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BIOCHEMICAL STUDIES OF BLACK GRAM SEEDS INFECTED WITH *RHIZOCTONIA BATATICOLA* AND *FUSARIUM OXYSPORUM*

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Vigna is one of the important genera growing among the pulses forming a part of Indian people`s diet. Various seed-borne fungi found to attack the pulses during storage and deteriorate chemical contents to some extent.

Keywords: - Seed-borne pathogens, storage conditions, biochemical.

INTRODUCTION

Pulses constitute important proteinaceous crops. Several Seed-borne fungi are known to cause considerable loss in the seed content. Bilgrami *et.al.* (1976) studied the deterioration of black gram seed in storage conditions by *Aspergillus flavus*. They recorded loss in sugars and organic acid contents but a few amino acids and organic acids were found to be synthesized by *Aspergillus flavus*. Deterioration of proteins, total sugars and protein contents was also noted in black gram seeds during infestation by *A. flavus* in thirty-eight cultivars of pulse crops by Premlata *et.al.* (1990). Ibraheem, Okesha and Mhathem (1987) reported a marked decrease in the protein contents of soybean seeds by associated seed-borne fungi. Contrary to this, an increase in protein contents in rice seeds due to helminthosporiose infection was reported by Vidhyasekaran *et.al.* (1973). Shukla *et.al.* (1988) recorded a decrease in protein contents of Arhar seeds infested with different Aspergilli. The amount differed from species to species. *A. flavus* and *A. niger* brought maximum and *A. awamori* and *A. nidulans* minimum alterations in protein contents.

Black gram contains 59.6% carbohydrates. Bilgrami *et.al.* (1976) recorded the disappearance of sugars within 5 days of the incubation period. But it again appeared on the 10th and 15th days of incubation. Vidhyasekaran and Govindaswamy (1968) also observed the accumulation of reducing sugars due to seed-borne fungi in paddy seeds. Changes in starch contents of Arhar seeds by fungal infection were also studied by Sinha *et.al.* (1981). Maheshwari *et.al*. (1984) observed depletion in starch contents of Coriander sativum infected with *Protomyces macrosporus*.

Faulty storage conditions make the seeds more vulnerable to fungal attacks. Several storage fungi produce aflatoxins which cause significant health hazards in human beings. Aflatoxin production in water-soaked black gram seeds by four isolates of *A. flavus* was observed by Reddy and Subbaya (1981). Premlata *et.al.* (1990) screened thirty-eight different pulse cultivars against aflatoxin production by *A. flavus*. Two cultivars were found highly resistant against aflatoxin elaboration. They also recorded a more incredible amount of total phenol and proteins in resistant varieties than susceptible ones of the same pulse crop.

MATERIALS AND METHODS

Essential constituents of black gram seed *viz.* proteins, starch and total soluble sugars were studied in symptomatic seeds infected with *R. bataticola* and *F. oxysporum*. Standard biochemical techniques were followed for analysis. Healthy seeds were taken as control.

Extraction

100 mg of dry seed powder was extracted in 10 ml. of 80% ethanol and kept overnight. The extracted mass was centrifuged at 1000 rpm for 30 min. The supernatant so obtained was used for the estimation of total soluble sugars whereas the residue was collected for starch estimation.

Protein Estimation

Total soluble proteins were estimated by the method of Lowry, Rosebrough, Farr and Randale (1951). 100 mg of seed powder was mixed in 10

ml. of ethanol-ether (2:1, v/v) solution and was left overnight. It was centrifuged for 5 min. at 2500rpm, the supernatant was discarded. The residue after drying was used for further extraction. The dried residue was mixed in 10 ml. of 10% TCA (Tri Carboxylic Acid), ground properly and centrifuged for 5 min. The supernatant was discarded and residue was further treated by the same practice. Later the residue was mixed with 10 ml. of 1N NaOH, boiled for 10 min, cooled aliquot was taken and mixed with 5 ml alkaline solution. To this 0.5 ml Folian reagent was added and shaken properly. After 30 min absorbance was taken on a spectronic-20 spectrophotometer at 750 nm using the filter. The alkaline solution was prepared by mixing Reagent B (0.5% copper sulphate in 1% sodium potassium tartrate). A standard curve was prepared by using Bovine Serum Albumin protein. The same method was employed and adopted for infected seeds.

Total Soluble Sugars Estimation

Total soluble sugars were estimated according to the method as described by Dubbois, Giller, Hailton, Rebers and Smith (1951).

0.5 ml of supernatant per sample was taken and 1 ml of 5% phenol was added to this. To the final solution, 5 ml of 96% sulphuric acid was rapidly mixed and allowed to cool under running tap water for 20 min. The optical density was determined at 490 nm. A reference curve was prepared by using glucose.

Starch Estimation

Starch contents were estimated by following the method of McCready, Guggols, Silviera and Owens (1950). The residue was utilized for starch estimation. It was suspended in 5 ml of distilled water and subsequently, 6.5 ml of 52% perchloric acid was added to this. The final volume was centrifuged at 1000 rpm for 30 min. This process was repeated three times and the supernatant was collected and the final volume was made 100 ml by adding deionised water. This mixture was filtered through Whatman filter paper no. 42. 0.5 ml of this filtrate was used for estimating starch contents by following the same method adopted for total soluble sugars. Starch contents were calculated by using a conversion factor of 0.9 to convert the values of glucose to starch.

RESULTS AND DISSCUSSION

Biochemical estimation of total soluble proteins, total soluble sugars and starch in healthy and *R. bataticola* and *F. oxysporum* infected seeds were carried out.

Total soluble proteins

The amount of protein was 211.3 mg/gm in healthy seeds whereas in *R. bataticola* and *F. oxysporum* infected seeds it was 233.6 and 215.0 mg/gm, respectively. The increase was found insignificant.

Total soluble sugars

No significant difference in sugar content was observed. The amount was 55.6 mg/gm in healthy seeds and 51.4 mg/gm in *R. bataticola* and 530.0 mg/gm in *F. oxysporum,* infected seeds.

Starch

Starch was lower in infected seeds than the healthy ones but the difference was insignificant. It was recorded 518.20 mg/gm in healthy seeds whereas 510.00 mg/gm in *R. bataticola* and 486.52 mg/gm in *F. oxysporum* infected seeds.

TABLE 18 : Quantitative changes in various metabolites of seeds of black gram naturally infected with *R. bataticola* and *F. oxysporum*.

Seeds	Metabolites(mg/gm) of dry weight*		
	Total soluble proteins	Total soluble sugars	Starch
Healthy seeds	211.3	55.6	518.20
R. bataticola	223.6	51.4	510.00
F. oxysporum	215.0	53.0	486.52
C.D. at 5% level	N.S.	N.S.	N.S.

* = Average of three replicates.

An attempt was made to study the changes in important primary products of black gram seeds naturally infected with *R. bataticola* and *F. oxysporum*.

No significant difference was observed in starch contents in infected and normal seeds. Histochemical analysis revealed strong reaction in infected cells similar to healthy tissues Maheshwari *et. al.* (1981) in "Arhar" seeds. Starch content were found to be decreased in seeds of pigeon pea as a result of infection of *R. bataticola* (Sharma, 1996)

During present investigation lipids were found to show negative reaction both in healthy and infected tissues.

The presence of cellulose and lignin was demonstrated in cell walls of cotyledons of both the infected and uninfected seeds.

In the present study tannins and phenols both were found to reveal negative reaction to the stain. Many authors have correlated the presence of tannin contents with the degree of resistance shown by host (Bhatia, Uppal and Bajaj, 1972 and Chopra, Jhooty and Bajaj, 1972).

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