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Polyphenol oxidase activity in phytoplasma associated sesame phyllody transmitted by leafhoppers

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Abstract
Polyphenol oxidase content was assessed in controlled and open field condition and results shows that there was a increased trend from 58 to 72 days after sowing in open field and compare to controlled condition genotypes in open field condition were having higher content of polyphenol oxidase and also resistant genotypes were significantly different from susceptible ones.

Keywords: *Sesamum indicum*, sesame phyllody, phytoplasma, polyphenol oxidase leafhoppers

Introduction
The diseases associated to phytoplasmas are important to many different crops around the world. Studies related to biochemical and physiological changes in plants caused by these microorganisms are rare. Some results have shown that phytoplasma infection can change the level of some compounds in plant tissues. In diseased sesame chlorophyll synthesis was altered during plant growth (Chang 1977).
Development of an antioxidant defense system in plants protect them against oxidative stress damage, by Polyphenol oxidase is a tetramer that contains four atoms of copper per molecule and binding sites for two aromatic compounds and oxygen. In this study, polyphenol oxidase activities assessed in healthy and infected leaves of sesame plants.

Materials and Methods
Polyphenol oxidase assay: 3ml of mixture contained: 0.1M phosphate buffer (pH 7.0), 45mM Pyrogallol (substrate) and 25 µl diluted enzyme extract was added. The reaction was incubated in 5min at 25 ^{0}C in incubator. Without substrate in enzyme mixture was considered as control.
The amount of purpurogallin from substrate was measured at 420 nm in spectrophometer (Spectronic-20D) (Sadasivam and Manickyam, 1996) [7].
The polyphenol oxidase (PPO) activity gradually enhanced in phyllody infected sesame genotypes than healthy plants controls. Nearly two fold higher enzyme activity was observed in infected plant leaves than healthy plant leaves in all genotypes tested.
Among the resistant genotypes *viz.*, OSC-207, VS-07-023 and RT-363 at 72 DAS the PPO activity were 4.79, 6.33 and 6.36 units of purpurogallin/ ml/min, respectively and were significantly different from each other. Similarly, in susceptible genotypes *viz.*, GT-1 and DS-5 at 72 DAS the PPO activity were 3.83 and 3.82 units of purpurogallin/ ml/min, respectively and were on par with each other in controlled condition.
In open field condition among the resistant genotypes *viz.*, OSC-207, VS-07-023 and RT-363 at 72 DAS the PPO activity were 8.16, 8.05 and 8.60 units of purpurogallin/ ml/min, respectively and were on par with each other. Similarly, in susceptible genotypes *viz.*, GT-1 and DS-5 at 72 DAS the PPO activity were 4.31 and 5.31 units of purpurogallin/ ml/min, respectively and were on par with each other (Table 1).
For instance the polyphenol oxidase activity of all the genotypes in controlled condition shows increased trend from 58, 65, 72 DAS i.e., 1.84, 2.10 and 3.12 units of purpurogallin/ml/min, respectively in resistant genotype OSC-207, where as in susceptible AT-249 also followed the same pattern i.e., 2.91, 3.67 and 6.67 units of purpurogallin/ ml/min, respectively at 58, 65, 72 DAS (Table 1).
Compared to genotypes controlled condition polyphenol oxidase (PPO) activity observed high in open field condition genotypes i.e., increased from 52, 65, 72 DAS (2.47, 3.84 and 5.10 units of purpurogallin/ ml/min) in resistant genotype OSC-207 and in susceptible much more and maximum PPO activity was observed i.e., 4.14, 7.52, 11.00 units of purpurogallin/ ml/min at 58, 65, 72 DAS, respectively (Table 1).

Overall PPO activity of genotypes in controlled condition ranged from 1.84 to 2.71 units of purpurogallin/ ml/min in resistant varieties and in susceptible 2.91 to 4.04 units of purpurogallin/ ml/min and in open field condition PPO content ranges from 1.91 to 5.74 units of purpurogallin/ ml/min in resistant varieties and 4.14 to 11.00 units of purpurogallin/ ml/min in susceptible genotypes (Table 1) (Fig. 1).

Table 1: Polyphenol oxidase activity in different genotypes of sesame at different days of sowing in controlled and open field condition

Sl. No.	Genotypes	Category	Polyphenol oxidase (units of purpurogallin/ ml/min)						
			Controlled			Open field			Per cent increase from controlled to open field (%)
			58 DAS	65DAS	72 DAS	58 DAS	65DAS	72 DAS	
1	OSC-207	HR	3.56^{c}	3.91^{ab}	4.79^{bcd}	4.79^{bc}	6.15^{bc}	8.16^{c}	41.30
2	VS-07-023	HR	3.26^{d}	3.61^{abc}	6.33^{ab}	4.49^{cd}	5.85^{bc}	8.05^{c}	21.37
3	RT-363	R	3.8^{b}	4.66^{a}	6.36^{ab}	5.03^{ab}	6.39^{bc}	8.6b^{c}	26.05
4	JLS-9707-2	R	3.15^{d}	4.16^{ab}	5.26^{abc}	4.38^{d}	5.74^{c}	7.95^{c}	33.84
5	G-TIL-2	MR	4.15^{a}	4.82^{a}	6.09^{ab}	5.38^{a}	6.74^{ab}	9.4^{b}	35.21
6	RT-367	MR	2.91^{e}	3.67^{de}	6.67^{a}	4.14^{d}	7.52^{a}	11^{a}	39.36
7	RT-366	S	1.84^{i}	2.1^{de}	3.12^{e}	2.47^{g}	3.84^{ef}	5.1^{def}	38.82
8	RT-368	S	1.84^{j}	2.11^{de}	3.49^{de}	2.47^{g}	3.85^{ef}	4.87^{ef}	28.34
9	AT-231	S	2.71^{f}	2.97^{bcd}	4.04^{cde}	3.34^{e}	4.71^{d}	5.74^{d}	29.62
10	GT-1	HS	1.28^{j}	1.55^{e}	3.83^{cde}	1.91^{h}	3.29^{c}	4.31^{f}	11.14
11	DS-5	HS	2.28^{i}	2.55^{cd}	3.82^{cde}	2.91^{f}	4.29^{ab}	5.31^{de}	28.06
12	AT-249	HS	2.43^{g}	2.69^{cd}	4.04^{cde}	3.06^{ef}	4.43^{a}	5.46^{de}	26.01
S.Em±			0.03	0.11	0.13	0.09	0.12	0.18	
CD 5%			0.25	0.30	0.42	0.33	0.38	0.61	

Polyphenol oxidase activity changes in OD at 420 nm (units of purpurogallin/ ml/min), Means in the column followed by same letters are not significantly different at p=0.05(F-test), DAS-days of sowing

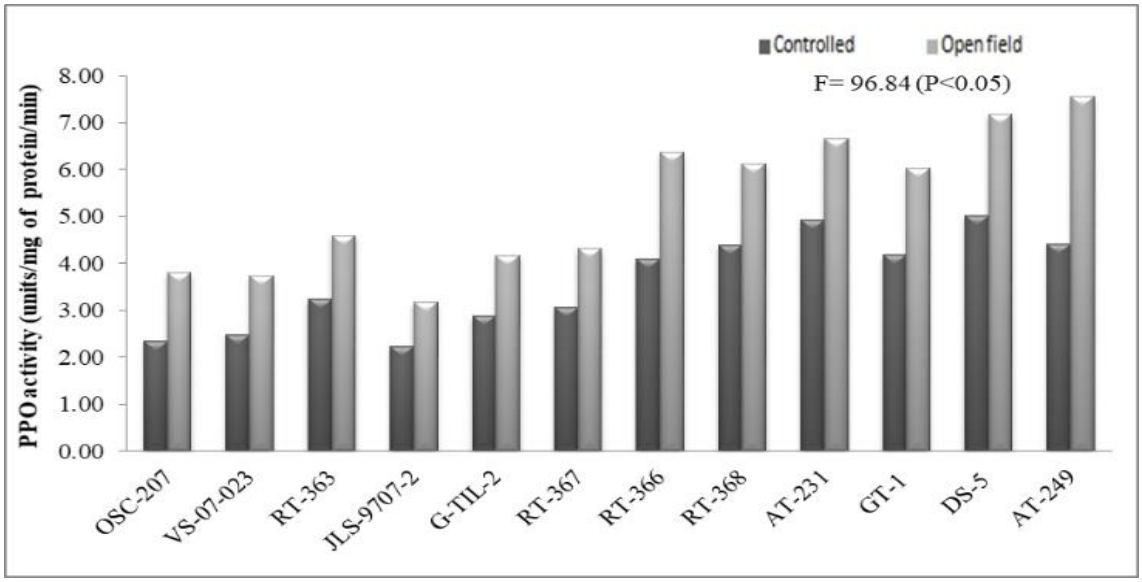

Fig 1: Polyphenol oxidase activity of sesame genotypes under controlled and open field condition

The present findings are in conformity with Youssef *et al.* (2018) [8] who observed activity of polyphenols oxidase in phyllody infected sesame (5.70 units/mg protein) was higher than in the healthy (3.20/mg protein) sesame plant. The results of present study also in agreement with Beltagi *et al.* (2013) [1] who found the activity of peroxidase and polyphenol oxidase increased in infected plants compared to that of healthy plant.

The results of present study were similar with findings of many workers who reported enzyme content across different viruses and mycoplasma (Farkas *et al.*, 1960) [3]. Higher activity of this enzyme naturally results in the formation of greater amounts of phenolics in the diseased tissues of cotton. Similar increase in the phenolics contents of diseased plants has been reported by Parthasarathy and Ramaswamy (1961) [5].

Similarly, Zafari *et al.* (2012) [9] reported in lime plants infected by the *Candidatus phytoplasma aurantifoliae* that activities of polyphenol oxidase (PPO), peroxidase (POX) and superoxide dismutase (SOD) were observed to be greater in infected leaves than the healthy control.

According to Ray *et al.* (1998) [6] antioxidative enzymes (POX and PPO) may participate in the responding defense reaction by inducing plant resistance against pathogenic agents. The results of present investigation are in close agreement with the findings of Lin and Kao (2001) [4] who found a correlation between increased antioxidant enzyme activities and pathogen resistance in plants.

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