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Estimation of protein and its related enzyme in cluster bean plant parts infected with *Fusarium solani* caused wilt disease

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Abstract
Estimation of alteration in protein content due to the infection of *Fusarium solani* causing wilt disease was carried out in various plant parts of *Cyamopsis tetragonoloba*. The infection of *Fusarium solani* is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. Symptomatic seeds carried white fungal growth of the pathogen. Estimations of protein content and its related enzyme were carried out in leaves, stem and seeds of healthy and infected counter plant parts. The alteration in protein contents were recorded in plant parts. Protein content was higher in case of healthy plant parts 0.296, 0.236 and 0.290 mg/gm in leaves, stem and seeds respectively while in wilt infected counterparts viz. leaves, stem and seeds it was 0.172, 0.132 and 0.206 mg/g. Protease activity was higher in case of wilt infected plant parts 0.017, 0.007 and 0.008 units/ sec/mg of fresh weight of tissue of leaves, stem and seeds whereas it was 0.008, 0.005 and 0.006 units/ sec/mg of fresh weight of tissue in healthy counterparts.

Keywords: *Cyamopsis tetragonolola*, dwarfing, *Fusarium solani*, protein, protease, wilt disease.

Introduction

Fusarium solani (Mart.) Sacc. Was first described by Martius [19] as *Fusisporium solani* from rotted tubers of potato (*Solanum tuberosum*). The species was transferred to the genus *Fusarium* by the Italian mycologist Saccardo [24]. *F. solani* was emended by Snyder and Hansen [34] to comprise a complex group of species that are widely distributed in soils and cause tuber, root, and stem rots of plants worldwide [13, 18]. Guar is an important legume crop belongs to family Fabaceae. It is drought tolerant crop suitable for cultivation under rainfed conditions of kharif season in arid and semiarid regions of India [20, 21]. The guar seed comprises three parts: the seed coat (14-17%), the endosperm (35-42%), and the germ (43-47%). The guar gum which is the prime marketable product derived from endosperm of guar[31]. The green and tender pods of guar are cooked as favorite vegetables in many parts of country including South India. The green pods of cluster bean are rich source of protein contents (3.7%) which is essential components of human consumption[12]. Guar meal is the main by-product of guar gum production contains about 40% proteins and utilized as feed ingredient. The removal of the gum from seeds enhances the protein contents (51%) of guar meal in comparison to seed. The fungal infection causes variations in protein contents of plant parts.

The cluster bean plants infected with several disease viz. root rot, wilt, blight and anthracnose diseases. *Fusarium solani* caused a severe wilt, root rot and damping off and resulted a significant loss of crop [26]. The seed-borne nature of *F. solani* was reported on guar crop by Dwivedi, Dubey and Dwivedi [14]. Wilt disease of cluster bean is characterized by yellowing of lower leaves and stunting or dwarfing of plant growth. The margins of the cotyledonary leaves curl downward and inward. The stem near the soil line and transition zone may be slightly thickened and brittle. Seeds showed white mycelial growth. Brown to black discolouration appears on stem affected with wilt disease.

The present study carried out to observe the alteration in physiological properties of infected cluster bean plant parts. Quantification of total proteins and its related protease enzyme was carried out.

Materials and Methods

Healthy and *Fusarium solani* infected cluster bean plant parts, grown in Sikar (ac. no. CB70) districts of Rajasthan were collected and their biochemical studies were carried out. Estimation of total protein contents in healthy and diseased leaves, stem and seeds done by employing Lowry's method[17]. Protein reacts with folin ciocalteau reagent to give a coloured complex. This colour is produced by the reduction of phosphomolybdate by tyrosine and tryptophan of protein by the action of alkaline of copper.

(i) Extraction of Protein

For protein extraction 500 mg of normal and diseased infected leaves, stem and seeds samples were grind in pestle and mortar in 10 ml of 80% ethanol. It was centrifuged at 2000 rpm for 20 minutes. The supernatant was discarded and residue was suspended in 5% perchloric acid to remove sugars and soluble nitrogen fractions. Again centrifugation was done at 2000 rpm for 20 minutes. In order to remove acid soluble frictions and lipids the residues collected were re-suspended in a mixture of ethanol, ether and chloroform (2:1:1). The residue was dissolved in 1.0 ml of 1N NaOH. 0.1 ml of solution was taken and subsequently made up 1.0 ml by adding distill water.

(ii) Estimation of Protein contents

To 1 ml of dissolved residue, 5.0 ml of alkaline copper reagent was added and allowed to stand for 10 minutes. To this, 0.5 ml of Folin- Ciocalteaus's reagent (diluted with equal volume of distilled water) was rapidly added, mixed thoroughly and incubated at room temperature for 30 minutes, which resulted in development of blue colour. The optical density was measured at 750 nm in a UV spectrophotometer (UV-vis-

systronics 118). For blank ethanol was used. The amount of protein in healthy and infected samples of leaves, stem and seeds was calculated from a standard curve prepared from BSA (Bovine Serum Albumin). Protein content was expressed in terms of mg/gm fresh weight of the tissue.

(iii) Protease enzyme activity

2 ml casein solution (1% Casein dissolved in Phosphate buffer pH 7) was mixed with 1 ml of 0.1 M phosphate buffer pH 7.0 and 1 ml of enzyme extract. The mixture was incubated and kept at 30ºC in water bath for 1/2 hr. 1 ml enzyme substrate mixture was taken in centrifuge tube and 1 ml TCA was added. Then it was allowed to stand at room temperature for an hour and then centrifuged at 2000 rpm for 20 minutes. 1 ml of supernatant was pipetted and add 1 ml of folin ciocalteau's reagent and 2 ml of 20% sodium carbonate. Then the test tube was placed for 1 minute in boiling water bath cooled under tap water and made upto 10 ml with distilled water. Estimation of protease activity was done and absorbance was read at 650 nm [7].

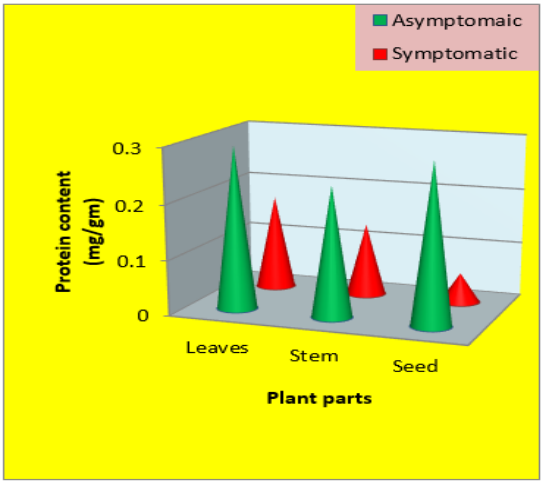
Results and Discussion

The alteration in protein contents and its related enzyme were observed in diseased and normal leaves, stem and seeds of cluster bean. The protein content were observed higher in healthy plant parts viz. leaves, stem and seeds as compared to wilt infected counterparts (Table:- 1 and Fig. 1A & C). However protease enzyme activity was found to be higher in infected seed, stem and leaves than healthy counterparts as shown in (Table: 1 and Fig. 1B & C).

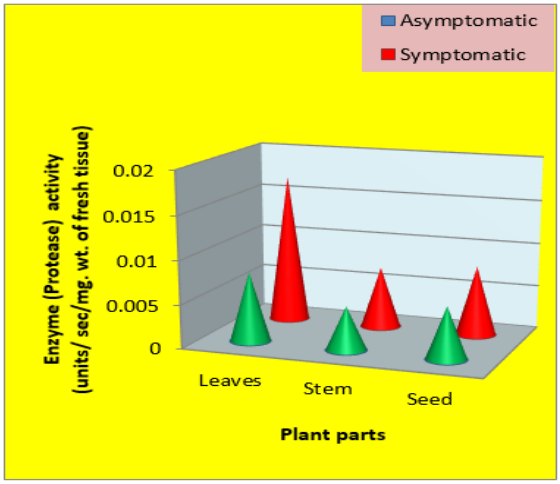
Table 1: Quantification of total Protein contents and their related enzyme (protease) activity in *Cyamopsis tetragonoloba* (L.) Taub.

Concentration (mg/g)	NSd	ISd	NS	IS	NL	IL
Protein contents (mg/g)	0.290±	0.206±	0.236±	0.132±	0.296±	0.172±
Protease activity (units/sec/mg. weight of fresh tissue)	0.006±	0.008±	0.005±	0.007±	0.008±	0.017±

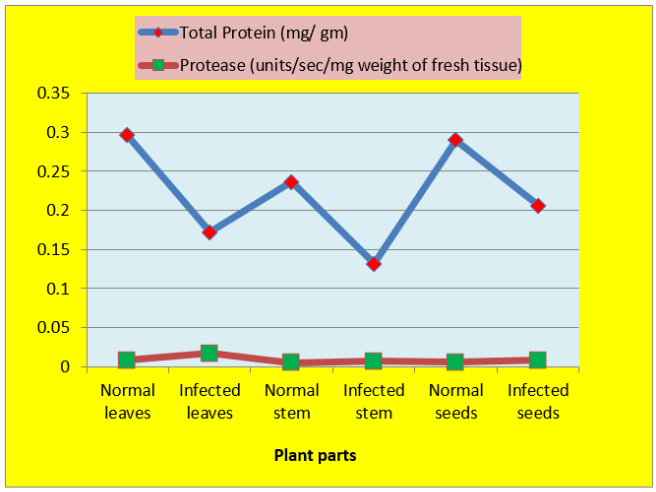
NSd = Normal seeds ISd = Infected seeds,
NS =Normal stem IS= Infected stem
NL = Normal leaves IL= Infected leaves



A



B



C

Fig 1: Comparison of protein and its related enzymes in guar.

A. Protein in healthy and wilt infected counterparts of guar.
B. Enzyme (Protease) activity in healthy and wilt infected plant parts.
C. Protein and protease activity in healthy and infected plant parts.

In the present study higher total protein content were observed 0.296, 0.236 and 0.290 mg/gm in healthy leaves, stem and seeds respectively where as it was observed 0.172, 0.132 and 0.206 mg/g in wilt infected counterparts.

Higher Protease activity was 0.017, 0.007 and 0.008 units/ sec/mg of fresh weight of tissue in case of infected plant parts viz. leaves, stem and seeds than the 0.008, 0.005 and 0.006 units/ sec/mg of fresh weight of tissue in healthy counterparts. Reduction in protein contents after inoculation with *Phytophthora drechsleri f. sp. cajani* in the resistant cultivar of pigeon pea [2]. Similar results were observed in sesame [28], in taramira and safflower [29], in sunflower seeds [22], in arhar seeds [32], in soybean seeds [35], in sugarcane [10], in chilli by [4], in tomato [6], in lentil by [27] and in chickpea by [30].

Yadav *et al*. [36] studied the Changes in protein content of mustard plant parts infected with *Albugo candida*. The fungi deteriorated biochemical qualities of seed due to change of nutritional profiles such as protein content and decrease the value for sowing, as food or feed.

Higher protein contents was observed in *Colletotrichum orbiculare* infected *Luffa cylindrica* plant parts viz. seed, leaf, stem and fruit than the healthy counterparts whereas protease enzyme activity was higher in case of healthy seed, leaf, stem and fruit than the anthracnose infected counterparts [25]. Similar observations were reported at early stages of infection followed by subsequent decline at later stages in *Taphrina maculans* infected leaves of *Curcuma longa* [3], *Brassica juncea* infected *Macrophomina phaseolina* [33], pearl millet infected by *Sclerospora graminicola* [23], lemon fruit infected by *Colletotrichum gloecosporioides* [15].

Storage fungi cause deterioration of the seeds by affecting planting and edible value. Both asymptomatic seeds and symptomatic seeds of soybean infected with *Rhizoctonia bataticola* showed initial enhancement of protease activity. Later it declined continuously in symptomatic seeds [35].

Alteration in protein metabolism of plant tissue during pathogenesis has been reported in cumin [9, 37] and in *Braassica* juncea [11].

Insignificant increase in protein contents were observed in unhealthy seeds of melon than the healthy seeds [1]. This may be due to the increased activity of the hydrolytic enzymes protease, in diseased tissue by the enhanced state of the host metabolism after infection or by the pathogen itself [16]. The fungi has strong ability to infect all components of seeds and therefore as reflected by reduction of seed quality parameters [8].

Akhtaruzzaman *et al*. [5] were isolate and characterize the protease from 7 leguminous seeds viz. soyabean, lentil, black gram, bengal gram, groundnut and pea. Highest concentration was observed in groundnut and lowest concentration was observed in lentil.

Conclusion

Present investigation suggests that infection of fungal pathogen in plants cause alteration in their biochemical composition. Related enzyme activities were also altered due to the heavy fungal invasion. In case of cluster bean present study also revealed that the total protein contents were higher in healthy plant parts than the *Fusarium solani* infected counterparts whereas related protease enzyme activity was observed higher in wilt infected plant parts than the healthy counterparts. The maximum total protein contents were found in healthy leaves and maximum protease enzyme activity was observed in wilt infected leaves. However, present study is helpful to understand the alteration in biochemical contents like total protein and related enzymes like protease in various plant parts infected with fungal pathogens.

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