

Protease activity by common and dominant seed-borne fungi of Green gram.

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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 common and dominant seed-borne fungi of test pulses on seed health of Green gram are evaluated. Total seventeen fungi recorded from all four test pulses. Out of these seventeen seed-borne fungi, six, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Drechslera tetramera* and *Rhizopus stolonifer*, 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.

Keywords: seed-borne fungi, pulses, Green gram

Introduction:

Pulses are the second most important group of food plants belonging to family Leguminosae. They form an important and indispensable part of our 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. The pulses like Green gram (*Vigna radiata* L.), Black gram (*Vigna mungo* L), and Chickpea (*Cicer arietinum* L.) 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. Usmani et al. (1998) reported *Fusarium oxysporum* caused maximum reduction in sugars compared to other fungi. While, *Macrophomina phaseolina* reduced protein content with respect to control. 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:

i) 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.

ii) Green gram flour medium:

Green gram flour: 10 g
KNO₃ : 2.5
KH₂PO₄: 1.0 g
MgSO₄.7H₂O: 0.5 g
Distilled water: 1000 ml.

iii) Basal medium for protease assay:

Agar: 2g
Gelatin: 1g
Distilled water: 100 ml

iv) Protease production:

Protease production was done by growing common and dominant seed-borne fungi of pulses on liquid seed flour media of Green gram. Twenty five ml of Green gram 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 °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 enzyme preparations.

v) 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. PH 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°C for 24 hrs. 15% HgCl₂ 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 common and dominant seed-borne fungi of pulses showed protease activity in variable degree. The seed-borne fungus *Fusarium moniliforme* showed maximum proteolytic activity (23 mm activity zone); whereas minimum activity was recorded by *Drechslera tetramera* (13 mm activity zone).

Similar findings were recorded by Yike (2011) 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: Protease activity of common and dominant seed-borne fungi of Green gram (After ten days of incubation, by cup plate method).

Sr. No.	Common and dominant seed-borne fungi	Protease activity (activity in mm zone)
		Glucose-gelatin medium (GGM)
1	<i>Aspergillus flavus</i>	15
2	<i>Aspergillus fumigatus</i>	14
3	<i>Aspergillus niger</i>	15
4	<i>Drechslera tetramera</i>	13
5	<i>Fusarium moniliforme</i>	23
6	<i>Rhizopus stolonifer</i>	22

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