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Original Research Article

Biochemical Factors Imparting Resistance to Asian Soybean Rust (*Phakopsora pachyrhizi*)

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A B S T R A C T

An experiment was conducted to study the different biochemical constituents imparting resistance and susceptibility to Asian soybean rust (ASR) disease caused by *Phakopsora pachyrhizi*. For this, resistant (R) and susceptible (S) genotypes of soybean to ASR were inoculated at 30 days after sowing (DAS) and studied at both 15 and 30 days after inoculation (DAI). Reduction in the chlorophyll a, chlorophyll b, total chlorophyll and phenol contents was found in inoculated S genotypes as compared with inoculated R genotypes at both the stages. However, reduction in chlorophyll content was less in inoculated R genotypes than inoculated S genotypes at both the stages. The total phenol content and enzymatic activities *viz*., peroxidase and polyphenol oxidase were increased in R as well as S genotypes when challenged with ASR, while increased levels of phenol, peroxidase and polyphenol oxidase activity were found more in R genotypes than S genotypes at both 15 and 30 DAI.

Introduction

Soybean [*Glycine max* (L.) Merrill] is a short-duration crop with high economic value and grown commercially in more than 35 countries including USA, Brazil, Argentina, and China. Although the cultivated area under this crop has been increasing every year in India, it has low productivity and unstable yield and low resistance to diseases and abiotic stresses. Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi* is a polycyclic foliar disease causing grain yield losses from 15-80%. Since last two decades the ASR assumed epiphytotic form and caused substantial losses particularly in the parts of Maharashtra, Karnataka and Madhya Pradesh. In order to minimize losses caused by ASR, cultivation of resistant cultivars is one of the cheaper and suitable options over the use of chemicals. Therefore, identification of resistant sources and the factors imparting resistance to ASR are needed to be studied thoroughly. Comparative studies on biochemical constituents in R and S genotypes of soybean during pathogenesis has often helped in understanding the nature and mechanism of resistance, which could be

used as basis for identification of R genotypes. Now, a little information is available regarding factors imparting rust resistance and their activities. The present study attempts to identify the biochemical factors which help in identification of traits responsible for resistance to ASR.

Materials and Methods

Estimation of biochemical components such as chlorophyll (chlorophyll 'a', chlorophyll 'b' and total chlorophyll) content, total phenols, peroxidase activities and polyphenol activities was carried out in three rust resistant (EC-241778, EC-241780 and Phule Agrani) and two rust susceptible (JS 335 and JS 93-05) genotypes of soybean. An experiment was carried out during 2013-14 at the Department of Plant Pathology and Agriculture Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri under the controlled glass house condition. Seed of the each genotype were sown in the plastic pots. Three seed were sown wide apart in each pot and ten pots were allotted for each genotype. All the plants were sprayed uniformly thrice with urediniospore suspension of *P. pachyrhizi* after 30 days of sowing (DAS) at an interval of two days during evening hours. Simultaneously, similar sets of all the five genotypes were sown in pots separately under rust free environment in another glasshouse for comparison. Rust severity was recorded at 45, 60 and 75 DAS using '0 to 9 scale' suggested by Mayee and Datar (1986). Further per cent disease index (PDI) was calculated using the formula given by Wheeler (1969).

Sampling for biochemical studies was done at 15 and 30 DAI from both the sets. Standard procedures were followed for estimation of different biochemical constituents from the leaf portion i.e. chlorophyll content (Arnon, 1949), total phenol by Folin-Ciocalteau reagent (FCR) method (Bray and Thorpe, 1954), peroxidase activity and polyphenol oxidase activity (Kumar and Khan, 1982).

Results and Discussion

Severity of ASR in different soybean genotypes: Rust severity assessed at 45, 60 and 75 DAS on all the genotypes revealed that it differed significantly as far as genotypes, crop growth stages (days) and their interaction are concerned. The genotypes EC 241778, EC 241780 and Phule Agrani were found free from rust. However, maximum rust severity was recorded in JS 335 and JS 93-05 at 75 DAS (100 %) than at 60 and 45 DAS (Table 5).

Biochemical studies

In the foregoing studies where in changes among several biochemical constituents in both R and S genotypes of soybean, due to infection by *P. pachyrhizi* was monitored, although no single constituent could pin pointed as the cause for resistance, yet they have thrown sufficient light on mechanism of resistance.

Chlorophyll

The results on chlorophyll 'a', chlorophyll 'b' and total chlorophyll content as influenced by ASR analyzed at 15 and 30 DAI are presented in Tables 1a and 1b. In general, levels of Chlorophyll content were higher at 15 and 30 DAI in healthy plants but lower under inoculated condition. Per cent decrease in all the three chlorophyll components over healthy leaf and R genotypes was observed in both R and S genotypes after inoculation.

The R genotypes had higher amount of 1.88, 0.62 and 2.51 mg g^{-1} fresh wt of

chlorophyll-a, chlorophyll-b and total chlorophyll content than susceptible one i.e. 1.66, 0.55 and 2.24 mg g^{-1} fresh wt, respectively under inoculated condition at 15 DAI. Similar trend of higher amount of all the chlorophyll contents was observed at 30 DAI in R genotypes (1.96, 0.63 and 2.65 mg g^{-1} fresh wt, respectively) than that of S genotypes (1.61, 0.46 and 2.05 mg g^{-1} fresh wt, respectively).

In R genotypes, per cent decrease over healthy leaf recorded at 15 and 30 DAI *w.r.t.* chlorophyll 'a' was 3.14 and 4.98; chlorophyll 'b' was 1.61 and 4.69; total chlorophyll was 0.40 and 0.38 while in S genotypes significantly higher levels of per cent decrease over healthy leaf i.e. chlorophyll 'a' was 19.57 and 33.16; chlorophyll 'b' was 16.67 and 55.56; total chlorophyll was 21.20 and 39.22 recorded at both the stages, respectively.

Earlier workers attributing to various reasons have reported the phenomenon of reduction of chlorophyll. Amongst them, Ellis *et al.,* 1981 reported decrease in chlorophyll content due to infection in several host pathogen systems and Heath, 1974 reported a change in the ultra-structure of chloroplast in rusted cowpea leaves. Ammajamma and Patil (2008) working with soybean rust observed reduction in chlorophyll 'a' and chlorophyll 'b' content in response to infection of *P. pachyrhizi*. Similar trend of reduction in chlorophyll contents was noticed in the present study at 15 and 30 DAI.

Total phenols

One of the most important components in imparting resistance to several plant diseases among all the biochemical components of different hosts are phenols. High concentration of the phenolic compounds causes an instant lethal action by a general tanning effect while, low concentration causes gradual effect on the cellular constituent of the parasite. If the concentration does not occur in toxic level, the inhibition will be obviously slow. Besides, the pathogen readily detoxifies low concentrations of the toxicants rather than high concentrations (Dasgupta, 1988).

It has been frequently observed that phenol accumulation takes place in all the infected plant tissues but more rapid accumulation of phenolics takes place in incompatible host pathogen complex than in the compatible ones. Newton and Anderson (1929) suggested that rust resistance in wheat was attributed by the liberation of phenolics in the host cells due to invasion of the fungus and these liberated phenols kill the host cell and inhibit the growth of the pathogen. Ammajamma and Patil (2008) were in conformity with earlier workers and reported soybean rust resistant genotypes had more per cent of total phenols than susceptible ones.

From the present investigation (Table 2), level of total phenols in the plant leaves was observed to be significantly higher in all the R genotypes (Phule Agrani, EC 241778 and EC 241780) than that of S genotypes (JS 93-05 and JS 335) at both 15 and 30 DAI. This indicates a significant positive correlation between phenolic content and disease resistance was at 30 DAI.

In this study, all R genotypes recorded the maximum mean total phenol content at both 15 and 30 DAI in healthy leaves (0.53 and 1.18 mg g^{-1} fresh weight, respectively) and inoculated leaves (0.55 and 1.25 mg g^{-1} fresh weight, respectively). However, minimum phenol content was recorded in S genotypes at both the stages in healthy and inoculated leaves as compared to R genotypes.

Table.1a Changes in chlorophyll a, chlorophyll b and total chlorophyll content (mg g^{-1} fresh wt) in soybean genotypes as influenced by infection of *P. pachyrhizi* at 15 DAI

Genotype	Chlorophyll 'a'				Chlorophyll 'b'				Total chlorophyll			
	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf
EC 241778	1.92	1.86	1.89		0.62	0.61	0.62		2.5	2.49	2.50	
EC 241780	1.90	1.89	1.90		0.63	0.61	0.62		2.52	2.51	2.52	
Phule Agrani	1.91	1.81	1.86		0.62	0.61	0.62		2.51	2.49	2.50	
Sub mean	1.91	1.85	1.88	-3.14	0.62	0.61	0.62	-1.61	2.51	2.50	2.51	-0.40
JS 335	1.77	1.45	1.61		0.60	0.49	0.55		2.50	1.94	2.22	
JS 93-05	1.92	1.51	1.72		0.60	0.51	0.56		2.50	2.01	2.26	
Sub mean	1.84	1.48	1.66	-19.57	0.60	0.5	0.55	-16.67	2.50	1.97	2.24	-21.20
% decrease over R genotype	-3.67	-20.00	-11.70	-	-3.22	-18.03	-10.56	-	-0.34	-21.20	-10.79	-
Mean	1.87	1.66	1.77		0.61	0.55	0.58		2.50	2.23	2.37	
Comparison between means of		SEm ±		CD at 5%		SEm ±		CD at 5%		SEm ±		CD at 5%
Genotypes (G)		0.029		0.088		0.012		0.037		0.019		0.057
Types (T)		0.047		0.139		0.012		0.058		0.031		0.090
GXT		0.066		0.197		0.028		NS		0.043		0.129

NS: Non significant

Table.1b Changes in chlorophyll a, chlorophyll b and total chlorophyll content (mg g^{-1} fresh wt) in soybean genotypes as influenced by infection of *P. pachyrhizi* at 30 DAI

Genotype	Chlorophyll 'a'				Chlorophyll 'b'				Total chlorophyll			
	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% decrease over healthy leaf
EC 241778	1.97	1.9	1.94		0.64	0.6	0.62		2.63	2.62	2.63	
EC 241780	2.02	1.91	1.97		0.63	0.6	0.62		2.66	2.65	2.66	
Phule Agrani	2.05	1.93	1.99		0.64	0.62	0.63		2.66	2.65	2.66	
Sub mean	2.01	1.91	1.96	-4.98	0.64	0.61	0.63	-4.69	2.65	2.64	2.65	-0.38
JS 335	1.84	1.24	1.54		0.63	0.28	0.46		2.46	1.52	1.99	
JS 93-05	2.02	1.33	1.68		0.63	0.27	0.45		2.64	1.57	2.11	
Sub mean	1.93	1.29	1.61	-33.16	0.63	0.28	0.46	-55.56	2.55	1.55	2.05	-39.22
% decrease over R genotype	-3.98	-32.46	-17.86		-1.56	-54.10	-27.20		-3.77	-41.29	-22.50	
Mean	1.97	1.60	1.78		0.63	0.44	0.54		2.60	2.07	2.35	
Comparison between means of		SEm ±		CD at 5%		SEm ±		CD at 5%		SEm ±		CD at 5%
Genotypes (G)		0.022		0.065		0.020		0.060		0.024		0.070
Types (T)		0.034		0.102		0.032		0.095		0.037		0.111
GXT		0.049		0.145		0.045		0.135		0.053		0.157

Table.2 Changes in total phenol (mg g^{-1} fresh wt) in soybean genotypes as influenced by infection of *P. pachyrhizi* at 15 DAI

Genotype	15 DAI				30 DAI			
	Healthy leaf	Inoculated leaf	Mean	% increase over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% increase over healthy leaf
EC 241778	0.53	0.56	0.55		1.18	1.26	1.22	
EC 241780	0.53	0.55	0.54		1.18	1.24	1.21	
Phule Agrani	0.53	0.55	0.54		1.17	1.24	1.21	
Sub mean	0.53	0.55	0.54	3.78	1.18	1.25	1.22	5.93
JS 335	0.44	0.44	0.44		0.87	0.90	0.89	
JS 93-05	0.42	0.44	0.43		0.86	0.89	0.88	
Sub mean	0.43	0.44	0.44	2.32	0.87	0.90	0.89	3.45
% increase over S genotype	23.26	25.00	24.14	-	35.63	38.89	37.29	-
Mean	0.48	0.50	0.49	-	1.03	1.08	1.05	-
Comparison between means of		SEm ±		CD at 5%		SEm ±		CD at 5%
Genotypes (G)		0.003		0.009		0.004		0.013
Types (T)		0.005		0.014		0.007		0.020
GXT		0.006		NS		0.010		0.039

NS: Non significant

Table.3 Peroxidase activity in R and S soybean genotypes as influenced by *P. pachyrhizi*

Genotype	Peroxidase activity (ΔA_{420} min^{-1} g^{-1} fresh wt)							
	15 DAI				30 DAI			
	Healthy leaf	Inoculated / Diseased leaf	Mean	% increase over healthy leaf	Healthy leaf	Inoculated / Diseased leaf	Mean	% increase over healthy leaf
EC 241778	0.25	0.31	0.28		0.24	0.32	0.28	
EC 241780	0.24	0.29	0.27		0.23	0.31	0.27	
Phule Agrani	0.24	0.29	0.27		0.24	0.30	0.27	
Sub mean	0.24	0.30	0.27	25.00	0.24	0.31	0.28	29.17
JS 335	0.19	0.23	0.21		0.19	0.23	0.21	
JS 93-05	0.19	0.24	0.22		0.19	0.23	0.21	
Sub mean	0.19	0.24	0.22	26.32	0.19	0.23	0.21	21.05
% increase over S genotype	26.31	25.00	22.72	-	26.31	34.78	33.33	-
Mean	0.22	0.27	0.24	-	0.22	0.27	0.24	-
Comparison between means of	SEm ±		CD at 5%		SEm ±		CD at 5%	
Genotypes (G)	0.009		0.026		0.002		0.007	
Types (T)	0.026		0.041		0.004		0.012	
GXT	NS		NS		0.006		0.017	

NS: Non significant

Table.4 Polyphenol oxidase activity in R and S soybean genotypes as influenced by *P. pachyrhizi*

Genotype	Polyphenol oxidase activity (Δ A_{420} min^{-1} g^{-1} fresh wt)							
	15 DAI				30 DAI			
	Healthy leaf	Inoculated leaf	Mean	% increase over healthy leaf	Healthy leaf	Inoculated leaf	Mean	% increase over healthy leaf
EC 241778	0.18	0.22	0.20		0.19	0.26	0.23	
EC 241780	0.18	0.21	0.20		0.19	0.25	0.22	
Phule Agrani	0.18	0.21	0.20		0.19	0.25	0.22	
Sub mean	0.18	0.22	0.20	22.22	0.19	0.25	0.22	31.58
JS 335	0.18	0.18	0.18		0.18	0.20	0.19	
JS 93-05	0.18	0.18	0.18		0.19	0.20	0.20	
Sub mean	0.18	0.18	0.18	0.00	0.18	0.20	0.19	11.11
% increase over S genotype	0.00	22.22	11.11	-	5.55	25.00	15.79	-
Mean	0.18	0.20	0.19	-	0.185	0.23	0.21	-
Comparison between means of	SEm $\pm$		CD at 5%		SEm $\pm$		CD at 5%	
Genotypes (G)	0.002		0.007		0.004		0.011	
Types (T)	0.004		0.011		0.006		0.017	
GXT	0.005		0.016		0.008		0.024	

Table.5 Rust severity in resistant and susceptible genotypes of soybean at 45, 60 and 75 DAS

Genotype	Per cent Disease Index		
	45 DAS	60 DAS	75 DAS
EC 241778	0	0	0
EC 241780	0	0	0
Phule Agrani	0	0	0
JS 335	51.11	77.77	100
JS 93-05	46.66	73.33	100

Increase in phenol content was observed in R over S genotypes in both healthy and inoculated condition at 15 DAI (23.26 % and 25.00 %, respectively) and at 30 DAI (35.63 % and 38.89 %, respectively). Also, it was noted that there was decrease in the per cent mean phenol content at inoculated condition in both R and S genotypes at both 15 DAI (3.78 % and 2.32 %, respectively) and 30 DAI (5.93 % and 3.45 %, respectively).

Susceptible genotypes of soybean showed slower rate of increase in total phenol content over the resistant genotypes in response to rust infection. This is in conformity with the earlier reports of Arora and Wagle (1985), Sivakumar and Sharma (2003), Ammajamma and Patil (2008) and Pawar *et al.,* (2012).

Peroxidase activity

The peroxidase enzyme is believed to be contributing to the resistance by oxidation of phenolic compounds to quinones, which are toxic to micro-organisms (Clark and Lorbeer, 1975; Sempio *et al.*, 1975 and Urs and Dunleavy, 1975).

Generally, the peroxidase and polyphenol oxidase enzymes have a defensive role against the invading pathogen, in that it is responsible for removal of toxic hydrogen peroxide in the host cells, thereby protecting the cells from getting damaged.

There was an increase in peroxidase activity from 15 to 30 DAI observed under inoculated condition in all the R and S genotypes (Table 3). Peroxidase activity till 15 DAI was found to be increased at slower rate which was non-significant but it was increased significantly at 15 to 30 DAI (22.72 % to 33.33 %).

At 30 DAI, both EC 241780 recorded maximum mean peroxidase activity (0.28 ΔA_{420} min^{-1} g^{-1} fresh wt) followed by both EC 241778 and Phule Agrani (0.27 Δ A_{420} min^{-1} g^{-1} fresh wt). However, minimum mean peroxidase activity was recorded in both the S genotypes JS 335 and JS 93-05 (0.21 ΔA_{420} min^{-1} g^{-1} fresh wt).

The mean peroxidase activity was found more in the R genotypes, at both healthy and inoculated conditions at both 15 (26.31 % and 25.00 %, respectively) and 30 DAI (26.31 % and 34.78 %, respectively) when compared with S genotypes.

Increased activity of peroxidase enzyme on inoculation was reported in tobacco against *Pseudomonas tabaci* by Lovrekovich *et al.*, (1968); in wheat against *Puccinia graminis* f. sp. *tritici* (Erikss and Henn) by Seevers and Daly (1970); in sorghum against *Peronosclerospora sorghi* (Watson) by Gowda *et al.,* (1989); in groundnut against *Phaeoisariopsis personata* by Jyosthna *et al.,* (2004); in teak against powdery mildew by Sankar and Sreeramulu (2009) and in

cotton against grey mildew by Pawar *et al.*, (2012). The present findings are in agreement with these earlier workers.

Polyphenol oxidase activity

Soybean genotypes (R and S) exhibited activity of polyphenol oxidase at increased rate under both inoculated and healthy conditions at both 15 and 30 DAI (Table 4). It was evident that significant difference existed among the genotypes at both the stages with gradual increase in polyphenol oxidase activity from 15 to 30 DAI.

Healthy (uninoculated) leaves exhibit minimum activities of polyphenol oxidase. At 15 DAI, maximum polyphenol oxidase activity in inoculated R genotypes (0.21 to 0.22 Δ A_{420} min^{-1} g^{-1} fresh wt) plants over inoculated S genotypes (0.18 Δ A_{420} min^{-1} g^{-1} fresh wt). Under inoculated condition at 30 DAI, activities of polyphenol oxidase found to be at higher level in the R genotypes as compared to S genotypes (0.25 to 0.26 and 0.20 Δ A_{420} min^{-1} g^{-1} fresh wt, respectively).

The per cent polyphenol oxidase activity increased over healthy leaf in the R genotypes was more as compared to S genotypes at both 15 and 30 DAI. Also, polyphenol oxidase activity was found to be increased over S genotypes in both R and S genotypes at both the stages i.e., 15 DAI (22.22 %) and 30 DAI (25.00 %).

Some of the past workers reported similar increased activity of polyphenol oxidase as in the present findings were Sivakumar and Sharma (2003) while working on banded leaf and sheath blight affected maize plants grown out of seeds treated with *P. fluorescens;* Gupta *et al.*, (1992) in groundnut as influenced by leaf spot pathogen; Jyosthna *et al.*, (2004) in groundnut genotypes challenged by *Phaeoisariopsis personata*; Sankar and Sreeramulu (2009) in teak leaves infected with powdery mildew and Pawar *et al.*, (2012) in cotton plant infected by grey mildew disease. Pawar *et al.*, (2012) observed that the uninoculated leaves of R and S cotton plants exhibited less polyphenol oxidase and peroxidase as compared to infected leaves as influenced by grey mildew disease.

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