Isolation and identification of Seed-Borne Fungi in Green gram Seeds

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Abstract: An experiment was undertaken to isolate and identify the seed borne fungi of Green gram. Seed samples of three Green gram varieties were collected from Pulse research station, NAU, Navsari. PDA plating method was employed for the isolation of the fungi and cultural, microscopic study was adopted for the identification. Altogether nine fungi were found in association *Viz*., *M. phaseolina*, *A. alternata*, *C. capsici*, *N. sphaerica*, *Chaetomium* sp., *Aspergillus* sp., *R. oryzae*, sterile aseptate and sterile septate fungus with all the three variety seed lots.

Key Words: Seed-Borne Fungi, Green gram, cultural, morphological study.

Introduction:

Green gram (*Vigna radiata* (L.) Wilczek) is one of the most important pulse crop of the arid and semiarid tropics (Chen *et al*., 1987). It is an excellent source of easily digestible protein. Several factors are responsible for low production of Green gram. Among them, diseases play an important role (Nene, 1980; Pal, 1996). Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productive capacity. However, published reports of seed-borne fungi of Green gram in India are very few. Hence the study was undertaken to isolate and identify the incidence of seed-borne fungi associated with Green gram.

Materials and Methods:

Green gram varieties, GM-3, GM-4 and K-851 are very popular in south Gujarat region and are grown extensively. The bulk of seed samples of these three varieties grown at Pulse Research Station, Navsari Agricultural University, Navsari were collected at harvest and stored for two months in polythene bags. The agar plate method (ISTA, 1999) was used for the isolation of seed mycoflora of green gram. Ten seeds of each variety were taken randomly and then transferred aseptically in Petri plates containing 20 ml solidified sterilized potato dextrose agar (PDA) medium sterilized in an autoclave at 1.2 kg cm^{-2} pressure for 20 minutes using laminar air flow system. The seeds were placed at equal distance per plate by keeping one seed at the centre and nine seeds at the periphery. These seeds were incubated under 12/12 hr alternating light and darkened period at 25± 2^0C for 8 days. Purification of the fungal culture was done from every new growth by hyphal tip culture. Five repetitions were kept for each of each variety and identification of the common fungi occurred in all the three varieties were done by microscopic observations and with the help of literature. These fungi were designated as isolate 1 to 9. Each of the purified isolate was identified on the basis of their cultural and morphological characteristics. The pure culture slants were also sent to ITCC, New Delhi for further confirmation.

Results and discussion:

Results revealed from the isolation and identification that there is association of nine different fungi. The results obtained from cultural and morphological characteristics were presented hereunder

Isolate number 1

The colonies of the fungus grew fast on PDA and attained full plate diameter of about 85mm, profused mycelial growth within 5 to 6 days at 25±20C temperature in incubation with sclerotial formation. The fungus produced initially white mycelial growth later changing to brown black in centre due to the formation of numerous small black sclerotia. The mycelium was hyaline to brown, branched, septate and 1.73 to 6.52 µm in width. The sclerotia formed in culture were black, hard and 67.90 to 195.14 µm in diameter. The pycnidia were not observed in the culture (Plate I-1). The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Macrophomina phaseolina* as described by Tandel (2004). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *M.phaseolina* (I.T.C.C. No.7811.10).Thus, the fungus under present study was identified as *Macrophomina phaseolina* (Tassi) Goid.

Isolate number 2

The colonies of the fungus grew fast on PDA and attained full plate diameter of about 85mm with profused mycelial growth within 8 days at 25±20C temperature in incubation with black to dark grey cloured zonations. The mycelium was irregularly branched at acute angle. The conidiophores were light brown, simple and septate bearing obclavate to oval, light to dark brown, muriform conidia with 1 to 4 transverse and 0 to 2 longitudinal septa, variable in size and shape with rudimentary beak and measuring from 10.80 to 58.57 x 5.41 to 16.20 µm (31.73 to 11.54 µm) in size (Plate I-2). The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Alternaria alternata* as described by Patel (2003). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *A. alternata* (I.T.C.C. No. 7820.10). Thus, the fungus under presentstudy was identified as *Alternaria alternata* (Fr.) Keissler.

Isolate number 3

The colony of isolated fungus was initially cottony white, which turned finally black in colour. The hyphae were initially hyaline, turning dark at maturity and septate. Conidia were single celled, hyaline, crescent shaped and curved at both ends, measuring 22 x 5 µm in size. Acervuli were brown to black in colour, irregular to oblong in shape and measured from 250 to 460 x 180 to 360 µm (av. 384 x 288 µm), producing hyaline, short cylindrical conidiophores and dark brown long setae. Setae were abundant, brown in colour, 1-4 septate, slightly tapered towards the apex and measuring from 58.6 to 156 x 5 to 6.7 µm (99.75 x 5.85 µm) in size (Plate I-3). The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Colletotrichum capsici* as described earlier by Agrawal (1991). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *C. capsici* (I.T.C.C. No. 7813.10). Thus, the fungus under present study was identified as *Colletotrichum capsici* (Syd.) Butler & Bisby.

Isolate number 4

The colony of isolated fungus was initially white later turned flat and grey coloured. The fungi produced dark brown, septate, branched mycelium and spherical to subspherical, single-celled conidia measuring 13 to 16 × 11 to 13 µm (14.5 x 12 µm) in size, which were borned on a hyaline vesicle at the tip of the conidiophores (Plate I-4).

The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Nigrospora sphaerica* as described earlier by Zang *et al*. (2011). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *N. sphaerica* (I.T.C.C. No. 7819.10). Thus, the fungus under present study was identified as *Nigrospora sphaerica* E. W.Masson.

Isolate number 5

Initial colony growth of the isolated fungus was appeared cottony white which later turned grey to olive coloured. However, colonies appeared brown to black on reverse. Brown coloured subglobose or ellipsoid perithecia were almost covered with curly blackish hair like structure in the upper surface. Asci appeared hyaline and clavate, which contained 8 ascospores. Ascospores were apiculate at both the ends. Ascospores appeared olive coloured, elliptical or somewhat lemon shaped (Plate I-5).

The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Chaetomium sp*. as described earlier by Kotgire (2009). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *Chaetomium sp.* (I.T.C.C. Numbers 7815.10). Thus, the fungus under present study was identified as *Chaetomium sp*.

Isolate number 6

The isolated fungus produced hyaline to white light mycelium on PDA, grew rapidly and turned into dirty white to black colony, which covered the entire Petri plate (90 mm) within four days of incubation at 25 ± 2oC. The hyphae was hyaline and septate.The conidiophores were erect, unbranched, straight, hyaline to light brown, long aseptate and darker near the vesicle. The vesicle was globose, thick walled and brown to black. The conidia produced in chain were globose, single celled, pale to dark brown on maturity. They measured 3.5 to 5 µm in size. (Plate I-6)

The studies on the cultural and morphological characters of isolated fungus showed its close identity with *Aspergillus* sp. as described earlier by Wable (2006), Moreover, the I.T.C.C., New Delhi also identified the same fungus as *Aspergillus* sp. (I.T.C.C. No. 7818.10). Thus, the pathogen under study was confirmded as *Aspergillus* sp.

Isolate number 7

The isolated fungus on PDA produced fast growing colonies and covered the entire Petri plate (90 mm) in three days at 25 + 2°C temperature of incubation. The fungal growth was white cottony, sporangiophores were smooth walled, non-septate, simple or branched, arising from stolons opposite to rhizoids usually in groups of 3 or more. Sporangia were globose with flattened base, grayish black, 150 µm in diameter with many spored. Sporangiospores were sub-globose with ridges on the surface (Plate I-7). The studies on the morphological and cultural characters of isolated fungus showed its close identity with *Rhizopus oryzae* as described earlier by Wable (2006). Moreover, the I.T.C.C., New Delhi also identified the same fungus as *R. oryzae*. (I.T.C.C. No. 7817.10). Thus, the fungus under present study was confirmded as *Rhizopus oryzae* Went & Prins.Geerl.

Isolate number 8

The isolated fungus on PDA produced fast growing white colonies and covered the entire Petri plate (90 mm) in 4 days (25 + 2oC) of incubation. The mycelium was aseptate, hyaline and there was no production of conidia or spores (Plate I-8).The fungus thus identified as sterile fungus as it was also reported earlier by Tandel (2004). The I.T.C.C., New Delhi also identified the same fungus as sterile aseptate fungus (I.T.C.C. No. 7814.10).

Isolate number 9

The isolated fungus on PDA produced fast growing white colonies later turned to faint red and covered the entire Petri plate (90 mm) in 4 days (25 + 2oC) of incubation. The mycelium was septate, hyaline and there was no production of conidia or spores (Plate I-9).Thus, the fungi was tentatively identified as sterile fungus as it was earlier isolated and identified by Tandel (2004).Moreover, the I.T.C.C., New Delhi also identified the same fungus as sterile septate fungus (I.T.C.C. No. 7816.10). Thus, the fungus under present study was confirmed as septate sterile fungus.

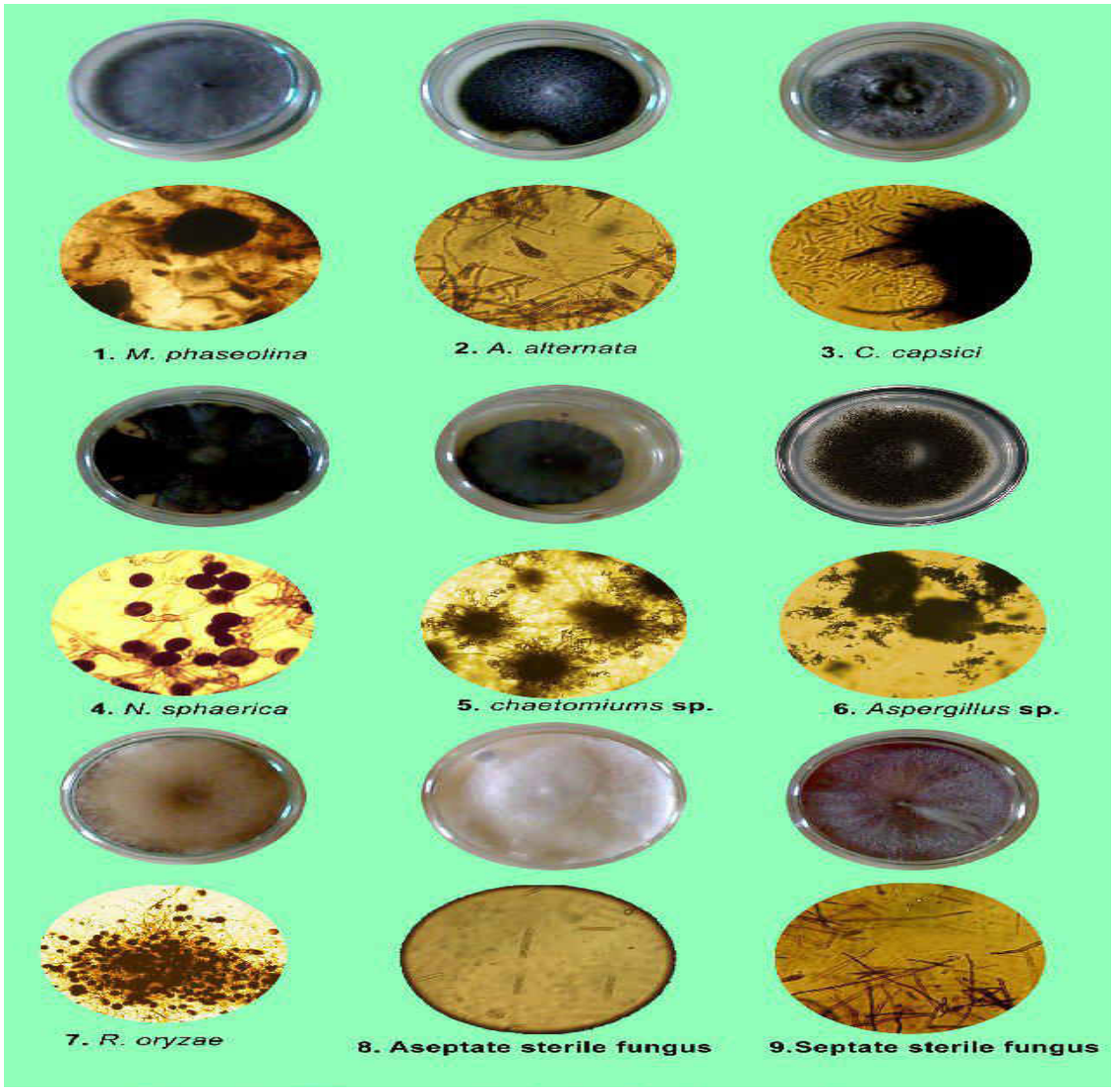

Plate I: Different seed infecting fungi in plate and microscopic view isolated from Green gram

Conclusion:

The seed samples of all the three varieties revealed the association of nine different fungi Viz., *M. phaseolina*, *A. alternata*, *C. capsici*, *N. sphaerica*, *Chaetomium* sp., *Aspergillus* sp., *R. oryzae*, sterile aseptate and sterile septate fungus

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