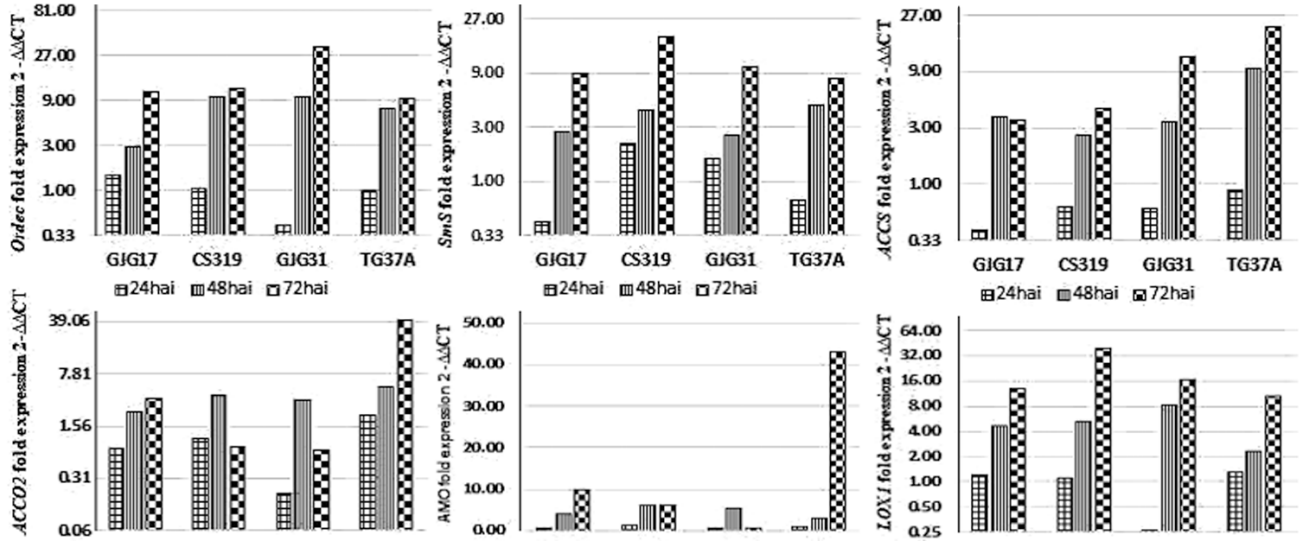

Fig. 2 — Comparative fold change expression of genes in groundnut genotypes in response to *Sclerotium rolfsii* infection at 24, 48 and 72 h time interval. Data were analyzed by two factor (genotype x time) factorial experiment and values were compared P <0.05 for significance (A) ornithine decarboxylase (*Ordec*), P <0.05: 0.335; (B) spermine synthase (*Sms*) genes, P <0.05: 0.49; (C) 1-aminocyclopropane-1-carboxylate synthase (*ACCS*), P <0.05: 0.425; (D) 1-aminocyclopropane-1-carboxylate oxidase, P <0.05: 662; (E) lipoxygenase 1 (*LOX1*), P <0.05: 0.629; and (F) amine oxidase (*AMO*) gene, P <0.05: 0.859.

Thus higher expression of polyamine biosynthesis genes in stem rot tolerant genotypes resulted in higher polyamine accumulation particularly putrescine which exhibited enhanced tolerance to stem rot pathogen. Similarly, reductions in the activities of ornithine decarboxylase and arginine decarboxylase were also observed in tomato infected with the fungus *Rhizopus stolonifer*, and these changes were accompanied by reduced polyamine concentrations[16]. Overexpression of heterologous *adc*[29] *or ordc* cDNAs leads to elevated putrescine levels in plant[30].

Expression analysis of ethylene biosynthesis genes

The expression of 1-aminocyclopropane-1-carboxylate synthase (*ACCS*) gene was observed higher in stem rot susceptible genotype TG37A at all time intervals (Fig. 2C). Tolerant genotype (GJG 17 and CS319) showed lower expression compared to TG37A. The expression of 1-aminocyclopropane-1-carboxylate oxidase 2 (ACCO2) gene was lower at early stage, but stem rot susceptible genotype (TG37A) exhibited highest expression at later stages of pathogen attack. On the other hand, tolerant genotype (CS319 and GJG31) showed significantly less expression than that of TG37A and GJG17 (Fig. 2D). These results clearly differentiate tolerant and susceptible genotypes based on ACCO expression pattern. At 48 h after infection, all genotypes showed little expression of ACCO2 gene which might start signals for defense reaction but higher expression of ACCS and ACCO2 genes may leads to sudden burst of ethylene in TG37A at 72 h afer infection and subsequent necrosis, senescence and death of plants. Our results suggested that a sub-optimal level of ethylene may require initiating ethylene mediated defense reactions but higher levels may cause plant death.

Increased ethylene evolution was also observed in tomato plant infected with *Rhizopus stolonifer*[31]. These results are further supported by observation of Brown and Lee[32]; they observed that ethylene production induces susceptibility to stem-end rot of citrus. A significant increase in ACCO gene expression was also observed in the *Xanthomonas campestris* pv. *vesicatoria* challenged tomato plants[34]. Ethylene mediated disease reaction was further confirmed by incorporation of ACC deaminase producing bacteria, *Paenibacillus lentimorbud* B-30488 in soil. These bacteria ameliorated deleterious enhanced ethylene level in *S. rolfsii* infected tomato plants. Modulated activities of *ACCO* and *ACCS* were also observed in *S. rolfsii* infected plants. These results concluded that ACC deaminase producing bacteria (B-30488) diminishing the ethylene production thereby control the southern blight disease caused by *S. rolfsii*[34]. Since the pathways for the biosynthesis of polyamines and ethylene share a common precursor S-adenosyl-methionine (SAM), an increase in ethylene evolution should, theoretically, lead to a reduction in polyamine biosynthesis. These results suggest complex regulation of SAM homeostasis[35]. New insights also placed SAM homeostasis and transmethylation in relation to promote plant virus infections, during which biosynthesis of ethylene is also important[36]. Moreover, overexpression of ethylene may inhibit jasmonic acid mediated defense response[37] which may lead to stem rot disease induction in susceptible genotypes.

Expression analysis of lipoxygenase1 (*LOX1*) and amine oxidase (*AMO*) genes

LOX1 expression induced with time dependent manner in all genotypes. However, highest expression of LOX1 gene observed in stem rot tolerant genotype CS319 at 48 and 72 h after infection (Fig. 2E). While, in susceptible genotype (TG37A) LOX1 expressed 4 times lesser than that of CS319. Similarly, in groundnut, the gene coding for PnLOX1 is induced in mature seeds infected with *Aspergillus* spp. The products of reactions catalyzed by PnLOX1 conferred a role in plant-fungus interaction to this particular lipoxygenase. The PnLOX1 product namely (13S)-hydro-peroxy-(9*Z*,11*E*)-octadecadienoic (13-HPOD) is an inhibitor and (9S)-hydroperoxy-(10*E*, 12*Z*)-octadecadienoic acid (9-HPOD) is an inducer of mycotoxin synthesis[38].

Moreover, overexpression of pepper *CaLOX1* gene in Arabidopsis (*Arabidopsis thaliana*) conferred enhanced resistance to *Alternaria brassicicola*, *Pseudomonas syringae* pv *tomato* and *Hyaloperonospora arabidopsidis*. In contrast, mutation of the Arabidopsis *CaLOX1* ortholog *AtLOX1* significantly increased susceptibility to these three pathogens. Together, these results suggest that *CaLOX1* and *AtLOX1* positively regulate defense and cell death responses to microbial pathogens[39]. The remarkable involvement of *CaLOX1*, *AtLOX1* and *RcLOX5*[40] in mediating resistance to pathogen attack suggests that these *LOX* genes are highly conserved for disease resistance in plants. Plant *LOXs* have been proposed to play a role in gene activation during wound response and necrotrophic fungal pathogen infection[40]. Higher expression of LOX1 gene in stem rot tolerant genotypes further confirmed the role of

lipoxygenase in disease resistance. Higher expression of LOX may contribute for jasmonic acid signaling pathway[41] which was identified as a possible defense mechanism in groundnut against stem rot disease[6].

Highest upregulation of amine oxidase (*AMO*) gene was observed in stem rot susceptible genotype TG37A while minimum in GJG31 at 72 h after infection. *AMO* gene expression was observed higher in tolerant genotypes at 48 h after infection, which further increased in all genotypes at 72 h after infection. However, expression of this gene was remarkably induced (about 4.5 fold) in TG37A at 72 h after infection (Fig. 1F). Polyamine oxidase is important in producing H_2O_2 *in-vivo* during host-pathogen interaction, cell growth and differentiation[420]. H_2O_2 is a mediator of several physiological events such as lignification and wall stiffening and programmed cell death. In present investigation, higher expression of PAO gene in stem rot susceptible genotype (TG37A) may cause more accumulation of H_2O_2 which leads membrane leakage and ultimately plant death. These results suggest that excess expression (beyond a threshold limit) of *AMO* and ethylene biosynthesis genes may harmful during *S. rolfsii*- groundnut interaction, however optimal level of *AMO* and *ACCS* and *ACCO* expression is essential for H_2O_2 and ethylene mediated defense reactions.

Polyamine profiling

Agmatine and putrescine content was increased in the leaves of all groundnut genotypes at 24 h after infection but concentration of agmatine was higher in stem rot susceptible genotype TG37A (Fig 3).

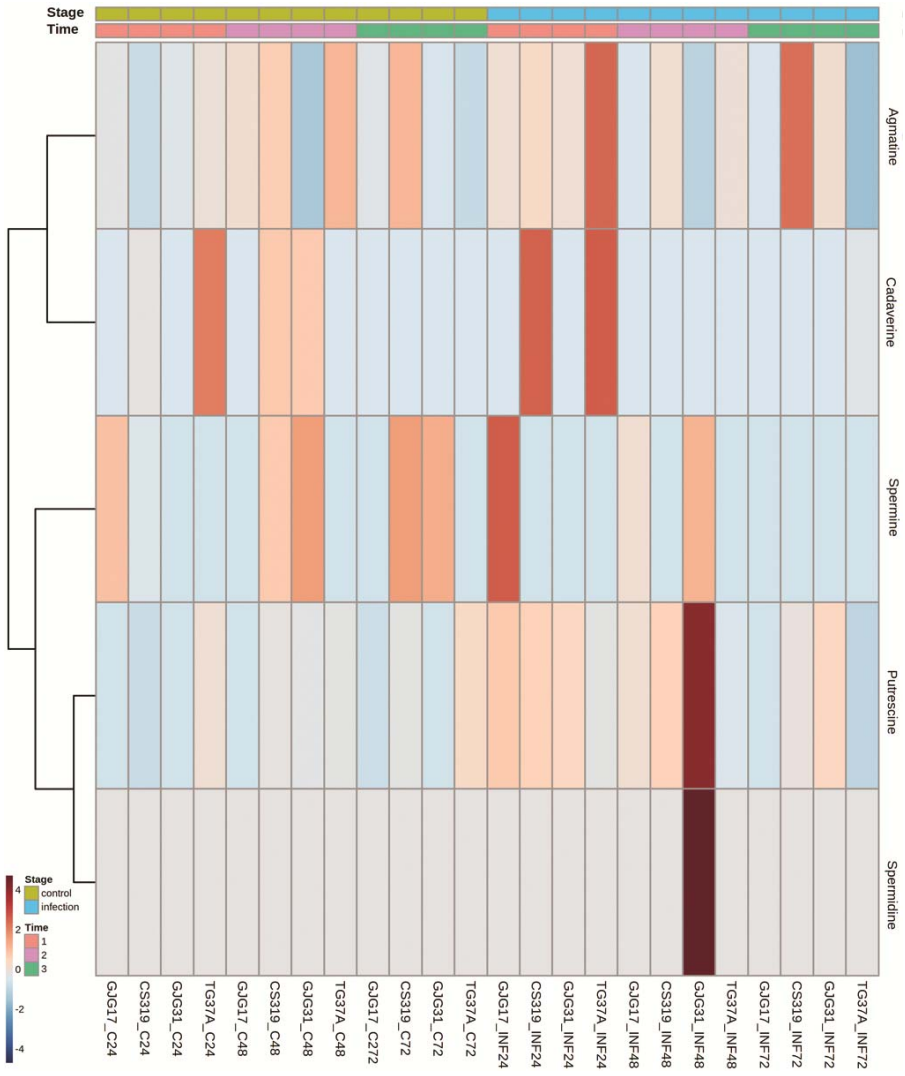

Fig. 3 — Heat map of polyamine in groundnut genotypes during *Sclerotium rolfsii*infection at 24, 48 and 72 h time interval. [Time series analysis was done using MetaboAnalyst 5.0 online software where C= control, INF= infected]

Constitutive level of putrescine and cadaverine also observed higher in TG37A. Cadaverine was increased at 24 h after infection in TG37A. However, TG37A could not maintain the level of any polyamines except agmatine at later stages of infection (48 and 72 h after infection). On the other hand, concentration of agmatine and putrescine increased in the stem rot tolerant genotypes (GJG17, GJG 31 and CS 319) at 48 and 72 h after infection. Though, the levels of polyamines were varied in genotype to genotype. Interestingly, spermine was not observed in TG37A at any stage of analysis. However, spermidine was detected only in GJG31 at 48 h after infection.

These results of polyamines profiling are supported by expression analysis of arginine decarboxylase and spermine synthase gene. Furthermore, our results are corroborated with a previous report where increased resistance against *Fusarium* wilt was observed in over-expressing arginine decarboxylase gene in transgenic eggplant[43]. Higher spermine content was also reported after *Fusarium* infection in wilt resistant genotypes of castor[18]. Higher content of spermine plays a role as a mediator in defense signaling against wilt disease caused by *Fusarium oxysporium*[43] and tobacco mosaic virus[44]. Therefore, higher levels of polyamines in resistant genotypes after infection (48 h) suggest their involvement in fungal disease resistance.

Conclusion

This study generated some basic understanding of the signals mediated defense mechanism of groundnut against *Sclerotium rolfsii* infection. These results implied that tolerant genotypes induced higher polyamine biosynthesis which may involve in plant defense and impart resistance/tolerance. While in susceptible genotype (TG37A), excess expression of ethylene biosynthesis genes at 72 h after infection may utilize higher flux of S-adenosyl-methionine for ethylene biosynthesis instead of polyamine biosynthesis. Expression of hydroperoxides producing enzymes *LOX1* was higher in tolerant genotype leads to induce hypersensitive reaction. Higher expression of LOX may contribute in JA signaling pathway which has been recently identified as a possible defense mechanism in peanut against stem rot disease. However, higher expression of amine oxidase in susceptible genotype may exert oxidative stress and higher membrane-lipid peroxidation due to pathogen infection which ultimately cause plant death. Thus, stem rot resistant genotypes may be developed through genetic manipulation of polyamine biosynthesis pathway.

Acknowledgement

One of the authors, Rakesh Nogiya is grateful to the Indian Council of Agricultural Research for awarding JRF to leading the M.Sc. (Ag) degree in the discipline of Plant Molecular Biology and Biotechnology. We thank Dr. PP Thirumalaisamy for providing *S. rolfsii* culture and Dr. SK Bera for providing seeds of CS319 genotype.

Conflict of interest

Authors declare no competing interests.

References

1 FAOSTAT 2019. http://www.fao.org/faostat/en/#data/QC/visualize, accessed on 17.07.2021

2 Bera SK, Kamdar JH, Kasundra SV & Thirumalaisamy PP, Identification of groundnut genotypes and wild species resistant to stem rot using an efficient field screening technique. *Electronic J Plant Breed*, 7 (2016) 61.

3 Dodia SM, Joshi B, Gangurde SS, Thirumalaisamy PP, Mishra GP, Dayama NK, Soni P, Rathanakumar AL, Dobaria JR, Sangh C, Chitikineni A, Chanda SV, Pandey MK, Varshney RK & Thankappan R, Genotyping-by-sequencing based genetic mapping reveals large number of epistatic interactions for stem rot resistance in groundnut. *Theor Appl Genet*, 132 (2019) 1001.

4 Mahatma MK, Thawait LK, Jadon KS, Thirumalaisamy PP, Bishi SK, Jadav JK, Khatediya, N & Golakiya BA, Metabolic profiles of groundnut (*Arachis hypogaea* L.) genotypes differing in *Sclerotium rolfsii* reaction. *Eur J Plant Pathol*, 151 (2018) 463.

5 Spoel SH & Dong X, Making sense of hormone cross talk during plant immune responses. *Cell Host Microbe*, 3 (2008) 348.

6 Bosamia TC, Dodia SM, Mishra GP, Ahmad S, Joshi B, Thirumalaisamy PP, Kumar N, Rathnakumar AL, Sangh C, Kumar A & Thankappan R, Unraveling the mechanisms of resistance to Sclerotium rolfsii in peanut (*Arachis hypogaea* L.) using comparative RNA-Seq analysis of resistant and susceptible genotypes. *PLoS ONE*, 15 (2020) e0236823.

7 Nambeesan S, AbuQamar S, Laluk K, Mattoo AK, Mickelbart MV, Ferruzzi MG, Mengiste T & Handa AK, Polyamines attenuate ethylene-mediated defense responses to abrogate resistance to *Botrytis cinerea* in tomato. *Plant Physiol*, 158 (2012) 1034.

8 Van Loon LC, Geraats BPJ & Linthorst HJM, Ethylene as a modulator of disease resistance in plants. *Trends Plant Sci*, 11 (2006) 184.

9 Lund ST, Stall RE & Kleea HJ, Ethylene Regulates the Susceptible Response to Pathogen Infection in Tomato. *Plant Cell*, 10 (1998) 371.

10 Walters DR, Polyamines and plant disease. *Phytochemistry*, 64 (2003) 97.

11 Torget R, Lapi L & Cohen SS, Synthesis and accumulation of polyamines and S-adenosyl methionine in Chinese cabbage infected by turnip yellow mosaic virus. *Biochem Biophys Res Commun*, 87 (1979) 1132.

12 Greenland AJ & Lewis DH, Amines in barley leaves infected with brown rust and their possible relevance to formation of green-islands. *New Phytol*, 96 (1984) 283.

13 Edreva A, Tobacco polyamines as affected by stresses induced by different pathogens. *Biol Plant*, 40 (1997) 317.

14 Chen D, Shao Q, Yin L, Younis A & Zheng B, Polyamine Function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. *Front Plant Sci*, 9 (2019) 1945.

15 Fluhr R & Mattoo AK, Ethylene-biosynthesis and perception. *Crit Rev Plant Sci*, 15 (1996) 479.

16 Bakanashvili M, Barkai-Golan R, Kopeliovith E & Apelbaum A, Polyamine biosynthesis in *Rhizopus*-infected tomato fruits: possible interaction with ethylene. *Physiol Mol Plant Pathol*, 31 (1987) 41.

17 Kumar SV & Rajam, MV, Polyamine-ethylene nexus: A potential target for post-harvest biotechnology. *Indian J Biotechnol*, 3 (2004) 299.

18 Mhaske SD, Mahatma MK, Jha S & Ahmad T, Polyamine metabolism and lipoxygenase activity during *Fusarium oxysporum* f. sp. *ricini* -Castor interaction. *Physiol Mol Biol Plants*, 19 (2013) 323.

19 Cona A, Rea G, Angelini R, Federico R & Tavladoraki P, Functions of amine oxidases in plant development and defence. *Trends Plant Sci*, 11 (2006) 80.

20 Croft KPC, Juttner F & Slusarenko AJ, Volatile products of the lipoxygenase pathway evolved from *Phaseolus syringae* pv *phaseolicola*. *Plant Physiol*, 101 (1993) 13.

21 Tatmiya RN, Ambalam P & Tomar RS, Metabolomics of groundnut (*Arachis hypogaea* L.) genotypes during groundnut- Sclerotium rolfsii interaction at different stages of infection. *Res J Biotechnol*, 16 (2021) 101.

22 Smith VL, Punja ZK &Jenkins SF, A histological study of infection of host tissue by *Sclerotium rolfsii*. *Phytopathology*, 76 (1986) 755.

23 Morgante CV, Guimarães PM, Martins AC, Araújo AC, Leal-Bertioli SC, Bertioli DJ & Brasileiro AC, Reference genes for quantitative reverse transcription-polymerase chain reaction expression studies in wild and cultivated peanut. *BMC Res Notes*, 4 (2011) 339.

24 Livak KJ & Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C (T)) method. *Methods*, 25 (2001) 402.

25 Flores HE & Galston AW, Analysis of polyamines in higher plants by high performance liquid chromatography. *Plant Physiol*, 69 (1982) 701.

26 Redmond JW & Tseng A, High pressurrliquid chromatographic determination of putrescine, cadaverine, spermidine and spermine. *J Chromatogr*, 170 (1979) 479.

27 Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques PE, Li S & Xia J, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, *Nucleic Acids Res*, 49 (2021) W388.

28 Antoniou C, Zarza X, Gohari G, Panahirad S, Filippou P, Tiburcio AF & Fotopoulos V, Involvement of polyamine metabolism in the response of *Medicago truncatula* genotypes to salt stress. *Plants* (*Basel*), 10 (2021) 269.

29 Capell T, Escobar C, Lui H, Burtin D, Lepri O & Christou P, Over-expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns *in vitro* and results in putrescine accumulation in transgenic plants. *Theor Appl Genet*, 97 (1998) 246.

30 Bhatnagar P, Glasheen BM, Bains SK, Long SL, Minocha R, Walter C & Minocha SC, Transgenic manipulation of the metabolism of polyamines in poplar cells. *Plant Physiol*, 125 (2001) 2139.

31 Barkai-Golan R & Kopeliovitch E, Induced ethylene evolution and climacteric-like respiration in *Rhizopus*-infected rin and nor tomato mutants. *Physiol Plant Path*, 22 (1983) 357.

32 Brown GE & Lee HS, Interaction of ethylene with citrus stem-end rot caused by *Diplodia natalensis*. *Phytopathology*, 83 (1993) 1204.

33 Yim JS, Kim KY, Lee WY, Sundaram SP, Lee Y & Sa TM, Real time expression of ACC oxidase and PR-protein genes mediated by Methylobacterium spp. in tomato plants challenged with *Xanthomonas campestris* pv. v*esicatoria*. *Plant Physiol*, 171 (2014) 1064.

34 Dixit R, Agrawal L, Gupta S, Kumar M, Yadav S, Chauhan PS & Nautiyal CS, Southern blight disease of tomato control by 1-aminocyclopropane-1-carboxylat (ACC) deaminase producing *Paenibacillus lentimorbus* B-30488. *Plant Signal Behav*, 11 (2016) 1113363e.

35 Pattyn J, Vaughan-Hirsch J, & Van de Poe B, The regulation of ethylene biosynthesis: a complex multilevel control circuitry. *New Phytol*, 229 (2021) 770.

36 Makinen K & De S, The significance of methionine cycle enzymes in plant virus infections. *Curr Opin Plant Biol*, 50 (2019) 67.

37 Ziqiang Z, Molecular basis for jasmonate and ethylene signal interactions in *Arabidopsis*, *J Exp Bot*, 65 (2014) 5743

38 Burow GB, Gardner HW& Keller NP, A peanut seed lipoxygenase responsive to *Aspergillus* colonization. *Plant Mol Bio*l, 42 (2000) 689.

39 Hwang IS & Hwang BK, The pepper 9-lipoxygenase gene *CaLOX1* functions in defense and cell death responses to microbial pathogens. *Plant Physiol*, 152 (2010) 948.

40 Mhaske SD, Mahatma MK, Jha S, Singh P, Mahatma L, Parekh VB, & Ahmad T, Castor (*Ricinus communis* L.) Rc-LOX5 plays important role in wilt resistance. *Ind Crop Prod*, 45 (2013) 20.

41 Chauvin A, Caldelari D, Wolfender J-L & Farmer EE, Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded *Arabidopsis thaliana* leaves: a role for lipoxygenase 6 in responses to long-distance wound signals. *New Phytol*, 197 (2013) 566

42 Cervelli M, Cona A, Riccardo A, Polticelli F, Federico R & Mariottini P, A barley polyamine oxidase isoform with distinct structural features and subcellular localization. *Eur J Biochem*, 268 (2001) 3816.

43 Prabhavathi VR & Rajam MV. Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. *Plant Biotechnol*, 24 (2007) 273.

44 Yamakawa H, Kamada H, Satoh M & Ohashi Y, Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. *Plant Physiol*, 118 (1998) 1213.