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Quantification of Total Lipid and Related Enzymes in Fusarium Wilt Infected Lens Culinaris Medic

Singh Anita

Department of Botany, School of Basic and Applied Science, Career Point University, Kota Rajasthan, India

Abstract: ***Quantification of lipid and their related enzymes was studied in Lentil (lens culinaris medic) infected with Fusarium oxysporum F. Sp. lentis causing wilt disease. The contents of total lipid and lipase activities in healthy and diseased counter parts of lentils were measured. Different plant parts showed variation in their lipid contents were recorded higher in wilt infected plant then healthy leaf, stem, fruit and seeds while lipase activities were recorded higher in normal leaf, stem, fruit and seeds as compared to infected plant.***

Keywords: Lensculinarismedic, Total lipid, *Fusarium oxysporum* F. Sp. lentis

1. Introduction

Lentil is an increasingly important pulse crop in the prairie regions of North America where it is grown in rotation with cereals and oilseeds. Canada, India, Australia, the USA and Turkey are the main producers of lentil and world production of lentil in 2013 was 4.95 Mt [1].

Lentil (*Lens culinaris* L.) is the second most important cool - season legume crop in India (8). It covers an area of 1.51 million ha with a production of 1.56 million tons and productivity of 1, 032 kg ha^{-1} (3). Lentils are a good source of protein, carbohydrates, dietary fiber components, minerals, vitamins, and secondary metabolites that include phenolic compounds [2].

Disease such as Ascochytablight is caused by *Ascochyta Lentis* Bond G vassil and wilt is caused by *Fusarium oxysporum* f. Sp. Lentis play a major role in reducing lentil yield 4. Wilt disease appears in the field in patches at both seedling and adult stages. Seedling wilt is characterized by sudden drooping followed by yellowing and drying of leaves and the whole seedling and apparently healthy roots with reduced proliferation.

Present study was undertaken to understand physiological changes of disease plant parts. The biochemical estimation of total lipid and their related enzymes were estimated.

2. Materials and Methods

Extraction of Lipids

One g of each of the dried and milled test sample was macerated with 10 ml distilled water (Jayaraman, 1981). To this, 30 ml of chloroform - methanol (2: 1, v/v) was added and mixed thoroughly. Each mixture was left overnight at room temperature; 20 ml of chloroform and the equal volume of distilled water was added and centrifuged. Out of the three layers, a clear lower layer of chloroform containing all lipids was collected in pre - weighted beaker, the solvent evaporated completely and weighed, which was taken as the weight of total lipids/g of the dried tissue sample.

Assay of lipase activity

Lipase activity was determined with p - nitrophenyl palmitate (pNPP) by the method reported by Licia et al. (2006) the substrate for this reaction was composed of solution A and solution B. Solution A contained 40 mg of pNPP dissolved in 12 ml isopropanol. Solution B contained 0.1 g of gum arabic and 0.4 ml of triton X - 100 dissolved in 90ml of water. The substrate solution was prepared by adding 1 ml of solution A to 19 ml of solution B drop wise with constant stirring to obtain an emulsion that remains stable for 2 h. The assay mixture contained 1 ml of the substrate, 0.5 ml of buffer (glycine - NaOH, pH 11, 0.5 M) 0.1 ml of enzyme (the filtrate) and the volume was made up to 3 ml with distilled water. This was incubated at 40°C for 45 min. The enzyme activity was stopped by adding 0.2ml of isopropanol. The absorbance was measured at 410 nm against substrate free blank. The standard graph was prepared by using para - nitrophenol (0.4 to 4 µmoles). One lipase unit (U) is defined as the amount of enzyme that liberated 1 µmol p nitrophenol per min under the assay conditions described (Maia et al., 1999).

3. Results and Discussion

Biochemical Studies of *F. oxysporum* and *R. solani* Infected Leaf, Stem, Seeds

Biochemical estimations to total lipid and their related enzyme in healthy and *Fusarium oxysporum* and *Rhizoctonia solani* infected lentil Leaf, stem, seeds were carried out (Table - 1& Figs. - 1).

I. *Fusarium oxysporum* and *Rhizoctonia solani*
I. Fusarium oxysporum

Lipid (Table - 1; Fig. - 1)

Slight increase in lipid was observed in infected leaf. It was 0.06 mg/gm in healthy leaves whereas 0.28 mg/gm in infected sample studied.

Slight increase in lipid was observed in infected stem. It was 0.07 mg/gm in healthy stem whereas 0.18 mg/gm in infected the sample studied.

Slight increase in lipid was observed in infected seeds. It was 0.11 mg/gm in healthy seeds whereas 0.2 mg/gm in infected the sample studied.

Lipase (Table - 1; Fig. - 1)

Slight increase in lipase enzyme was observed in infected leaf. It was 10.51 mM PNP/gm / /Min in healthy leaves whereas 10.43 mM PNP/gm / Min in infected sample studied.

Slight increase in lipase enzyme was observed in infected stem. It was 2.69 mM PNP/gm / /Min in healthy stem whereas 1.75 mM PNP/gm / /Min in infected the sample studied.

Slight increase in lipase enzyme was observed in infected seeds. It was 3.26 mM PNP/gm / /Min in healthy seeds whereas1.71 mM PNP/gm / /Min in infected the sample studied.

Generally the oil content decrease when associated with fungi (Kamble and Gangewane, 1987), but increase was observed in seeds infected with Rhizoctonia bataticola (Lalithakumari, Govindaswamy and Vidyasekaran, 1971). Angelo and Ory (1983) have reviewed lipid degradation during seed deterioration. Lipid peroxidation processes may cause a reduction in the orgenoleplic properties of food (Villaume et al.1993). Unsaturated fatty acids are by nature more prone to become rancid by the action of lypoxygenase enzyme present in lentil (Maccarrone et al., 1997).

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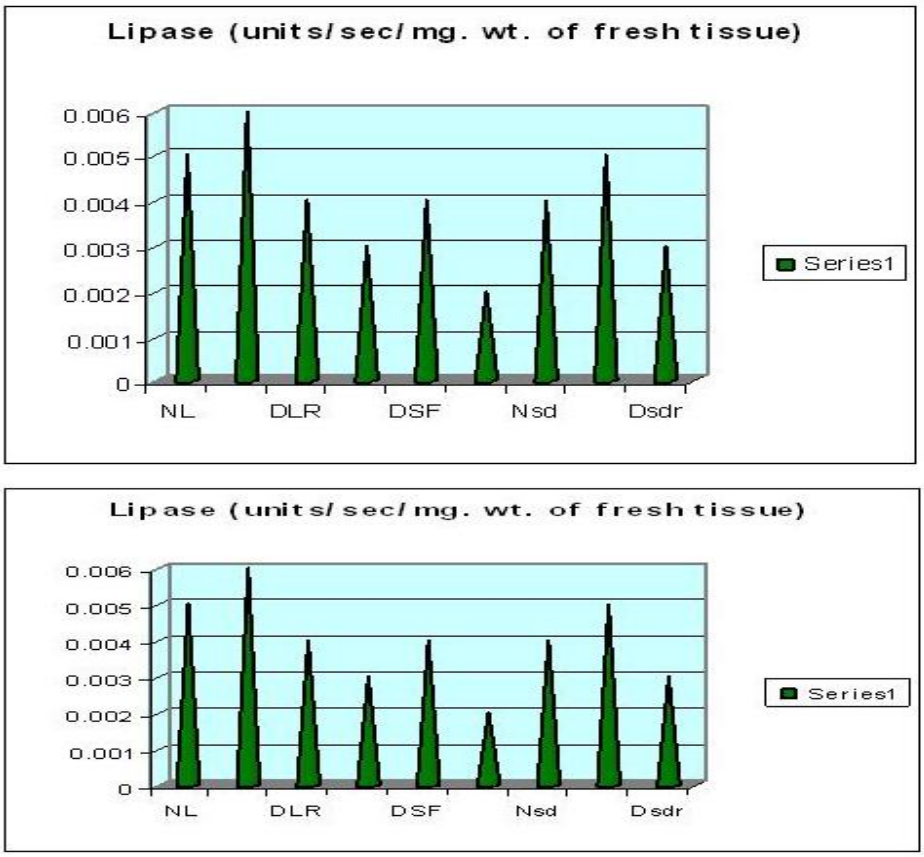
Table 1: Quantification of Lipid and their Related Enzymes

Concentration	NL	DLF	DLR	NS	DSF	DSR	Nsd	DsdF	DsdR
Lipid (mg/g)	0.06	0.28	0.2	0.07	0.18	0.1	0.11	0.2	0.17
Lipase activity (units/sec/mg. wt. of fresh tissue)	0.01	0.01	0	0	0	0	0	0.01	0

NL= Non infected leaves, DLF = Diseased leaves of F. oxysporum, DLR= Diseased leaves of R. solani, NS = Non infected stem, DSF= Diseased stem of F. o., DSR = Diseased stem of R. s., Nsd = Non infected seeds, DsdF = Diseased seeds of F. o., DsdR = Diseased seeds of R. s.



NL=Non infected leaves, DLF= Diseased leaves of F.*oxysporum*, DLR=Diseased leaves of *R.solani*, NS= Non infected stem, DSF= Diseased stem of *F.oxysporum*, DSR=Diseased stem of *R.solani*, Nsd=Non infected seeds, Dsdr=Diseased seeds of *F.oxysporum*, Dsdr = Diseased seeds of *R.solani*

Figure 1: Lipid lipase distribution levels