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DEFENCE RESPONSE TRIGGERED BY SCLEROTIUM ROLFSII IN GROUNDNUT (ARACHIS HYPOGAEA L.) PLANTS

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ABSTRACT

Total phenols and *ortho*-dihydroxy phenols from healthy and *Sclerotium rolfsii* inoculated hypocotyls regions of groundnut plants were estimated at different stages of disease development after 10 days of inoculation. The contents of total phenol and *ortho*-dihydroxy phenols were increased throughout the sampling period of the disease compared to healthy plants. Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and Peroxidase (PEO) activities were also increased at different stages of disease development. The results showed that the changes in the levels of phenols, *ortho*-dihydroxy phenols and the enzymes like PAL, PPO and PEO form an important part in the resistance mechanism of the groundnut plants against infection with *S.rolfsii*.

Keywords: *Sclerotium rolfsii*, phenols, defence response, phenyl ammonia lyase, peroxidase, polyphenol oxidase, groundnut

INTRODUCTION

Among the soil-borne fungal diseases of groundnut, stem rot or southern blight caused by *S.rolfsii* (Doidge and Bottomly, 1931) is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions (Tiwari *et al*., 2004; Ganesan *et al*., 2007). *S.rolfsii* causes severe damage during any stage of crop growth (Cilliers *et al*., 2000; Ganesan *et al*., 2007) and attacks all parts of the plant but stem infection is the most common and serious and yield losses over 25% have been reported (Mayee and Datar, 1988).

Resistance in plants towards pathogens depends on various factors. Several authors have demonstrated a distinct correlation between the degree of plant resistance and phenolics present in plant tissues (Nicholson and Hammerschmidt, 1992; Bhagat and Chakraborty, 2010). The role of phenolic compounds in the host-pathogen interaction is well established (Sarma *et al*., 2002) and constitutive phenolics are known to confer resistance indirectly through activation of post-infection responses in the host. Several studies have shown that some phenolics are inhibitors associated with non-host resistance (Nicholson and Hammerschmidt, 1992) whereas others are formed or increased in response to the pathogen infection, and are considered to be an important component in the defence response of the host to the pathogen (Punja *et al*., 1985; Nicholson and Hammerschmidt, 1992) Present investigation was undertaken to study the role of phenolics during the progression of disease development.

MATERIALS AND METHODS

Isolation of pathogen:

During the survey of groundnut fields around Srikalahasti and Tirupati areas of Chittoor District, and also other Rayalaseema Districts of A.P. *Sclerotium rolfsii* was found to be associated with the infected hypocotyls region of groundnut

variety TMV-2 at early stages of growth and development. The plants showing stem rot or southern blight symptoms were brought to the laboratory for making isolation according to the tissue segment method on PDA, pure culture was obtained by transferring the sclerotia to PDA plates. The stock culture was maintained on PDA slants in a refrigerator at 10ºC and subcultured for every two months.

Disease development

Method of raising plants: High quality seed material (average 95% germination) of groundnut variety TMV-2 was obtained from the local Agricultural Research. Sound seeds were surface sterilized with 0.1% mercuric chloride for 2-3 min followed by repeated washings with sterile water and sown in sterilized soil contained in seed pans. The seeds germinated and emerge in 4-5 days. One week old seedlings were used for inoculation purposes.

a) Inoculation:

Ten days old oatmeal-sand culture of *S.rolfsii* was thoroughly mixed with sterilized soil at 10%. This inoculum-soil mixture was then distributed in 12" diameter earthenware pots and left undistributed for two days. After this period, one week old seedlings grown in seed pans were lifted carefully without causing much damage to the root system and transplanted into the pots. They were watered on alternate days and kept in an open atmosphere.

c). Disease indexing: The plants were periodically examined for the progress of the disease. Samples were collected at random from four pots each time at '0' hours, 2 days, 5 days, 9 days and 11 days after inoculation. The pots, from which samples drawn were rejected. Almost all the seedlings collapse by 11 days after transplantation. The progress of disease in the hypocotyls of the seedlings could be differentiated into the following five fairly distinct stages, on the basis of lesion development.

Stage 1: (0 hours, i.e. immediately after inoculation): Healthy seedlings

Stage 2: (2 days after inoculation): The early or young phase, characterized by water-soaked appearance of invaded portions of hypocotyls which remained almost colourless or were light brown in colour.

Stage 3:(5 days after inoculation): The intermediate stage, in which the lesion surface become brown to dark brown in colour.

Stage 4: (9 days after inoculation): Well developed, dark necrotic lesions often girdling hypocotyls. This marks the final stage in lesion maturation. The lesions also show sunken appearance.

Stage 5:(11 days after inoculation): Characterized by dry appearance of the lesion surface. Downward destruction or rotting of the tap root occurs and then the seedlings wilt and die.

Symptoms characteristic of each of the above stages of lesion maturation are shown in Figure 1.

Collection of host tissue

For extraction of phenolics, hypocotyl regions with lesions and the corresponding hypocotyl portion of healthy seedlings were collected at the five different stages of lesion development cited above. They were washed thoroughly with distilled water to remove adhering soil particles and used immediately for extraction.

Extraction and estimation of phenolics:

The method of extraction was similar to that described by Mahadevan *et al*., (1965). The plant material was chopped into small pieces and one gram of the material was transferred to about 10 ml of boiling 80% ethanol, extracted for five minutes on a boiling water bath by refluxing and cooled in tap water. The material was homogenized in a mortar with pestle and squeezed through a wet cheese cloth. The material was transferred back to a small quantity of fresh boiling 80% ethanol and reextracted for five minutes. Both the extracts were pooled and centrifuged. The supernatant was concentrated on boiling water bath to 5 ml.

Estimation of *ortho*-dihydric phenols:

Ortho-dihydric phenols (OD) were estimated by employing Arnow's reagent, which is specific to *ortho* groups (Johnson and Schaal, 1957). To one ml of the ethanol extract in a test tube, one ml of 0.5 HCl, one ml of Arnow's reagent and 2 ml of 1N NaOH were added. The volume was raised to 12.5 ml with distilled water and the light pink colour which developed immediately was read in Spectrnic-20 colorimeter at 522 nm. A reagent blank was obtained with one ml of distilled water instead of ethanol extract. The quantity of OD phenols in the sample was calculated from a standard curve prepared for an authentic sample of catechol.

Estimation of total phenols:

To one ml of ethanol extract in a test tube, one ml of Folin-Ciocalteu reagent and 2 ml of 20 per cent sodium carbonate were added. The mixture was heated on a boiling water bath for exactly one minute and cooled, resulting in the development of blue colour. It was diluted to 25 ml with distilled water and absorbance was determined in Spectronic-20 colorimeter at 725 nm. A blank was maintained with one ml of distilled water in the place of ethanol extract. Total phenols were calculated from the standard curve plotted for catechol.

Extraction and assay of Polyphenol oxidase and peroxidase:

The procedure adopted for determining the activities of polyphenol oxidase (PPO) and peroxidase (PEO) was essentially that of Fahrmann and Dimond (1967).In a chilled glass mortar (0^0C) 0.5 g of freshly harvested material was ground with 20 ml of 0.1M ice cold phosphate buffer and centrifuged at 2000 rpm for 10 minutes. The supernatant was made up to 25 ml and used for assay. The enzyme extracts were prepared in buffer of pH 6.1 for PPO and pH 7.2 for PEO.

To determine the PPO activity, catechol (0.1 M) was used as the substrate. The reaction mixture consisted of, 0.5 ml of enzyme extract, 0.2 ml of 0.1 M catechol, 2.3 ml of 0.1 M phosphate buffer pH 6.1. Change in absorbance at 400 nm at intervals of 15 seconds from zero time of reaction, was determined using a Spectronic-20 colorimeter. For PEO activity the reaction mixture was 1ml of enzyme extract, 0.1 ml of 0.2M pyrogallol and 0.5 ml of 0.01M hydrogen peroxide. Activity was measured by the change in absorbance at 430 nm at 15 sec intervals from zero time. Specific activity of the enzyme was calculated according to the method of Fric and Fuchs (1970) and expressed as per mg protein.

Extraction and assay of Phenylalanine ammonia lyase (PAL-ase) activity:

The activity of phenylalanine ammonia – lyase (PAL-ase) was determined according to the method of Biehn *et al.,* (1968) with slight modifications. One gram of freshly harvested material was ground in a chilled mortar with 10 ml of ice cold 0.1M borate buffer, pH 8.8, at 4-5^0C in cold room. The homogenate was filtered through several layers of muslin cloth. The extract was then centrifuged in the cold at 10,000 g for 30 min. The supernatant was made up to 15 ml and used for the assay of PAL-ase activity. The reaction mixture consisted of 2 ml of enzyme extract, 1 ml of 0.05M L-phenylalanine and 2 ml of 0.1M borate buffer pH 8.8. The reaction mixture was incubated for 1 hr in a test tube at 40^0C and then the reaction was stopped by adding 0.1 ml of 5 N HCl. A zero time control prepared as described above was assayed at the same time. The acidified reaction mixture was evaporated to dryness, the residue dissolved in 0.05 N NaOH and the absorbancy were measured at 268 nm in Hilger and Watts Spectrophotometer. The values, in terms of cinnamic acid formed were calculated from a standard curve prepared for known amounts of cinnamic acid.

RESULTS

Ortho-dihydroxy phenols of healthy and *S.rolfsii* inoculated hypocotyls of different stages of disease development were extracted after 10 days of inoculation. Results (Figure 2) showed that

ortho-dihydroxy phenols (OD phenols) in healthy groundnut hypocotyls increased gradually throughout the sampling period. The inoculated plants contained higher concentrations of OD phenols, as compared with healthy ones, from the time of initiation of the disease. At later stages of disease development, when the rotting developed fully, the extent of increase was significant.

Similarly results revealed that the total phenol content also increased slightly with age, in healthy plants. Infection with *S.rolfsii* was associated with more increase in total phenol content of the infected hypocotyls.

Changes in enzyme activities

Polyphenol oxidase (PPO)

The results (Figure 3) PPO activity was increased with increasing the stages of disease development. The activity of the enzyme showed slight and gradual increase in the healthy hypocotyls. Infection had a pronounced effect on PPO activity and resulted in steep increase at various stages of disease development.

Peroxidase (PEO):

Infection resulted in considerable increase in the activity of peroxidase at all stages of disease development (Fig. 3) compared with the corresponding healthy controls. The activity of peroxidase showed a slight and gradual increase in the corresponding infected hypocotyls.

Phenylalanine ammonia-lyase activity (PAL-ase):

Results (Fig.3) showed that PAL-ase activity of in healthy plants, the activity was increased gradually throughout the experimental period. In infected plants, its activity was also higher as compared with healthy controls. The increase was more pronounced from stage 3 onwards.

DISCUSSION

In the present study, total and *ortho*-dihydric phenols increased with the disease development compared to healthy seedlings. Increased synthesis of existing phenolic compounds, synthesis of new compounds and increased activity of phenolase are typical symptoms of diseased plant tissues. Perturbations in phenolic metabolism due to infection by a wide range of fungal pathogens have long been recognized and have been covered in a number of reviews (Rohringer and Samborskui, 1967; Gupta *et al*., 1987).

The role of phenolic compounds in the host-pathogen interaction is well established and constitutive phenolics are known to confer resistance indirectly through activation of post-infection responses in the host (Sarma *et al*., 2002). Several studies have shown that some phenolics are inhibitors associated with non-host resistance (Nicholson and Hammerschmidt, 1992) whereas others are formed or increased in response to the pathogen infection, and are considered to be an important component in the defense response of the host to the pathogen (Hammerschmidt *et al*., 1982; Punja *et al*., 1985; Nicholson and Hammerschmidt, 1992; Bhagat and Chakraborty., 2010) Present investigation revealed marked increase in total phenol content in the infected tissues than in the healthy ones. This suggests an increased synthesis of phenols not only with aging of plants but also their further stimulation as a result of infection. Increase in total phenol content in infected plants was also observed by several workers in various host-pathogen interactions (Farkas and Kiraly, 1962; Reddy and Rao, 1974; Brahamchari and Kolte, 1983; Mahto *et al*., 1987; Sindhan and Jaglan, 1987; Padmanabhan *et al*., 1988).

The content of *ortho*-dihydric phenols also increased predominantly with infection. Similar results were also observed by others in many host pathogen interactions (Sharma *et al*., 1983; Gupta *et al*., 1985; Sindhan and Jaglan, 1987). These phenols are resistant factors because they become highly reactive upon oxidation and may result in the formation of substances toxic to the pathogen or which inactivate enzymes including hydrolytic enzymes produced by plant pathogenic fungi (Byrde, 1963; Patil and Dimond, 1967).The increased accumulation of phenols in the infected

tissues may be explained by their release from the glycosides or esters by enzymatic (β-glycosidase) activity of the pathogen and /or the host tissues (Noveroske *et al*., 1964 a; Pridham, 1965), enhanced synthesis by the host, through shikimic acid pathway (Neish, 1964), production of phenolic compounds by the parasite (Farkas and Ledingham, 1959) or migration from uninfected tissues (Rubin and Artsikhovskaya, 1964).

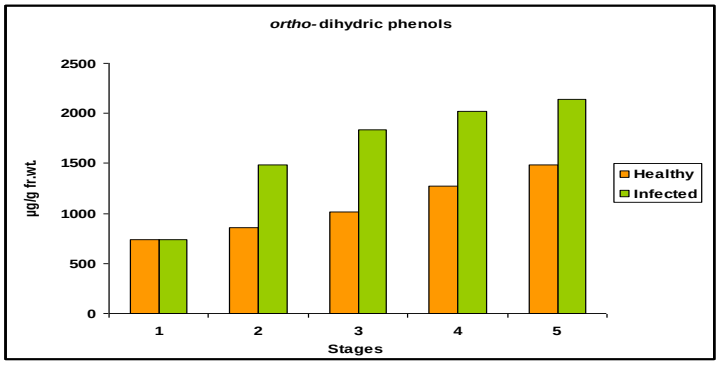
REFERENCES

1. Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. *N. C. Agri. Exp. Stn. Tech. Bull.*174.
2. Biehn, W. L., Kuc, J. and Williams, E.B. 1968a. Accumulation of phenols in resistant plant-fungi interactions. *Phytopathology* 58:1255-1260.
3. Brahmachari, B.K. and Kolte, S.T. 1983. Total phenols, chlorophylls and reducing sugars in the leaves are generally considered to be responsible for disease resistance in different crops. *Indian Phytopathology* 36:149-150.
4. Byrde, R.J.W. 1963. Natural inhibition of fungal enzymes and toxins in disease resistance. Pages 31-47. In: S. Rich (ed.), Perspectives of biochemical plant pathology. *Connecticut Agr. Exper. Sta. Bull*: 663.
5. Cilliers, A.J., Herselman, L. and Pretorius, Z.A.2000. Genetic viability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. *Phytopathology* 90 (9): 1026-1031.
6. Doidge, E.M. and Bottomly, A.M. 1931. A revised list of plant diseases occurring in South Africa. *Bot. Surv. S. Afr. Mem*.11.
7. Farkas, G.L. and Kiraly, Z. 1962. Role of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopathology* Z 44: 105-150.
8. Farkas, G.L. and Ledingham, G.A. 1959. Studies on the polyphenol-polyphenol oxidase system of wheat stem rust uredospores. *Canadian Journal of Microbiology* 5:37-46.
9. Fehrnann, H. and Dimond, A.E. 1967. Peroxidase activity and *Phytophthora* resistance in different organs of potato plant. *Phytopathology* 57:69-72.
10. Fric, F. and Fuchs, W.H.1970. Veranderunger der aktivital eninger enzyme in Weizenblatt in Abhangig keit vonder temperature labilen vestraglichkeit fur *Puccinia graminis tritici. Phytopathology:* Z .67:161-174.
11. Ganesan, S., Ganesh, K.R. and Sekar, R. 2007. Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.*)* using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). *Turk. J. Agric. For* 31: 103-108.
12. Gupta, P.P., Gupta, S.K., Kaushik, C.D. and Yadav, T.P. 1985. Biochemical changes in leaf surface washing of groundnut due to tikka disease (*Cercospora personatum*). *Indian Phytopathology* 30:339-340.
13. Gupta, S.K., Gupta, P.P., Kaushik, C.D. and Saharan, C.S. 1987. Biochemical changes in leaf surface extract and total chlorophyll content of sesame in relation to Alternaria leaf spot disease (*Alternaria sesame n*.). *Indian Journal of Mycology and Plant Pathology* 17(2):165-168.
14. Hammerschmidt, R., Nuckles, E.M. and Kuc, J. 1982. Association of enhanced peroxidase activity with induced systematic resistance of cucumber to *Colletotrichum lagenarium. Physiological Plant Pathology* 20: 73-82.
15. Indramani Bhagat and Bishwanath Chakraborty.2010. defence response triggered by Sclerotium rolfsii in tea plants. Ecoprint 17:69-76.
16. Johnson, G. and Schaal, L.A.1957. Chlorogenic acid and other *ortho*-dihydric phenols in scab resistant Russet Burbank and scab susceptible Trium potato tubers of different nutrients. *Phytopathology*, 47:253-258.

17. Mahadevan, A., Kuc, J. and Williams, E.B. 1965. Biochemistry of resistance in cucumber against *Cladosporium cucumerinum* 1. Presence of pectinase inhibitor in resistant plant. *Phytopathology* 55:1000-1003.
18. Mahto, B.N., Singh, R.N., Awasthi, C.P and Abidi, A.B.1987. Sugars and phenolic compounds in rice leaves in relation to varietal resistance to bacterial blight pathogen. IRRN 12(4):12-13.
19. Mayee, C.D. and Datar, V.V. 1988. Diseases of groundnut in the tropics. *Review of Tropical Plant Pathology* 5: 169-198.
20. Neish, A.C.1964. Major pathways of biosynthesis of phenols. Pages 295-359. In: Harborne, J.B. (ed.), Biochemistry of phenolic compounds. *Academic Press*. New York. 618 p.
21. Nicholson, R. L. and Hammerschmidt, R. 1992. Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* 30: 369-389.
22. Noveroske, R.L., Kuc, J. and Williams, E.B. 1964a. Oxidation of phloridzin and phloretin related to resistance of Malus to *Venturia inaequalis. Phytopathology* 54: 92-97.
23. Padmanabhan, P., Alexander, K.C. and Shanmugam, N. 1988. Some metabolic changes induced in sugarcane by *Ustilago scitaminea*. *Indian Phytopahtology* 41(2): 229-232.
24. Patil, M.B. and Rane, M.S. 1983. Studies on host range effect of plantage on susceptibility and varietal reaction of groundnut to *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology* 13(2):183-186.
25. Patil, S.S. and Dimond, A.E. 1967. Inhibition of *Verticillium* polygalacturonase by oxidation products of polyphenols. *Phytopathology* 57: 492-496.
26. Pridham, J.B.1965. Low molecular weight phenols in higher plants. *Annual Review of Plant Physiology* 16:13-36.
27. Punja, Z.K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23:97-127.
28. Punja, Z.R., Smith, V.L., Campbell, C.L. and Jenkins, S.F. 1985. Sampling and extraction procedure to estimate numbers, spatial pattern and temporal distribution of sclerotia of *Sclerotium rolfsii* in soil. *Plant diseases* 69: 469-474.
29. Reddy, M.N., Rao, A.S. and Rao, K.N. 1975. Production of phenolic compounds by *Rhizoctonia solani*. *Trans. Brit. Mycol. Soc.* 64: 146-148.
30. Rohringer, R. and Samborski, D.J. 1967. Aromatic compounds in the host-parasite interaction. *Annual Review of Phytopathology* 5: 77-86.
31. Rubin, B.A. and Artsikhovskaya, E.V. 1963. Biochemistry and physiology of plant immunity. 358p. Pergamon Press. Oxforde.
32. Sarma, B.K., Singh, U.P. and Singh, K.P.2002. Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia* 94 (6): 1051-1058.
33. Sharma, O.P., Khatri, H.L. and Bansal, R.D. 1984. Effect of cucumber mosaic virus and /or *Sphaerotheca fuliginea* on phenolics, peroxidase and polyphenol oxidase content in muskmelon. *Indian Journal of Mycology and Plant Pathology* 14(2):107-110.
34. Sindhan, G.S. and Jaglan, B.S.1987. Role of phenolic compounds and carbohydrates in resistance of groundnut to tikka leaf spot. *Indian Journal of Mycology and Plant Pathology* 17(2):141-144.
35. Tiwari, R.K.S., Tiwari, R.B. and Ashok Singh.2004. Effect of irrigation schedules on collar rot (*Sclerotium rolfsii*) incidence and yield in groundnut. *Journal of Mycology and Plant Pathology* 34(2): 470-472.

Fig. 1: Groundnut seedlings inoculated with S.rolfsii. Stage-1 (immediately after inoculation), 2, 3, 4 & 5 of disease development on stems until the maturation of lesions.

Fig.2:Effect of *S.rolfsii* infection on *ortho*-dihydric phenols and total phenols of groundnut hypocotyls at different stages of disease development. Results are expressed as mg/g fresh weight material in both healthy and infected tissues.



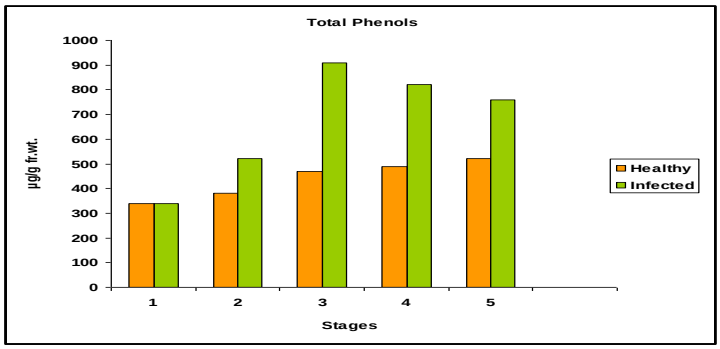


Fig.3 Effect of *S.rolfsii* infection on the activity of enzymes (polyphenol oxidase, peroxidase and phenylalanine ammonia – lyase, of groundnut hypocotyls at different stages of disease development