Seed Transmission Studies on Seedborne Fungi of Soybean

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Abstract- The present investigation was undertaken with the main objective to determine studies on seedborne fungi of soybean were conducted at DSST and Plant Pathology, College of Agriculture, Rajendranagar, Hyderabad. A total of 120 representative soybean seed samples (cv. JS - 335) were collected from major soybean growing districts of Andhra Pradesh *viz.*, Adilabad (60 samples) and Nizamabad (60 samples) during *Rainy* season 2012. Per cent total incidence of seed mycoflora in Nizamabad and Adilabad districts ranged from 30 to 49.2 % and 23.6 to 45.0 % by blotter method, 14.8 to 28.1% and 11.6 to 22.1% by 2, 4 - D blotter method, 11.8 to 19.3 % and 9.5 to 16.2 % by deep freeze blotter method, 13.1 to 37% and 15.4 to 26.4 % by agar plate method, respectively. Out of nine fungal species recorded, *Macrophomina phaseolina* was found predominant in the samples analysed from two districts (8.5 to 28.5 %), while the occurrence of *Cladosporium* sp. (0.3 to 0.5%) was least. Seed transmission of *M. phaseolina* in apparently healthy soybean seeds (cv. JS 335) was 6 % and 8 % and in artificially inoculated soybean seeds (38.5 % and 49 %) and in naturally infected soybean seeds (32 % and 43.1 %). Germination in the above seed samples ranged from 75% to 72%, 55% to 46% and 59.3 % to 50.5 % in test tube water agar method (*in vitro*) and in glasshouse conditions.

Index Terms- Soybean, Fungi, Seed mycoflora, Seed samples and Seed transmission.

I. Introduction

Soybean (*Glycine max* (L.) Merrill) the "golden bean" is one of the fore most important oil seed crop known for its excellent protein (42-45%), oil (22%) and starch content (21%). It is good source of vitamin - B complex, thiamine and riboflavin. Soybean protein is rich in valuable amino acids like lysine (5%) in which, most of the cereals are deficient. Soybean can substitute for meat and to some extent to milk (Endres *et al.*, 2013)[1]. Inspite of phenomenal increase in area and soybean production, its productivity remains low because of lack of quality seeds. Low yield and productivity of soybean in India is mainly due to various diseases and pests occurring in the field and causing yield losses. One of the major constraints in the endeavour of increasing productivity of soybean is its susceptibility to a large number of diseases caused by fungi, bacteria, viruses and nematodes. In India, although 40 fungal pathogens have been identified in soybean crop, but only a few of them are economically important (Sarbhoy and Agarwal, 1983)[2]. Seeds of soybean are known to harbour several species of seed borne fungi *viz.*, *Cercospora kikuchi, Alternaria alternata, Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Colletotrichum dematium, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium* sp. and *Rhizopus stolonifer* were found in germinating seeds and seedlings of soybean (Shovan *et al.*, 2008)[3]. Disease free quality seed production in soybean is utmost important to sustain the productivity and maintain the quality of the crop**.** The infected seeds failed to germinate or seedlings and plants developed in the field from infected seeds may escape the early infection but often may be infected at the later stages of the crop growth. Besides, pathogens can spread over a longer distance and uninfected field may be infected by the seeds in which different pathogens are present. The frequency in occurrence of such potentially pathogenic fungi on soybean cultivars poses a potential threat in crop production programme.Transmission of the pathogen through seed is also known as a means of spread of disease into new areas and new countries. *M. phaseolina* in soybean transmits from seed to seedlings in a systemic manner. The reduction in seed germination and increase in seed rot and seedling mortality were noticed. Another adverse effect of seed borne pathogens is that it contaminates the areas which were disease free previously. So, it necessitates the eradication of seed borne inoculum through various seed treatments and through the enforcement of proper domestic and international quarantine acts and procedures (Arya *et al*., 2004)[4]. Keeping this in view, the present investigation was taken up.

II. Materials And Methods

Scope of the Study

The present experiment was carried out at Department of Seed Science and Technology and Plant Pathology, Rajendranagar, Hyderabad during *rainy* season, 2012. Soybean seed samples (cv. JS - 335) collected from different soybean growing districts of Andhra Pradesh *viz.*, Nizamabad and Adilabad districts of Andhra Pradesh.

Collection of soybean seed samples

One hundred and twenty soybean seed samples were collected from the major soybean growing districts of Andhra Pradesh *viz.*, Adilabad (60 Nos) and Nizamabad (60 Nos) for

assessment of seed mycoflora. The collected seed samples were shade dried and stored in paper bags at ambient storage temperatures of 28 + 2 ºC for further studies.

Isolation of seed mycoflora

Four different seed health testing methods *viz.*, standard blotter method, 2, 4 -D blotter paper method and agar plate method as described by ISTA (1996)[5], and deep freeze blotter method developed by Limonard (1968)[6] were employed for estimation of seed mycoflora associated with soybean seed samples. Four hundred seeds were tested in different detection methods.

Data analysis

The data were statistically analyzed by using Completely Randomized Design (CRD) as suggested by Gomez and Gomez (1984)[7]. The data pertaining to percentage were angular transformed wherever necessary.

III. Results And Discussion

Isolation of the pathogen

Different seed health testing methods *viz.,* standard blotter paper method, 2, 4 - D blotter paper method, deep freeze blotter paper method and agar plate method were employed to detect the predominant seed borne fungi from soybean seed samples. Among the seed borne fungi, *M. phaseolina* was found predominant and it was used as test pathogen for further studies. The pathogen appeared as greyish mycelial growth on incubated soybean seeds in different detection methods.

Seed Inoculation of *Macrophomina phaseolina*

Seeds of soybean (cv. JS – 335) were artificially inoculated with conidial suspension (^{106} conidia ml^{-1}) of *M. phaseolina* exhibited the symptoms of seed rot and seedling blight. First symptoms were noticed in the form of seed rot (5 days) and seedling blights and charcoal rot 15 days after sowing. More than 90 to 95 % seedling mortality was observed. Control pots kept with healthy seeds in sterilized soil in isolation did not exhibit any symptoms up to 15 days after sowing. Similar observation was made by Kunwar *et al*. (1986) [8] and Arya *et al*. (2004)[4] who reported that similar type of symptoms on soybean seedlings inoculated with *M. phaseolina.* It was also confirmed earlier by Raut (1985)[9] in sunflower seeds due to *M. phaseolina*

Seed transmission studies were carried out by test tube water method (seedling symptom test) under *in vitro* conditions and also in pot culture under glasshouse conditions.

Seedling symptom test (TWA)

Seeds of soybean cv. JS - 335 were tested by test tube water agar method under laboratory conditions. The results indicated (Table 1) that artificially inoculated soybean seeds with *M. phaseolina* showed reduction in seed germination (55 %) and increased in seed rot and seedling blight (18 % and 20.5 %), respectively. Whereas naturally infected soybean seed samples recorded germination of 59.3 %, seed rot of 15.5 % and seedling blight of 16.5 %. On the contrary, apparently healthy seed samples recorded high germination (75 %), less seed rot (2.5 %) and seedling blight (3.5 %). The germinated seedlings from the infected seed sample exhibited the symptoms of seed rot, seedling blights, discolouration of roots and production of spots on cotyledons and true leaves after 15 days of incubation. Similar findings were reported earlier by Arya *et al*. (2004)[4] in soybean and Raut (1985)[9] in sunflower.

Seed transmission studies in pot culture under glasshouse conditions

The results indicated (Table 2) that artificially inoculated seeds of soybean cv. JS - 335 with seedborne *M. phaseolina* exhibited reduction in seed germination (46 %) and increased seed rot and seedling mortality (25.8 % and 23.2 %), respectively. Whereas naturally infected soybean seed samples recorded germination (50.5 %), seed rot (24 %) and seedling mortality (19.1 %). On the contrary apparently healthy seed samples recorded high germination (72 %), less seed rot (4.2 %) and seedling blight (3.8 %), respectively. Seedlings raised from naturally infected and artificially inoculated seeds recorded seed rot and seedling blights and discoloration of basal stem at the above soil level with the numerous production of sclerotial bodies on the stem surface. The present findings are inconformity with the earlier findings of Arya *et al*. (2004)[9] in soybean who reported that *M. phaseolina* transmits from seed to seedling in a systemic manner. The germination in naturally infected soybean seeds was 52 % and 45%, respectively as against 75 % and 72 % germination in healthy seeds under laboratory and glasshouse conditions, respectively. In naturally infected seeds, 30 % and 25 % seed rot, 18 % and 8 % seedling mortality were recorded in laboratory and glasshouse conditions, respectively. Similar findings was reported earlier by Kunwar *et al*. (1986)[8] in sunflower, Anwar *et al*. (1995)[10] and Mandhare *et al*. (2009)[11] who reported that *M. phaseolina* causing charcoal rot in soybean transmits from infected seed to seedlings.

IV. Conclusion

Seed transmission of *M. phaseolina* in soybean (cv. JS - 335) was found high in artificially inoculated and naturally infected seeds over apparently healthy seeds in test tube water agar and under glasshouse conditions revealed that significant differences in seed rot, seedling blight and per cent emergence of seedlings was observed at 35 days after sowing. Studied the transmission process of *M. phaseolina* from root to upward growth of the soybean and development of fungus *M. phaseolina* established in the seedlings within 48 hours of entering in the host tissue. At cotyledon stage, seedling gets infected 3-7 days after sowing. Variation of host in disease severity is due to genetic variation, geographical origin or source of the isolate. Due to high degree of genetic variation in the pathogen, cultivation of resistant varieties is the most economical and practical approach.

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References

[1] Endres, J., Barter, S., Theodora, P and Welch, P. 2013. Soybean enhanced lunch acceptance by preschoolers. Journal of American Dietetic Association. 103: 346-351.

[2] Sarabhoy AK and Agarwal DK. (1983). Fungal diseases of soybean and their management. Int. J. Trop. Pl. Diseases. 1:13-19.

[3] Shovan, L.R., Bhuiyan, M.K.A., Sultana, N., Begum, J.A and Pervezi, Z. 2008. Prevalence of fungi associated with soybean seeds and pathogenecity tests of the major seed borne pathogens. International Plant Protection. 72-75.

[4] Arya, V.K., Vishunavat, K and Himanshu, N. 2004. Detection, location and transmission of seedborne inoculums of Macrophomina phaseolina in charcoal rot in soybean. Journal of Mycology Plant Pathology. 34: 319-328.

[5] ISTA. (1996). International rules for seed testing rules. Seed Science and Technology. 13: 299-513.

[6] Limonard J. (1968). Ecological aspects of seed health testing. International Proceedings of Seed Testing Association. 33: 1-8

[7] Gomez KA and Gomez AA. (1984). Statistical procedures for agricultural research (second edition) John Wiley and Sons, New York.

[8] Kunwar, I.K., Singh, T., Mechado, C.C and Sinclair, J.B. 1986. Histopathology of soybean seed and seedling infection by Macrophomina phaseolina. Phytopathology. 76: 532-535.

[9] Raut, J.G. 1985. Transmission of seed borne Macrophomina phaseolina in sunflower. Seed Science and Technology. 11: 807 – 814.

[10] Anwar, SA, Abbas SF, Gill MM, Rouf CA, Mahamood S and Butta AR. (1995). Seed borne fungi of soybean and their effect on seed germination. Pakistan J. Phytopathology. 7: 184-190.

[11] Mandhare, V.K., Gawade, S.B and Suryawanshi, A.V. 2009. Detection and transmission of seed borne infection of Macrophomina phaseolina causing charcoal rot in soybean. Journal of Plant Disease Sciences. 4:130-131.

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Table 1: Seed transmission of *M. phaseolina* in soybean (cv. JS – 335) by test tube water agar method (TWA)

S.N.	Treatment	*Germination (%)	*Seed rot (%)	*Seedling blight (%)
1	**Apparently healthy seed sample**	75.5 (59.9)	2.5(9.09)	3.5(10.7)
2	**Naturally infected seed sample**	59.3 (50.3)	15.5 (23.1)	16.5 (24.1)
3	**Artificially inoculated seed sample**	55.0(47.8)	18.0 (25)	20.5 (26.9)
	S.Em (±)	**0.62**	**0.18**	**0.22**
	CD at 5%	**2.19**	**0.64**	**0.80**

Figures in parentheses indicate angular transformed values. * Average of three replications

Table 2: Seed transmission of *M. phaseolina* in soybean (cv. JS – 335) under glasshouse conditions

S.N.	Treatment	*Germination (%)	*Seed rot (%)	*Seedling blight (%)
1	**Apparently healthy seed sample**	50.5 (45.2)	24.0 (29.3)	19.1 (25.9)
2	**Naturally infected seed sample**	46.0 (42.6)	25.8 (30.5)	23.2 (28.7)
3	**Artificially inoculated seed sample**	46.0 (42.6)	25.8 (30.5)	23.2 (28.7)
	S.Em (±)	**0.38**	**0.35**	**0.36**
	CD at 5%	**1.34**	**1.25**	**1.30**

Figures in parentheses indicates angular transformed values. * Average of three replications

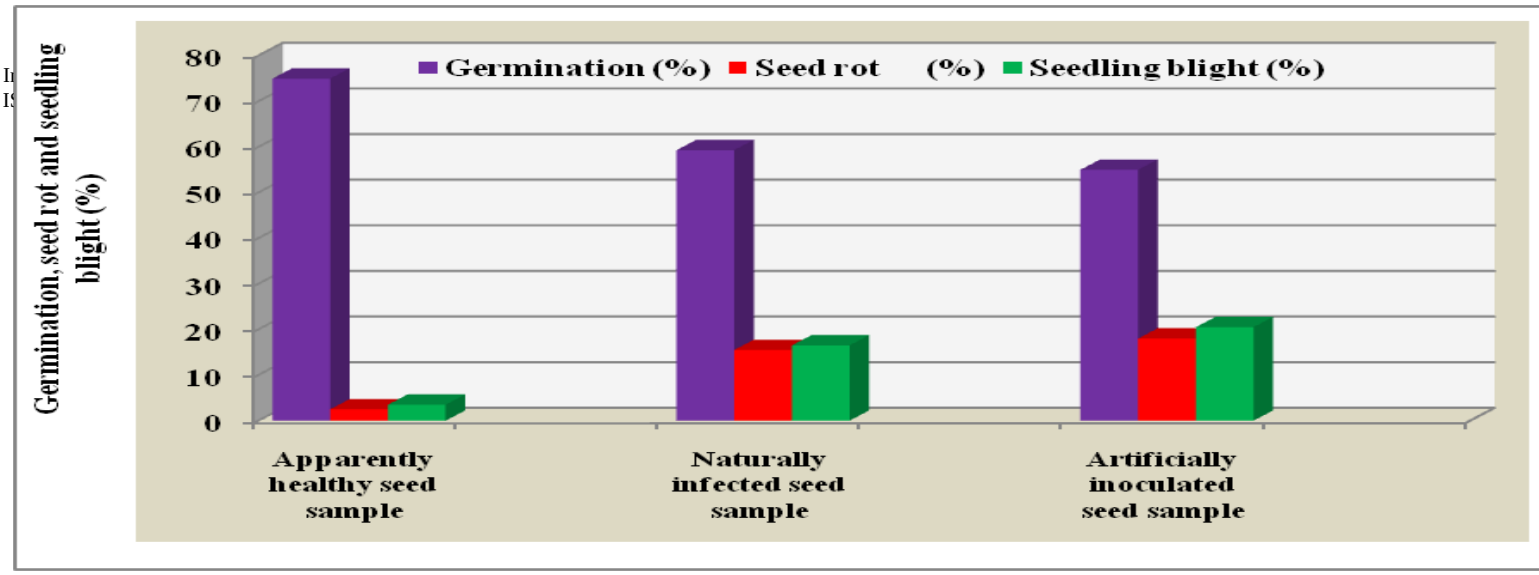

Figure1. Seed transmission of *M. phaseolina* in soybean cv. JS-335 by test tube water agar method (TWA)

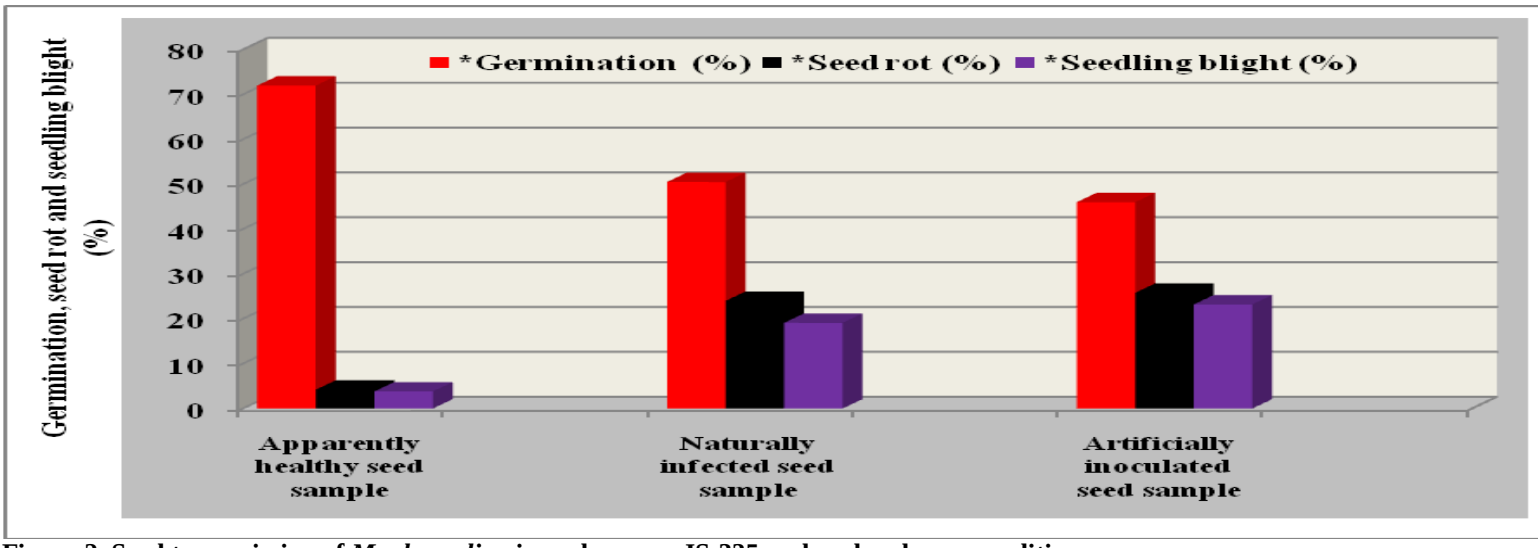

Figure 2. Seed transmission of *M. phaseolina* in soybean cv. JS-335 under glass house conditions

Plate 1: Seed rot and seedling blight of soybean cv. JS-335 due to *M. phaseolina*