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Biochemical Changes in Groundnut Genotypes Consequent to Infection with the Rust Pathogen *Puccinia arachidis*

R. Sudhagar, D. Sassikumar and V. Muralidharan[1]

Centre for Plant Breeding and Genetics
Tamil Nadu Agricultural University
Coimbatore-641003, India

ABSTRACT. Changes in the activities of the defense enzymes viz., phenyl alanine ammonia lyase and peroxidases as well as phenolics were studied at 80th and 90th day in resistant and susceptible genotypes along with cross derivative segregants of groundnut consequent to infection with the rust pathogen, Puccinia arachidis Speg. Increase in the contents of both total phenol and ortho-dihydroxy phenol as well as phenyl ammonia lyase was observed in resistant genotypes whose activity prolonged even up to 90th day. However, the susceptible genotypes showed an initial increase followed by a decrease in their contents. On the contrary the susceptible genotypes showed increased level of peroxidase at both the stages as against an initial increase followed by a decrease in resistant genotypes.

INTRODUCTION

Low productivity of groundnut *Arachis hypogeae* L. is well attributed by the incidence of a few foliar fungal diseases. Among them, *Puccinia arachidis* Speg. assumes greater significance as it reduces the yield up to 50% besides, reducing the seed quality, oil and protein contents (Sanders *et al.*, 1989). The role of phenolic compounds and oxidative enzymes in resistance to other rust fungi has been studied extensively by many workers (Hofferek and Wolffgang, 1975; Velazhahan and Vidhyasekaran, 1994). Identification of resistance and susceptible genotypes to such fungal pathogens can be done through extrapolation of alterations in the levels of phenols, peroxidase and phenyl ammonia lyase contents in response to pathogenic infection. Though such studies are well documented in many crops (Arora, 1983; Srivastava, 1987; Whetten *et al.*, 1998) not much work was done on groundnut with reference to rust infection. The present paper reports the studies on the changes in phenols, activity of peroxidase and phenyl ammonia lyase in susceptible and resistant varieties consequent to the infection with the *Puccinia arachidis* L.

MATERIALS AND METHODS

Three groundnut cultivars with difference in resistance to *Puccinia arachidis* Speg. were selected; TMV1, VRI2–Susceptible and ICG1697– Resistant. Each susceptible parent was crossed with the resistant parent. The F_2 of these cross combinations along with their parents were raised in Rabi 1999. Uredospores from infected leaves were collected

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1 Department of Oilseeds, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641 003, India.

from the pustules by gently scraping with a sterile needle into distilled water containing 0.01% Tween–80 and adjusted to a concentration of 1×10^5 ml^{-1} (Velazhahan and Vidhyasekaran, 1994). To affirm the incidence of rust, 70 day old plants were inoculated with uredospore suspension using a plastic atomizer. Biochemical changes consequent to rust infection in F_2 segregants along with their parents were studied at 80 and 90 days after sowing (DAS). The parents and the segregants were scored for rust incidence fifth day after uredospore inoculation using 1–9 scale as suggested by Subrahmanyam *et al.* (1980a). Five plants in each of susceptible and resistant reaction classes were selected at random. Leaves were collected from bottom, middle and top portions of the plants, pooled together. From each plant, one gram leaf sample was taken for the assay. The plants raised under protected condition and free of rust disease served as control.

Estimation of phenols

Fresh leaves were extracted with 80% boiling ethyl alchohol for 10 min ground in a porcelain mortar with pestle. The extracts were pooled, filtered through Whatman No. 41 filter paper and the final volume was adjusted to represent five ml for every gram of tissue used (Mahadevan *et al.*, 1965). Total phenolics and ortho-dihydroxy phenols (OD phenol) in the alcohol extracts were estimated following the methods of Bray and Thorpe (1954) and Johnson and Schaal (1957) respectively.

Assay of oxidative enzymes

Leaf samples were homogenised at –15°C with pre chilled acetone. The homogenates were filtered through Whatman filter paper and rinsed with chilled acetone. The residues which settled on the filter paper were dried for 1 h at room temperature, stored at –15°C and used for further assays (Rahe *et al.*, 1970). The end product *i.e.*, the cinnamic acid in the assay mixture was measured using the Beckman spectrophotometer (Vidhyasekaran *et al.*, 1992). The enzyme peroxidase was extracted from the acetone powder with ice-cold phosphate buffer (Hammerschmidt *et al.*, 1982) and enzyme activity was determined at 470 nm in a Beckman spectrophotometer. Enzyme activity was expressed as changes in absorbance of the reaction mixture in the specified time of reaction. Boiled enzyme was used as control in all cases.

RESULTS AND DISCUSSION

Host resistance to any pathogen is mostly channelized through phenols. The plant respond to an infection of a plant pathogen through the release of several phenolic compounds, whose oxidation products (quinones) are highly toxic to the invading fungi. The accumulation of phenolics is high after infection in a resistant cultivar than in a susceptible cultivar (Velazhahan and Vidyasekaran, 1994). In the present study, the donor and the resistant segregants contained more phenolics than the susceptible genotypes.

The data presented in the Table 1 shows that the total phenol and ortho-dihydroxy phenol contents increased after infection in both resistant and susceptible varieties as

observed in 80th DAS and showed an increasing trend at 90 days after sowing. The increase in the levels of phenols in response to infection by the pathogen might be either due to its increased synthesis or translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosidase to yield free phenols (Luthra *et al.*, 1988). The increase in OD phenols become highly reactive upon oxidation and produce substances that are highly toxic to the hydrolytic enzymes of plant pathogens (Patil and Diamond, 1967). Hence, the inhibition of growth of pathogen and the concentration of OD phenols are directly related (Hunter, 1978). Prolonged accumulation of OD phenols in resistant types indicates the inhibitory role of phenols on the establishment and growth of the fungus.

Table 1. Changes in total phenols and OD phenol contents of groundnut leaves in response to inoculation with *Puccinia arachidis*.

Genotypes	Treatment	Total Phenol		OD Phenol	
		80 DAS	90 DAS	80 DAS	90 DAS
TMV1(s)	Healthy	288	246	54	43
	Infected	334	270	91	66
VRI2(s)	Healthy	151	169	46	45
	Infected	289	214	63	52
ICG1697(r)	Healthy	357	287	92	103
	Infected	563	596	144	192
TMV1 X ICG1697(s)	Healthy	296	283	69	100
	Infected	385	304	96	74
VRI2 X ICG1697(s)	Healthy	307	276	78	64
	Infected	392	310	117	83
TMV1 X ICG1697(r)	Healthy	303	234	71	104
	Infected	499	517	135	171
VRI2 X ICG1697(r)	Healthy	314	272	76	64
	Infected	510	538	152	156

S - Susceptible R - Resistant Data expressed in μg/g fresh weight of tissue

Phenolics are oxidized to quinones by several host enzymes. Of which, the peroxidase, an iron-containing enzyme is known to catalyze the oxidation of many mono and diphenols and aromatic amines to reactive oxygen species such as hydrogen oxides and quinones (Bolwell and Wojtaszek, 1997). It was also reported that peroxidase itself inhibits the rust pathogen under *in vitro* culture (Macko *et al.*, 1968).

In this study, the level of peroxidase activity increased and prolonged in both 80 and 90 DAS in susceptible genotypes while in resistant genotypes the levels were high

initially and declined at 90 DAS (Table 2). Similar observations were also made by Shimoni *et al.*, (1991); Velazhahan and Vidyasekaran (1994) and Luke RatnaKumar (1999). The prolonged increase of peroxidase in susceptible genotypes may be due to increase of reactive oxygen species such as super oxides radical (O_2^-) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) which are maintained at lowest level in plant system is instigated by pathogenic attack and accumulates to toxic concentrations and corroborated with the severity of infection (Bolwell and Wojtaszek, 1997).

Table 2. Changes in peroxidase activity and phenyl ammonia lyase activity in response to inoculation with *Puccinia arachidis*.

Genotypes	Treatment	Peroxidase		Phenyl Ammonia Lyase	
		80 DAS	90 DAS	80 DAS	90 DAS
TMV1(s)	Healthy	59	40	438	454
	Infected	86	92	687	532
VRI2(s)	Healthy	54	46	392	379
	Infected	81	95	576	525
ICG1697(r)	Healthy	91	44	518	478
	Infected	179	82	921	949
TMV1 X ICG1697(s)	Healthy	109	59	610	548
	Infected	138	168	783	637
VRI2 X ICG1697(s)	Healthy	118	54	489	450
	Infected	152	166	732	589
TMV1 X ICG1697(r)	Healthy	117	64	593	552
	Infected	292	91	714	987
VRI2 X ICG1697(r)	Healthy	128	54	463	478
	Infected	296	64	731	612

S - Susceptible R - Resistant
Peroxidase activity: (OD value of reaction mixture at 420 nm after 180 seconds)
AL : (μg of cinnamic acid/hour/g of fresh weight of tissue)

Phenyl ammonia lyase (PAL) plays a key role in the production of lignins from phenyl alanine through phenyl propanoid pathway. Lignin, a phenolic polymer acts as a barrier to the entry of pathogens and the lignification of cell walls in plants inhibits the growth of the fungus by preventing the haustoria to enter inside the cell (Whetten *et al.*, 1998). Several findings indicated that higher activity of PAL results in enhanced production of Lignin. In this study as shown in table 2, the PAL activity is high in resistant genotypes in both 80 and 90 DAS while the susceptible genotypes though recorded higher activities of PAL at 80 DAS manifested drastic reduction at 90 DAS.

Hence from this experiment, it is concluded that resistant genotypes to the fungal pathogen in groundnut can be identified through the aforesaid phenolics and enzymes

conforming to the explanations mentioned above. It is suggested that the resistant genotype selected based on such enzyme and phenolic compounds will not face "Vertifolia effect" with the emergence of new pathotypes as multiple resistance is operating.

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