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RESEARCH ARTICLE

FUNGAL PROTEASE ASSAY OF CHICKPEA AND PIGEON PEA

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ABSTRACT

Green gram, Black gram, Pigeon pea and chickpea are common pulses in diet rich in carbohydrates, proteins and minerals. Numerous fungi affect pulses adversely causing reduction in seed content and seed health. During present study, effects of enzyme metabolites of six common and dominant seed-borne fungi of test pulses on seed health of Chickpea and Pigeon pea are evaluated. Total seventeen fungi recorded from all four test pulses. Out of these seventeen seed-borne fungi, six were found to be common and dominant on all four test pulses. These common and dominant seed-borne fungi produced protease enzyme in variable quantity, which helped the fungi degrade the seeds and ultimately affected seed quality and yield of test pulses. Common and dominant seed-borne fungi of pulses tested for their protease activity against Chickpea and Pigeon pea.

INTRODUCTION

Pulses are the second most important group of food plants belonging to family Leguminosae. They form an important and indispensable part of daily diet. It is important source of dietary carbohydrates, proteins, essential amino acids and micronutrients such as calcium, phosphorus and iron. Therefore, pulses are important source of protein and essential amino acids for major vegetarians. Pulses like Black gram (*Vigna radiata* L.), Black gram (*Vigna mungo* L), Chickpea (*Cicer arietinum* L.) and Pigeon pea (*Cajanus cajan* L) etc are cultivated in Marathwada region of Maharashtra during Kharif and rabbi seasons, either as sole or intercrops, under rain fed or irrigated conditions.

Various seed-borne fungi affect pulses. Seventeen seed-borne fungi reported from all four test pulses i.e. Green gram, Black gram, Chickpea and Pigeon pea, of these six found to be common and dominant; these are *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera* and *Rhizopus stolonifer*. 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 quantity. Protease enzymes cause degradation of protein content of the seed and reduce its protein content affecting seed quality.

Chauhan and Nager (1979) studied seed mycoflora of groundnut and sunflower and found loss in protein content due to seed-borne fungi. Chary and Reddy (1982) found that, fungi isolated from decaying moong seeds showed protease production, especially *Fusarium oxysporum*, *Rhizoctonia solani* and *Phoma exigua* were proteolytic. Ivanov et al. (1989) studied loss in protein content due to seed-borne fungi in groundnut and sunflower. Usmalini et. al. (1998) reported *Fusarium oxysporum* caused maximum reduction in sugars. While *Macrophomina phaseolina* reduced protein content. Reduction in protein content due to *Fusarium oxysporum* and *Aspergillus flavus* was significant, functional role of proteolytic enzyme includes hydrolysis of macromolecular substrates, initiation and maintenance of pathogenesis (Kudryavtseva et.al. 2008). Virulence of *Aspergillus flavus* and *Penicillium* sp. Caused cell wall degradation due to production of enzymes (Oluyemisi et.al, 2006). Mirian et.al. (2011) found variation in P^H affects microbes enzymes quantitatively and qualitatively; 9.0 P^H was reported to be best for *Aspergillus ochraceus, Fusarium moniliforme* and *Fusarium solani*.

MATERIALS AND METHODS

Preparation of spore suspension

Spore suspension of common and dominant seed-borne fungi of pulses were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures of seed-borne fungi of pulses; maintained on PDA slants for seven days at room temperature. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

Table 1

Different medium for protease activity		
Chickpea flour medium:	**Pigeon pea flour medium:**	**Basal medium for protease assay:**
Chickpea flour : 10 g KNO_3 : 2.5 KH_2PO_4: 1.0 g $MgSO_4.7H_2O$: 0.5 g Distilled water: 1000 ml.	Pigeon pea flour: 10g KNO_3 : 2.5 KH_2PO_4: 1.0 g $MgSO_4.7H_2O$: 0.5 g Distilled water: 1000 ml	Agar: 2g Gelatin: 1g Distilled water: 100 ml

Protease production

Protease production was done by growing common and dominant seed-borne fungi of pulses i.e. *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera* and *Rhizopus stolonifer* on liquid seed flour media of Chickpea and Pigeon pea. Twenty-five ml of Chickpea and Pigeon pea seed flour media was poured in 100 ml conical flasks separately and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with spore suspension of test fungi prepared from 7 day old culture grown on PDA slants. These flasks were incubated for 10 days at 25 ± 1 ^{0}C and on 11th day, the contents were filtered through Whatman filter paper No.1 to remove fungal mat and the liquid part was collected in pre-sterilized bottles and used as crude protease enzyme preparations.

Protease assay by cup plate method

Determination of protease activity was done with the help of cup plate method, adopted by Hislop et.al. (1982) and Rajamani et al. (1988). A basal medium was prepared containing 2 % (w/v) agar and 1% (w/v) gelatin. P^H of the medium was adjusted at 5.6. The medium was sterilized at 15 lbs pressure for 20 minutes. 15 ml of medium was poured in presterilized Petriplates under aseptic conditions. 6 mm diameter cavities (cups) were made in the center of the solidified agar plate with No.4 cork borer. About 0.5 ml of culture filtrate (crude enzyme preparation) was poured in the cavity. The plates were incubated at 25^{0}C for 24 hrs. 15% $Hgcl_2$ in 7M HCl was added to the plates. After 10 minutes, a transparent zone indicating hydrolysis of gelatin by extra cellular proteolytic enzymes was observed. The diameter of the transparent zone was used as a measure (mm) of protease activity and non appearance of clear zone considered absence of protease in culture filtrate.

RESULTS AND DISCUSSION

The results in the Table clearly show that, all six common and dominant seed-borne fungi of pulses showed protease activity in variable degree. In case of Chickpea Maximum protease activity was shown by *Fusarium moniliforme* (22 mm activity zone). Minimum protease activity was reported in *Aspergillus niger* (12 mm activity zone), similarly *Aspergillus flavus* also showed 12 mm activity zone. Whereas, in case of Pigeon pea *Fusarium moniliforme* showed maximum activity among all seed-borne fungi (20 mm activity zone), followed by *Rhizopus stolonifer*. Minimum protease activity shown by *Drechslera tetramera* (12 mm activity zone).

Similar findings were recorded by Yike (2011) he stated that, proteolytic enzymes play an important role in fungal physiology and development. External digestion of protein substrates by secreted proteases is required for survival and growth of both saprophytic and pathogenic species.

Table 2 Protease activity of common and dominant seed-borne fungi of Chickpea and Pigeon pea (After ten days of incubation, by cup plate method).

Sr. No.	Common and dominant seed-borne fungi of pulses	Protease activity by Chickpea (activity in mm zone) Basal medium	Protease activity by Pigeon pea (activity in mm zone) Basal medium
1	*Aspergillus flavus*	14	14
2	*Aspergillus fumigatus*	15	15
3	*Aspergillus niger*	12	12
4	*Drechslera tetramera*	17	20
5	*Fusarium moniliforme*	22	15
6	*Rhizopus stolonifer*	18	17

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