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Biochemical analysis of phytoplasma infected sesame plant transmitted by *Orosius albicinctus* distant

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Abstract
Changes in different biochemical parameters *viz.*, total phenols, reducing sugar and total protein were compared in healthy and Phyllody infected plant samples of sesame (cv DSS-9) sown on different dates. Results indicated that the Phyllody infected samples contained more total phenols, reducing sugar and total protein compared to the uninfected samples. The total phenol content of diseased samples was found to be 30 and 25 mg/g however, the healthy samples consistently recorded low phenol content (18 and 22 mg/g) in the 1st and 2nd date of sowing respectively. Irrespective of the dates of sowing, diseased samples recorded higher reducing sugar (35 and 18 mg/g) compared to healthy samples (13 and 15 mg/g, respectively). The diseased sample from 1st and 2nd date of sowing recorded highest content of total protein (60 and 58 mg/g) while healthy samples recorded 46 and 39 mg/g of total protein respectively. The elevated levels of different biochemical parameters in diseased plants indicate that the reaction of plants to Phytoplasma infection is induced rather than constitutive.

Keywords: *Sesamum indicum*, sesame Phyllody, Phytoplasma, total phenols, reducing sugar, total protein

1. Introduction
Sesame (*Sesamum indicum* Linnaeus) is an important oilseed crop, grown mainly in tropics and sub-tropical regions of the world. The major sesame producing countries are India, China, Turkey, Myanmar, Pakistan, Egypt, Sudan, Greece, Venezuela, Argentina, Colombia, Nicaragua, Elsalvador, Mexico and USA. Among the sesame growing countries in the world, India ranks first in area (17.5 lakh ha) and production (8.93 lakh tonnes) with the productivity of 368 kg/ha. However, the productivity is quite low compared to world average (489kg/ha) [1]. The crop is affected by many pests and diseases and one of the important pests is leafhopper, *Orosius albicinctus* Distant which transmits Phyllody disease in sesame. The infected plants remain partially or completely sterile, resulting in the total loss of yield.

In nature, plants are constantly challenged by a wide range of pathogen and their protective mechanisms involve an inducible defense system. The ability of plants to invoke such defense reactions is presumed to be mediated by an initial recognition process that involves detection of certain unique signal molecules of incompatible pathogens by receptor-like molecules in plants, resulting in a cascade of biochemical events that leads to the expression of resistance and susceptibility to a disease [2]. Host-pathogen interactions are presumed to generate signals that activate nuclear genes involved in plant defense responses leading to the induction of stress-related enzymes, differential expression of proteins and release of free amino acids and the associated accumulation of high levels of phenolic compounds. Antimicrobial phytoalexins such as sesquiterpenoids, isoflavonoids, coumarins, acetylenic and phenolic compounds also contribute to multilayered plant defense systems [3]. Therefore, the present studies were taken up to know the changes in biochemical parameters *viz.*, total phenols, reducing sugar and total protein in Phytoplasma infected sesame plants.

2. Materials and Methods
Sesame (CV DSS-9) was sown two times at 15 days interval (June 6th and June 21st) in a plot size of 10m x 12m with 30 cm x 15 cm spacing in Entomology field, MARS, Dharwad starting from 6th June, 2012. Both healthy and Phyllody infected plant samples (20 plant each) were randomly collected from first and second date of sowing for estimation of biochemical parameters *viz.*, total phenols, reducing sugar and total protein.

2.1 Sample preparation: 2 gm of fresh leaf was weighed and cut into small pieces, ground thoroughly in a mortar and pestle with 20 ml of 80% alcohol. It was passed through muslin cloth twice. The filtrates were filtered through Whatman No. 41 filter paper and made up to 20 ml volume with 80% alcohol. Later the extract was stored in a refrigerator at 4 °C and used for further analysis.

2.2 Clarification of alcoholic extracts: Dark colour alcohol extracts create a great problem in analysis procedure. The interference due to coloured plant pigments like chlorophyll, carotenes, xanthophylls etc. is enormous which need to be eliminated prior to analysis. Heavy metals and salts were therefore employed to tackle the problem, excess of which is precipitated by disodium hydrogen phosphate. 2 ml of saturated lead acetate solution was added drop by drop to 25 ml of the coloured alcoholic extract with 3 ml of saturated solution of disodium hydrogen phosphate till the precipitation was completed. The above solutions were mixed thoroughly and kept overnight. Next day, it was filtered through Whatman No. 41 filter paper and made up to 25 ml volume with 80% alcohol and stored in a refrigerator at 4 °C for the analysis.

2.3 Estimation of Total Phenol: Estimation of total phenols present in plant samples was done following Folin-Ciocalteu Reagent Method [4].

2.4 Estimation of Reducing Sugar: Reducing sugar was estimated by following Nelson's modification of Somogyi's method [5].

2.5 Estimation of Total Protein: Total protein content was estimated by Lowry's method [6].

3. Results and Discussion

Irrespective of different dates of sowing, the biochemical analysis of healthy and diseased samples revealed elevated levels of total phenol, reducing sugar and total protein in the Phyllody infected plants than the healthy ones (Table 1). The total phenol content of diseased samples was found to be 30 and 25 mg/g however, the healthy samples consistently recorded low phenol content (18 and 22 mg/g) in the 1st and 2nd date of sowing (DoS) respectively (Fig.1). Irrespective of the dates of sowing, diseased samples recorded higher reducing sugar (35 and 18 mg/g from samples collected from 1st and 2nd DoS, respectively) compared to healthy samples (13 and 15 mg/g, respectively) (Table 1) (Fig. 2). The diseased sample from 1st and 2nd date of sowing recorded highest content of total protein (60 and 58 mg/g) while healthy samples recorded 46 and 39 mg/g of total protein respectively (Table 1) (Fig. 3). Irrespective of the dates of sowing, the diseased samples recorded higher total phenol, reducing sugar and total protein content than the healthy samples. Elevated level of different biochemical parameters in diseased samples indicate that the reaction of plants to Phytoplasma infection which is induced rather than constitutive. Present findings are in conformity with reports of [7] who reported that the increased in protein, reducing sugar and phenolic compound contents in leafhopper inoculated maize plants points towards changes in host metabolism due to the Phytoplasma infection. The quantity of phenols in infected leaves of *Cucumis sativa* (Linn.) affected by leaf spot disease caused by *Penicillium notatum* was also higher compared to healthy leaves [8].

Table 1: Biochemical parameters of healthy and Phyllody diseased sesame plants

Sample	Total Phenol (mg/g)		Reducing Sugar (mg/g)		Total Protein (mg/g)	
	1st DoS	2nd DoS	1st DoS	2nd DoS	1st DoS	2nd DoS
Diseased	30	25	35	18	60	58
Healthy	18	22	17	13	46	39

***Note:** DoS-Date of sowing

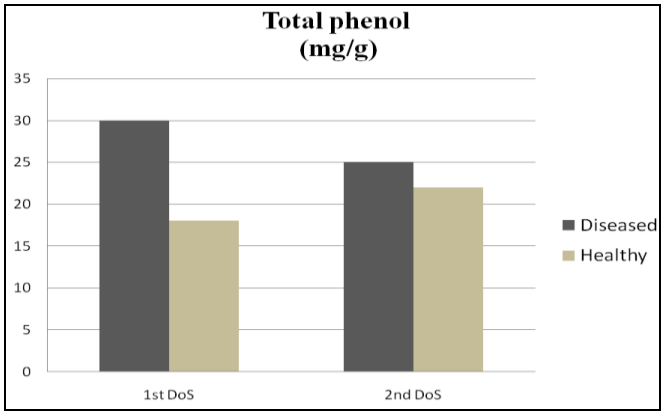

Fig 1: Concentrations of total phenol from diseased and healthy sesame plants

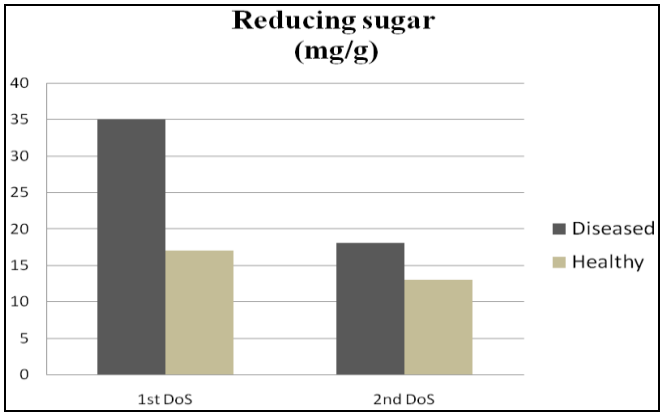

Fig 2: Reducing sugar concentrations from diseased and healthy sesame plants

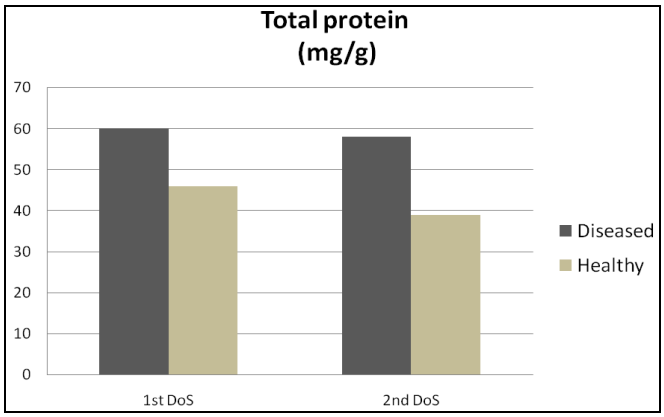

Fig 3: Total protein concentrations from diseased and healthy sesame plants

4. Conclusion

Host plant resistance is the result of interactions between the plant and the pathogens under the influence of various environmental factors. Among the secondary plant metabolites in general phenolics are the source of resistance against pathogens and are ubiquitous in plants. The elevated

level of different biochemical parameters in diseased plants indicates that the reaction of plants to Phytoplasma infection is induced rather than constitutive.

5. References

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