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Changes in Biochemical Composition of Soybean Leaves Infected With Resistant and Sensitive Isolates of *Phakopsora Pachyrhizi* Causing Soybean Rust

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Abstract

Various biochemical constituents viz., reducing sugar, total sugar, DNA, RNA, phenol, total ash and ascorbic acid were estimated from the healthy soybean plant, resistant and sensitive isolate of soybean rust. Phenols and ascorbic acid increased due to the infection of soybean rust and reducing sugar, total sugar, DNA, RNA, total ash were decreased due to the infection of soybean rust as compared to the healthy one.

Key words: *Glycine max, Phakopsora pachyrhizi*, biochemical composition, resistant and sensitive isolate.

Introduction:

Soybean is very important oil yielding plant. It is nature's precious gift to man kind and treated as golden bean on the account of its three dimensional utility viz., oilseed, pulses and vegetable. Soybean has now become a major substitute crop for sugarcane in the Maharashtra state, due to its high returns. But soybean plant is mainly suffered from rust disease which causes heavy loss of yield. The rust disease is caused by *Phakopsora pachyrhizi* Syd. Biochemical changes in many plant pathogen interactions are accompanied by the rapid increase in phenolic compounds and realted enzymes, often termed the hypersensitive response (Khan, 1983). Several types of biochemical changes associated with fungal diseases in plants have been reported such as changes in phenolic compounds (Siqueira et al, 1991), changes in catalase, peroxidase and polyphenol oxidase enzymes (Velazhahan and Vidhyasekaran, 1994; Wang et al., 1991), sucrose transport (Moussatos et al., 1993) and 4-hydroxycinnamic acid (Hipskind et al., 1993). Considering the economic importance of the crop, studies on the impact of disease on crop production is one of the important factors relevant to crop cultivation.When plant is affected by disease plants adopt a variety of biochemical defenses to ward off microbial attack. A variety of compounds synthesized by plants as a sort of defense mechanism. The metabolic changes in the plant tissue infected by fungal attack have been reported by many researchers (Naik *et al* (1988), Nema,A.G. (1991). This communication is deals with changes in the biochemical constituents estimated in healthy, resistant and sensitive isolate of soybean plant.

Material and Methods:

Soybean plants with symptoms of rust were collected from western Maharashtra and

north western Karnataka and brought to the laboratory and 18 isolates of *Phakopsora pachyrhizi* were obtained from them which were maintained in the glasshouse on *Glycine max* C.V. JS 335.

Soybean plants were grown in plastic pots, and thinned to 5 plants per pot. The sensitivity of *Phakopsora pachyrhizi* to 4 different fungicides (Tilt, contaf, byleton, and score) was tested *in vivo.* The different concentration of fungicides were sprayed on 21 days old soybean plants. After 24 hrs of fungicidal treatment urediniospore suspension was inoculated on the plants with the help of 0.5 mm Camlin brush. They were protected by polythene bags to maintain 100 % relative humidity. The plants which were sprayed with distilled water were treated as control. After 10 days of inoculation the % infection was recorded by using 0-9 scale (Mayee and Datar, 1986).

Among the 4 fungicides, tilt was most effective. Out of 18 isolates tested isolate Pp-9 was most sensitive to tilt with minimum inhibition concentration of 3 ppm while isolate Pp-4 was most resistant to tilt with minimum inhibition concentration of 36 ppm. The sensitive isolate Pp-9 and resistant isolate Pp-4 were used for further investigations.

Soybean plants were inoculated with sensitive and resistant isolate, after 10 days of inoculation the plants are dried in oven at 40^0 C and powder was obtained in grinder. The activity of phenols was estimated by following Mahadevan and Sridhar (1982). Total sugars and reducing sugars were estimated by Anthrone method (Morse,E.E., 1947)and Dinitrosalycylic acid method (Miller,G.L.,1972) respectively. Ascorbic acid content in the leaves was determined by the visual titration method based on the reduction of 2, 6-diclorophenol indophenol dye (Roe, 1954). The nucleic acids were estimated from the soybean leaf tissue using method of Cherry (1962).

Results:

The data present in the Table 1.clearly revealed that non reducing sugars, total ash, DNA and RNA contents were reduced due to the infection of *Phakopsora pachyrhizi* while phenols and ascorbic acids were higher in plants infected with resistant and sensitive isolate of *Phakopsora pachyrhizi* than healthy plants. Analysis of carbohydrate fraction revealed a significant decreased in non-reducing sugars and total sugars. Post infectional changes in carbohydrate content have been recorded in many host pathogen system. Changes in carbohydrate level are generally attributed to direct parasitic utilization and indirectly to the altered host metabolism (Reddy and Rao,1978). The increase in reducing sugars may be due to microbial enzymatic degradation of complex sugars in the host cell wall in to simple sugars (Hollighan,1974). Stress causes enhanced utilization and mobilization of ascorbic acid which is recognized as antistress factor (Kutsky, 1973) so increased ascorbic acid content may due to involvement of ascorbic acids in detoxifying mechanism. The phenol content increased in the infected leaf sample as a sort of defense mechanism against rust. These results were conformity with the reports of Ramadayal and Joshi (1968) in barley against leaf blight pathogen. The accumulation of phenolic compounds in infected host tissues may be related to their release from glycosidic esters by the enzymatic activity of host or pathogen (Noveroske et al., 1964).

Discussion:

Naik *et al.* reported that total sugar content of betelvine leaf decreased due to infection of *Colletotrichum gloesporiodes*. Khan et al. (2001) reported that chlorophyll, non-reducing sugars were decreased due to the infection of sorghum leaves by *Drechslera sorghicola* while there was increase in reducing and total sugars and phenols. Nagaraja (2007) reported that chlorophyll, nitrogen, reducing sugars, starch and carbohydrate contents were

considerably decreased in infected leaves and the activity of enzymes acid phosphatase and alkaline phosphatase were reduced but enhanced activity of enzymes peroxidase and superoxide dismutase in case of leaves of *Mappia foetida* infected by *Cylindrosporium mappiae.* Jite and Tressa (1999) reported that the polyphenol oxidase and peroxidase was enhanced in the infected plants as compared to healthy ones where as IAAO activity reduced in *Jasminum Grandiflorum* infected by *Uromyces hobsoni.* Increase in phenolic compounds was observed in groundnut infected with rust and apple scab disease (Mayr,1995; Velazhahan and Vidhyasekaran,1994). Considering the present results, it can be concluded that *Phakopsora pachyrhizi* during pathogenesis interferes with various physiological mechanisms of the host.

Conclusion:

Phenols and ascorbic acid increased due to the infection of soybean rust and reducing sugar, total sugar, DNA, RNA, total ash were decreased due to the infection of the infection of *Phakopsora pachyrhizi* soybean rust as compared to the healthy one. Carbohydrate fraction revealed a significant decreased in non-reducing sugars and total sugars.

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Table 1: Sensitivity of *Phakopsora pachyrhizi* isolates to four different fungicides on soybean leaves.

Sr.No	Locality	Isolate Number	Tilt	Contaf	Byleton	Score
1	Satara	Pp-1	10	37	5	10
2	Tandulwadi	Pp-2	10	10	15	25
3	Male	Pp-3	30	10	25	10
4	Vhanage	Pp-4	36	15	8	10
5	Kurli	Pp-5	15	12	20	20
6	Sankeshwar	Pp-6	30	20	10	25
7	Nipani	Pp-7	15	25	15	20
8	Petnaka	Pp-8	20	10	24	15
9	Tasgaon	Pp-9	3	4	10	5
10	Karad	Pp-10	25	25	20	25
11	Khochi	Pp-11	7	8	6	10
12	Khidrapur	Pp-12	10	7	25	20
13	Pimpalgaon	Pp-13	15	10	10	38
14	Vadgaon	Pp-14	20	20	30	25
15	Narsobachiwadi	Pp-15	10	15	40	7
16	Umbraj	Pp-16	30	20	15	15
17	Dattawad	Pp-17	25	10	15	22
18	Kasbe digraj	Pp-18	10	20	4	27

Table 2: Biochemical characteristics of the soybean leaves infected with tilt resistant and sensitive isolates of *Phakopsora pachyrhizi* after 10 days of inoculation.

Sr. no.	Estimations	Healthy	Sensitive	Resistant
1	Non reducing sugars (mg/g)	0.367	0.19	0.24
2	Reducing sugars (mg/g)	0.32	0.485	0.833
3	Total sugars (mg/g)	0.687	0.675	1.073
4	DNA (mg/g)	1.46	0.87	1.20
5	RNA (mg/g)	3.33	2.18	2.75
6	Phenols (mg/g)	50.6	88.7	97.6
7	Total ash (%)	4.0	2.5	3.5
8	Ascorbic acid (mg/g)	64	74.66	83.2