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Quantification of Total Protein and Related Enzymes in Fusarium wilt infected Lens culinaris medic

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Abstract

Quantification of protein 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 protein and protease activities in healthy and diseased counter parts of lentils were measured. Different plant parts showed variation in their protein contents were recorded higher in wilt infected plant then healthy leaf, stem, fruit and seeds while protiase activities were recorded higher in normal leaf, stem, fruit and seeds as compared to infected plant.

Keywords: Lens culinaris medic • Total protein

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]. Lentils are a good source of protein, carbohydrates, dietary fiber components, minerals, vitamins, and secondary metabolites that include phenolic compounds [2].

Disease such as Ascochyta blight is caused by *Ascochyta Lentis* Bond & 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 protein and their related enzymes were estimated.

Materials and Methods

Extraction of Proteins

A 60 mg of the dried test sample was macerated [3]. in 10 ml of cold TCA (10%) for 30 min kept at low temperature 4^0C for 24hr and then centrifuged. Each of the supernatants was discarded and the resultant pellet was re-suspended in 5% TCA (10 ml) and heated on a water bath at 80^0C for 30 min. Each of these samples was cooled, re-centrifuged and each time the supernatant discarded.

Later the pallet was washed with distilled water, centrifuged and each of the residues was dissolved in IN NaOH (10 ml) and left overnight at room temperature.

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Quantification of Proteins

In each of 1 ml extract, total protein content was estimated using the protocol of [4]. A stock solution (1 mg/ml) of bovine serum albumin (Sigma Chemicals) was prepared in 1 N NaOH, from which 0.1 to 0.9 ml of the solution was dispensed separately in a test tube. After this, the volume of each was raised to 1 ml by adding distilled water. To each test sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5%$CuSO_4$. $5H_2O$ in 1% sodium potassium tartrate) was added at room temperature and left undisturbed for a period of 10 min. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocaltcau reagent (CSIR centre for Bio-chemicals, Delhi: diluted with equal volume of distilled water just before use) was rapidly added and after half an hr, the OD of each was measured at 750 nm using a spectrophotometer against the blank. Three replicates of each concentration were taken and their mean values were used to compute a regression curve. The total protein contents in each sample were calculated by referring the ODs of test sample with the standard curve of BSA. Three replicates were examined in each case and their mean values were recorded.

Extraction of Protease

Plant material was homogenized in 0.1 M phosphate buffer (pH 7.0) in prechilled mortar and pestle at 4°C. The homogenate was centrifuge at 5000 rpm for 15 minutes. The supernatant was collected as enzyme extract and made up to 10ml with buffer solution.

Estimation of Protease: 2 ml casein solution (1% casein dissolved in phosphate buffer pH 7.0) was mixed with 1 ml of .1M phosphate buffer pH 7.0 and 1 ml of enzyme extract. Incubating mixture was kept at 30°C in water bath for ½ hr. 1ml enzyme substrate mixture was taken in centrifuge tube and 1ml TCA was added. Then it was allowed to stand at room temperature for an hour and then centrifuge at 2000 rpm for 20 minutes. 1ml of supernatant was pipette and added 1ml of folin ciocalteu's reagent and 2ml of 20% sodium carbonate. Tube was then placed in boiling water bath for one minute, cooled under tap and raised to 10 ml with distilled water.Absorbance was read at 650 nm [5]. Reference curves were drawn using tyrosine.

Results and Discussion

I. Fusarium oxysporum and Rhizoctonia solani

Total soluble proteins (Table-1; Fig.-1)

Protein in infected leaf was lower than the healthy leaf, in the samples studied. It was recorded 0.322 mg/gm in healthy leaves and 0.196 mg/gm in the infected sample respectively.

Table 1. Frequency of protein protease concentration.

Concentration	NL	DLF	DLR	NS	DSF	DSR	Nsd	DsdF	DsdR
Protein (mg/g)	0.322	0.196	0.191	0.116	0.0489	0.0451	0.0826	0.0122	0.0118
Protease activity (units/sec/mg. wt. of fresh tissue)	0.005	0.006	0.005	0.003	0.004	0.002	0.004	0.005	0.003

NL = Non infected leaves, DL= Diseased leaves, NS= Non infected stem, DS= Diseased stem,

NF= Non infected fruit, DF= Diseased fruit, Nsd = Non infected seeds, Dsd= Diseased seeds.

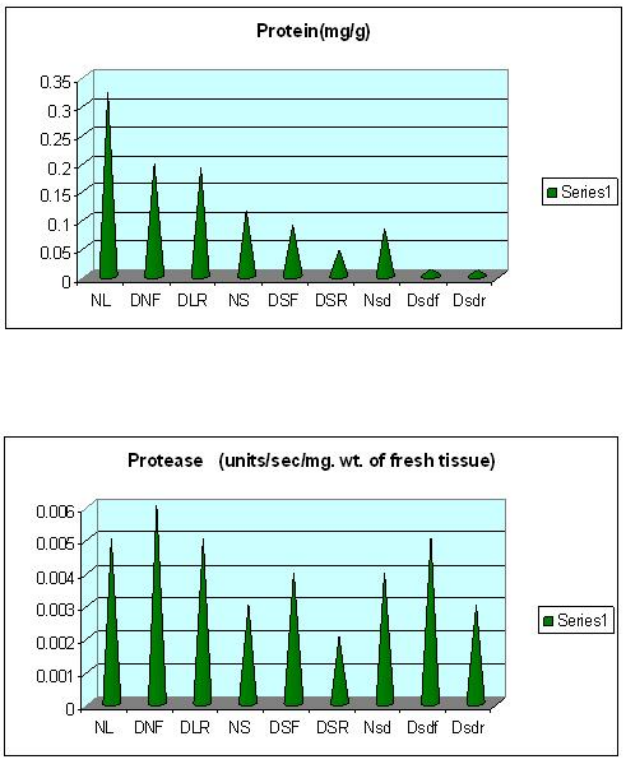

Figure 1: Protein protease distribution levels.

Protein in infected stem was lower than the healthy stem, in the samples studied. It was recorded 0.116 mg/gm in healthy stems and 0.0489 mg/gm in the infected sample respectively.

Protein in infected seeds was lower than the healthy seeds, in the samples studied. It was recorded 0.0826 mg/gm in healthy seeds and 0.0122 mg/gm in the infected sample respectively.

Protease (Table 1; Figure 1)

Protease enzyme in infected leaf was higher than the healthy leaf, in the samples studied. It was recorded 0.005 mg/gm in healthy leaves and 0.006 mg/gm in the infected sample respectively.

Protease enzyme in infected stem was higher than the healthy stem, in the samples studied. It was recorded 0.003 mg/gm in healthy stems and 0.004 mg/gm in the infected sample respectively.

Protease enzyme in infected seeds was higher than the healthy seeds, in the samples studied. It was recorded 0.004 mg/gm in healthy seeds and 0.005 mg/gm in the infected sample respectively.

The result is presented in Figure 1 [6] observed an increase in protein content of groundnut infected with *Cercospora personatum*. Higher total protein content in infected pods of T-9 variety of Vigna mungo susceptible to leaf crinkle virus was observed by [7]. It is a well known fact that enzymes are proteins and the increased synthesis of proteins during the infection may be due to activation of enzymes which are essential for the synthesis of various defense chemicals [8]. The increase in protein content may have resulted form synthesized proteins in the infected host. In addition to air classification, a new emerging dry fractionation method, triboelectrostatic separation has emerged recently as a novel solvent-free approach to separate protein isolates in the food industry by [9]. Methionine is typically lower in plant-based proteins compared with animal-based proteins by [10,11]. examined the physiochemical and functional properties of navy bean protein concentrated using triboelectrostatic separation.

[12] found that the protein content of arhar seeds with a mycelia mat was lower than of seeds from which the mycelial mat was removed. Protein contents were decreased after inoculation with *Phytophthora drechsleri f.sp. cajani* in the resistant cultivar of pigeon pea [13,14]. observed that *Macrophomina phaseolina* infected sesame seeds showed reduced protein content. [15] reported a decrease in protein content in sunflower seeds due to infection of *Macrophomina phaseolina*. [16] reported seed-borne fungi (*Alternaria brassicae*, *Aspergillus flavus*, *A. sydowi* and *Penicillium frequentens*) caused reduction in protein content to taramira and safflower. [17] observed higher amount of protein content in healthy stem, leaves and root as compared to *Erysiphe pisi* infected pea plants.

[18] studied aflatoxin elaboration and nutritional deterioration in some cultivars of black gram, pigeonpea, soybean, gram and green gram during infestation with *Aspergillus flavus* and found that the amount of proteins were greater in resistant varieties. Deterioration in protein was noted during infection in all samples except soybean. They found that in unautoclaved seeds reduction of protein content was caused by *Rhizcotonia solani*. *Curvularia lunata* and *Fusarium moniliforme* while it was increased by *A.niger* and *Rhizopus nigricans*, [19,20]. whereas in autoclaved seeds most of the fungi except *Pythium spp*. and *Fusarium moniliforme* Increase in perish ability of diseased tissue May facilate movement from the source to sink.

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References

1. Onyango B. O, Koech P. K, Anyango B, and Nyunja R. A, et al "Morphological, genetic and symbiotic characterization of root nodule bacteria isolated from Bambara groundnuts (Vigna subterranea L. Verdc) from soils of Lake Victoria basin, western Kenywaa". *Journal of Applied Biology and Biotechnology.* 3 (2015): 001-010.
2. Anchirinah V. M., Yiridoe E. K. and Bennett-Lartey S. O. "Enhancing sustainable production and genetic resource conservation of Bambara groundnut". *A survey of indigenous agricultural knowledge system. Outlook on Agriculture* 30 (2001): 281- 288.
3. Bryan C. S, Doku "Identification of Phytomonas, Azotobacter and

Rhizobium or Achromobacter upon initial isolation". *Soil Science* 45 (1938): 185-187.

4. Mohale C. K., Belane A. K. and Dakora F. D. "Why is Bambara groundnut able to grow and fix N2 under contrasting soil conditions in different agroecologies?" *A presentation at the 3rd international scientific conference on neglected and underutilized species*. on 26th September, 2013 in Accra Ghana. Pp. (2013). 13-16.
5. Sprent J. I., Odee D. W and Dakora F. T. "African legumes: A vital but under-utilized resource". *Journal of Experimental Botany*. 61 (2010): 1257 – 1265.
6. Atkinson E. M., Palcic M. M., Hindsgaul O. and Long S. R. "Proceedings of National Academy of Science" USA. 91 (1994): 8418–8422.
7. John M., Rohrig H., Schmidt J. and Wieneke U. et al "Proceedings of National Academy of Science" USA. 90 (1993): 625–629.
8. Branden J. Furseth,* Shawn P. Conley, and Jean-Michel Ané. "Enumeration of Soybean-Associated Rhizobia with Quantitative Real-Time Polymerase Chain Reaction" *Crop Science*. 50 (2010): 2591–2596.
9. Vincent J.M. "A Manual for the Practical Study of Root Nodule Bacteria." *Blackwell Scientific, Oxford.* (1970).
10. Geremia R. A., Mergaert, P., Geelen D.,Van Montagu M. and Holsters M. "Proceedings of National Academy of Science" *USA*.91: (1994). 2669–2673.
11. Toolarood A. A. Soltani, Alikhani H. A., Salehi Gh. , and Asadi-Rahmani H. et al "Genetic diversity and phylogeny of alfalfa nodulating rhizobia assessed by nifH and nodA genes International Research." *Journal of Applied and Basic Sciences.* 3 (2012): 1470-1476.
12. Triplett EW and Sadowsky Mj, Reeve "Genetics of competition for Nodulation of Legumes". *Ann Rev Microbiol* 46 1992: 399- 428.
13. Rohrig H., Schmidt J., Wieneke U., and Kondorosi E., et al *Proceedings of National Academy of Science USA*.91**:** (1994). 3122–3126.
14. Somasegaran, P. and H. J. "Hoben Handbook for rhizobia: methods in legume-Rhizobium technology", *Springer Science & Business Media.* (2012).
15. Spaink H. P., Wijfjes A. H. M., Van der Drift, and Haverkamp J., et al J. *Molecular Microbiology.* 13: (1994). 821–831.

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