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Peroxidase and polyphenol oxidase activities in healthy and viral infected sunflower (*Helianthus annuus* L.) leaves

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ABSTRACT

Sunflower (*Helianthus annuus* L.), a member of compositae family is the most important edible oilseed crop. *Sunflower necrosis virus* is one of the viral diseases in sunflower plants, The main symptoms of this disease chlorotic mosaic, leaf necrosis, stem necrosis, leaf distortion and plant death. In this study, plant enzymes, peroxidase, and polyphenol oxidase activities were assessed and chlorophyll content was measured in healthy and sunflower necrosis virus diseased plants. The peroxidase and polyphenol oxidase activities were enhanced, where as chlorophyll content was decreased in viral infected leaves than in healthy sunflower plants.

KEYWORDS

Sunflower plants;
SNVD;
Peroxidase;
Polyphenol oxidase;
Chlorophyll.

INTRODUCTION

Sunflower necrosis virus disease (*SNVD*) is one of the diseases affected by virus in sunflower plants. It is a strain of *Tobacco streak virus* belongs to genus *Ilarvirus,* Family *Bromoviridae*. Raw sunflower kernels are nutritious for humans. The kernels of sunflower contain 55 percent proteins. Other vitamins and minerals in sunflowers include B, E and A vitamins, phosphorus, nitrogen, calcium and iron. Sunflower oil is using in cooking, soaps, lubricants and candles preparation. It is also used as natural medicine in curing some of the diseases of human including sinusitis, hemorrhoids and leg ulcers in human beings. Peroxidase is an oxidoreductase enzyme, the presence of hydrogen peroxide it catalyzes the oxidation of organic and inorganic substrates[1,2]. The peroxidase was widely distributed in plants, animals and microorganism[3] The peroxidative damage of cell walls is controlled by the peroxidase of antioxidative enzyme system[4,5]. Peroxidase was one of the key enzyme, controlling plant growth and development. It involves in construction, rigidification and eventual lignifications of cell walls, protection of tissue from damage and infection by pathogenic microorganisms[3]. Polyphenol oxidase is a tetramer that contains four atoms of copper per molecule and binding sites for two aromatic compounds and oxygen[6]. In this study, peroxidase and polyphenol oxidase activities and chlorophyll contents were assessed in healthy and viral infected leaves of sunflower plants.

MATERIAL AND METHODS

Plant materials

Fresh sunflower leaves (both healthy and virus in-

FULL PAPER

fected.) were obtained from green house of Virology Department, Sri Venkateswara University, Tirupati, India.

Preparation of sunflower leaves homogenate

Healthy and virus infected plants were grown and maintained for further research. The sunflower leaves homogenate was prepared by Sakharov[7] method.

Enzyme assays

Both the enzyme assay were following with some changes according to Hemanta *et al*[8]

Peroxidase assay: The sample mixture containing 0.1M phosphate buffer (pH 7.0), 1 ml of 45mM Guicol and 22.5mM H_2O_2: From to this 25 µl diluted enzyme extract was added. The reaction was incubated in 5 min at 25 ^{0}C in incubator. The reaction was stopped by adding 0.5 ml 5% (V/V) H_2SO_4. Without substrate in enzyme mixture was considered as control. The amount of guicogallin was determined at 470 nm in spectrophometer (Spectronic-20D)

Polyphenol oxidase assay: 3 ml of mixture contained: 0.1M phosphate buffer (pH 7.0), 45mM Pyrogall (substrate) and 25 µl diluted enzyme extract was added. The reaction was incubated in 5 min 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)

Specific enzyme activity was calculated by following formula[9]

$$\text{Specific enzyme activit} = \frac{\text{Enzyme mix}}{\text{mg protein}} = \frac{\text{E1000V}}{\text{t mg protein}}$$

Where E indicates, Extinction coefficient, V indicate Volume of the reaction mixture and t indicates time in seconds.

Chlorophyll estimation

The Chlorophyll content in plant leaves was determined according the method of Arnon (1949)[10]. The young leaves of healthy, and sunflower necrosis virus disease infected leaves were collected from sunflower plants at Virology department Greenhou1e. Infected leaves were cut in to small pieces with well edged sharp blade. One gram of each healthy and virus infected leaf samples were taken separately and washed with tap water and fallowed by distilled water. Then the samples were macerated in 10 ml cold 80% acetone, squeeze with sterile absorbent cotton then centrifuged at 3000rpm for 20min and read the absorbance at 645nm and 663nm in Spectrophotometer (Spectronic-20D). The Chlorophyll content was estimated by following formula[11].

Total Chlorophyll = [20.2(OD645) + 8.02(OD663)] x V/ 1000xW

Chlorophyll*a* = [12.7(OD663) – 2.69(OD645)] x V/1000xW

Chlorophyll*b* = [22.9(OD645) – 4.68(OD663)] x V/1000xW

RESULTS

Enzyme assays

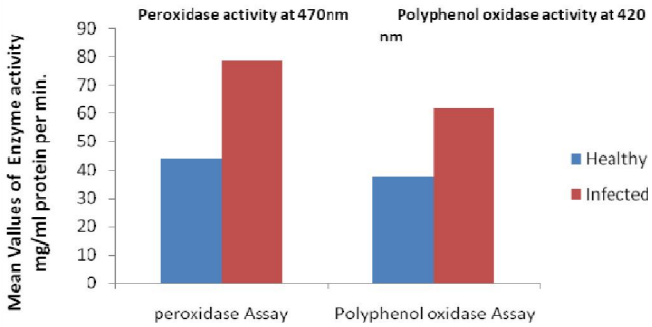
The peroxidase activity in healthy and sunflower necrosis virus infected sunflower leaves was measured and the results were represented in TABLE 1. The peroxidase activity was drastically reduced (nearly two folds) in healthy sunflower plants than the infected leaves (table.1and Figure1). For instance in healthy plant the peroxidase activity at 60seconds of incubation was 54.50 glycogualine/ml/min, where as in infected 105.80 glycogualine / ml/min The Similar observations were made in *S.lycopersicum*[12]. After completion of 180seconds of incubation period the Polyphenol oxidase activity in healthy 33.60 and 51.5 purpurogallin / ml/min, in virus infected sample was observed

TABLE 1 : Peroxidase activity in healthy and viral infected sunflower leaves.

Incubation period (in seconds)	Peroxidase activity *	
	Healthy	Infected
60	54.50	105.80
180	33.60	51.5

*** Values represented in above TABLE mean values of duplicates; *Activity measured in terms of liberation of mg of glycogualine / ml/min**

The polyphenol oxidase activity in healthy and sunflower necrosis virus infected sunflower leaves was measured and the results were represented in TABLE 2. The polyphenol oxidase activity gradually enhanced in virus infected sunflower plants than healthy plants controls. Nearly two fold higher enzyme activity was observed in infected plant leaves than healthy plant leaves. [TABLE 2. Figure1]. For instance the polyphenol oxidase activity in infected leaves was 58.24 purpurogallin



*** Values represented in above Figureure are mean values of duplicates**

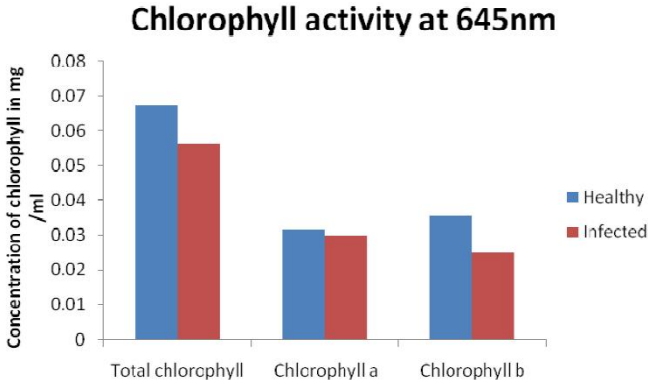
Figure 1 : Peroxidase and polyphenol oxidase activity in healthy and viral infected sunflower leaves.

/ ml/min where as in healthy leaves 29.86 purpurogallin / ml/min at 60 seconds of incubation. After completion of 180seconds of incubation period the Polyphenol oxidase activity in healthy 45.38 and 65.24 purpurogallin / ml/min, in virus infected sample was observed

TABLE 2 : Polyphenol oxidase activity in healthy and viral infected sunflower leaves.

Incubation period (in seconds)	Polyphenol oxidase activity*	
	Healthy	Infected
60	29.86	58.24
180	45.38	65.24

*** Values represented in above TABLE mean values of duplicates; *Activity measured in terms of liberation of mg of purpurogallin / ml/min**



Values represented in above Figureure are mean values of duplicates

Figure 2 : Chlorophyll content in healthy and viral infected sunflower laves

Chlorophyll estimation

The total chlorophyll content in healthy and infected sunflower plant leaves were estimated and shown in Figure 2 and TABLE 3. The Chlorophyll content in the virus infected plants were lowered than the health plants. Chlorophyll*a* and Chlorophyll*b* ratios also decreased in virus infected necrotic leaves compared to healthy. Similarly reduced chlorophyll content was observed in viral infected leaves[13,14].

TABLE 3 : Total chlorophyll, Chlorophyll*a* and Chlorophyll*a* content in healthy and infected sunflower leaves

Type of plants	Total Chlorophyll content (mg/ml)	Chlorophyll*a* content (mg/ml)	Chlorophyll*b* content (mg/ml)
Healthy	0.0672	0.0317	0.0353
Infected	0.0561	0.0298	0.0249

*** Values represented in above TABLE mean values of duplicates; * Activity was measured in liberation of end products mg/ml**

DISCUSSIONS

The cultivation of sunflower is assumed great impartment in the world. Sunflower is considered as a highly profitable crop for farmers, especially in Northern Karnataka, Maharashtra and Rayalaseema region of Andhra Pradesh in India. This crop is largely cultivated under rain fed conditions during late kharif/rabi season. The present work was undertaken to study the chlorophyll content and distribution pattern of peroxidase, and polyphenol oxidase in developing and senescing leaf tissues and to determine whether the increase in peroxidase and polyphenol oxidase activities during senescence is species specific. There was clearly a relationship between infection and POD activity, with plants containing the virus exhibiting greater enzymatic activity than in the healthy plants. The chlorophyll content was drastically decreased in total chlorophyll, Chlorophyll*a* and chlorophyll*b*. The total individual chlorophylls were reported to study the variation in diseases to healthy levels in different virus host combinations exhibiting chlorotic and mosaic symptoms[15]. In this study decreased Total chlorophyll, Chlorophyll*a*, Chlorophyll*b* were observed in virus infected sunflower leaves. Similarly higher POD activity was observed in many plant species. McKenzie *et al*[16] and Hamady *et al*[12] and analyzed the accumulation of defensive proteins, including peroxidase, in healthy and *tomato mottle virus* (ToMoV) infected plants, their findings indicated

that infected plants display greater POD activity than their healthy counter parts. In plants, virus infection has often been associated with a plethora of biochemical and metabolic changes[17]. For instance in cotton plants, *cotton leaf curl virus*[CLCuV] infection results in increases in catechol, phenols, carotenoids, protein contents[18,19].

The distribution of peroxidase and polyphenol oxidase activities in leaves of different physiological ages of 16 species varied to a considerable extent. Some species showed higher peroxidase and polyphenol oxidase activities toward the basal senescent leaves whereas other species showed higher activities in the middle mature leaves or the topmost leaves. Increase in enzyme activities during detached leaf senescence determined whether the tissue was capable of de novo synthesis in all of the plant species[20]. Improved enzyme activity was probably due to enzyme activation and detachment[21-24]. In the present study also it was observed that the enzyme activities such as peroxidase and polyphenol oxidase were higher in infected plant leaves when compared to healthy.An experiment was designed[23] to verify the changes in the enzymic levels during development and subsequent senescence of leaves which coincide with on attached leaves of rice at different developmental stages. Medhavi *et al[*2011] studied homology modeling in polyphenol oxidase by Modeller and Geno3D with a template sequence. The 3 D structure of the protein was evaluated and validated using PROCHECK and Verify_3D[25]. The enzyme polyphenol oxidase was converts polyphenolic compounds to quinine. The rate of oxidation of polyphenol is enhanced in tissue adjacent to the diseased lesions and this may creates inhibitory zone which is effective barrier to further speed of the pathogen. Where as in case of chlorophyll content, the infected leaves showed less amount of chlorophyll when compared to healthy leaves but there was no drastic reduction of chlorophyll in infected leaves.

CONCLUSION

In this study we conclude that the peroxidase and polyphenol oxidase activities were enhanced in infected sunflower plant leaves whereas the chlorophyll content was reduced than healthy. Decreased chlorophyll content in infected sunflower leaves is an indication of toxic nature of peroxidase and polyphenol oxidase activity increased in infected leaves of sunflower plants due to antioxidative mechanism.

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