ORIGINAL RESEARCH PAPER

Botany

EFFECT OF THE METABOLITES OF SEEDBORNE STORAGE FUNGI ON THE ACTIVITY OF PROTEASE OF THE SEED OF PIGEON PEA

KEY WORDS: Seed-borne fungi, Pigeon Pea

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ABSTRACT

Pigeon Pea are common dietary pulses rich in carbohydrate, proteins and minerals. Numerous fungi affect Pigeon Pea adversely causing reductions in seed content and seed health. During present study effect of enzyme metabolites of common and dominant seed-borne fungi of Pigeon Pea. Altogether, 52 spp. of seed borne storage fungi were isolated from red gram seed of which 6 spp. belong to Phycomycotina, 6 spp. belong to Ascomycotina and rest to Deuteromycotina. *Aspergillus* spp. dominated among the isolates. The frequency of *A. flavus* was highest followed by *A. niger* and *A. alternata*. These seed borne fungi produced protease enzyme in variable quantity, which helped the fungi degrade the seeds and ultimately affected seed quality and yield.

INTRODUCTION

Pigeon Pea *(Cajanus cajan)* is the most common pulses of Bihar belonging to family leguminosae. Pulses are important source of protein and amino acid for Indian Vegetarians. Pigeon Pea are cultivated in Bihar during the Kharif season.

Various seed borne fungi affect Pigeon Pea. Specially *Aspergillus* spp. dominated among the isolates. Protease enzyme is important metabolite of seed-borne fungi necessary for Pathogenesis. All the common and dominant seed-borne fungi produce protease enzyme in variable quantities. Protease enzymes cause degradation of protein content of the seed and reduce its protein content affecting seed quality.

MATERIALS AND METHOD

200g seeds of red gram cv stored with farmers was collected from different parts of Bihar state (Madhepura, Saharsa. Munger, Bhagalpur, Chapra) The collected seed was packed in sterilized polyethylene packets and stored at 5-6

Buffer:

I. Phosphate Buffer, pH 7.0

0.2M	Na_2HPO_4	61.0 mL
0.2M	KH_2PO_4	39.0 mL

These were mixed together.

ii. Citrate Buffer – 21 g of citric acid was dissolved in 50 mL of 0.1N NaOH

Reagents:

i. Ninhydrin: 250 mg in 6.25 mL of acetone
ii. Stannous Chloride: 10 mg in 6.25 mL of citrate buffer
iii. Diluents: Propanol : water (1:1)

Procedure:

Following were taken in a tube:

Casein Solution	2 mL
Phosphate Buffer	1 mL
Enzyme Extract	1 mL

The mixture was incubated at 30°C for 30 min. 1 ml of the aliquot was taken from the reaction mixture and 2 ml of ninhydrine reagent was mixed.

1 ml of mixture was diluted to 15 ml with dilutes and read at 570 nm. This reads the amount of total amino acid released from protein casein due to the activity of proteolytic enzyme extracted from the seeds treated with the metabolites of seed borne storage fungi.

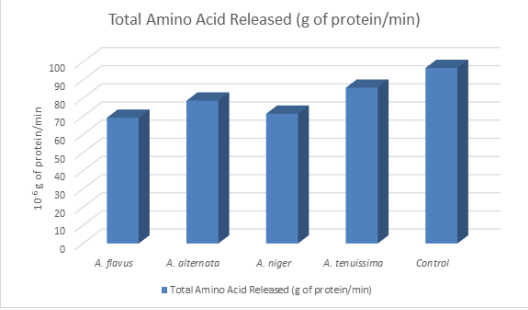
Calculation of the Results

The amount of amino acids released from casein was estimated by calibration curve prepared using 0.1 % solution of L–leucine. The result was recorded as gram amino acid released per gram protein per min.

Table

Effect of metabolite of seed borne storage fungi on the activity of proteolytic enzyme (expressed as g total amino acid released g/min of protein)

Storage Fungi	Total Amino Acid Released (g of protein/min)
A. flavus	69.3×10^{-6}
A. alternata	78.7×10^{-6}
A. niger	71.5×10^{-6}
A. tenuissima	85.8×10^{-6}
Control	96.7×10^{-6}



RESULT

i. It appears that the total amino acid released from casein was maximum from the control seedlot while the metabolite of the seed-borne storage fungi considerably suppressed the release of amino acid from casein as substrate.
ii. As regards the effect of the metabolite of storage fungi, *A. flavus* suppressed the release of amino acid to the maximum followed in succession by *A. niger*, *A. alternata* and *A. tenuissima*. Less amount of amino acid released indicates suppressed proteolytic enzyme activity and more amino acid released, the augmented activity, of proteolytic enzyme.

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