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Detecting Seed Borne Fungi of Soybean by Different Incubation Methods

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Abstract: A total number of 39 species of fungi belonging to 15 genera were isolated from 6-month old soybean seeds by four incubation methods from Sindh, a province of 14.1 m hac. out of 79.7 m hac. of land constituting Pakistan. Of the 4 methods employed for the isolation of seed borne fungi, incubation by PDA method yielded the highest number of fungal members either with or without the treatment of disinfectant. Incubation of seeds on blotting papers yielded lesser number of fungal species and the number of fungi gradually got reduced on component plating and the least number of fungi appeared by deep freezing method (-20°C).A comparative study of the literature on seed borne fungi of soybean revealed a taxonomic similarity among fungal isolates, in particular between Iran and India which are situated on the west and east of Pakistan respectively. Since the environmental factors for the growth and reproduction of fungi causing plant diseases in the 3 countries are more or less similar, a common strategy for the control of plant diseases on regional basis may prove useful on the pattern of locust control carried out by FAO.

Key words: Seed-borne fungi of soybean, isolated from Sindh, Pakistan

Introduction

Edible oil is obtained from traditional (mustard, raya, rapeseed etc) and non-traditional (sunflower and soybean) oil seed plants in Pakistan. The consumption of edible oil has been reckoned to be 1.09 million tons in 2000-2001 of which 29% was produced locally and the remaining 71% was imported (Badar, 2002). The consumption of edible oil is rising day by day and to meet up the increasing demand, mainly palm oil is imported from Malaysia and soybean from the USA. Presently edible oil is the second most important imported commodity after fossil fuel (petroleum) which requires dollar 8.56 million in its import. This has attained critical importance in the economy of Pakistan (Annual Report, 1999-2002). Since the traditional oil seed plants cannot meet up the demand because of the poor yield, therefore efforts are being made to promote the cultivation of non-traditional oil seeds such as sunflower and soybean. Both sunflower and soybean grow satisfactorily where as the agro climatic condition of Pakistan has not been found suitable for palm oil cultivation, therefore emphasis has been placed on soybean and sunflower cultivation.

Soybean (*Glycine max*(L)Merill) plants have the potentiality to raise edible oil production considerably. On an average soybean has been found to produce 1207 kg ha^{-1} and fetch a good economic return to farmers. The seeds of soybean have the advantage of its being cooked and

eaten as vegetable protein meat or it can be crushed for extracting edible oil. The meal of crushed soybean seeds is used as a fodder. One of the major factors that limit the cultivation of any crop plant is the seed borne fungi which cause reduction in germination. More than 40 fungi, bacteria and viruses have been reported to be actively associated with the seeds of soybean (Hartman ***et al.***, 1999). Infected seeds provide primary inocula for affecting new crop plants. Study of seed-borne fungi of a promising soybean variety William 82 was carried out by four different incubation methods with a view to find out the most commonly and persistently occurring fungi.

Materials and Methods

Seed samples of soybean variety William 82 were collected from Sindh and 400 seeds were drawn at random. The seeds were surface sterilized with NaOCl (1% available chlorine) for one minute followed by rinsing in sterile water (Kulik, 1981) and another lot of seed samples were left untreated. The 400 treated seeds of soybean variety William 82 were divided into four aliquot lots of 100 seeds. One lot of seeds was incubated on potato dextrose agar (PDA) by ISTA method (1966).Ten seeds were put on agar plate. Ten replicate plates were used and incubated for 5 days at 25(±1°C).A drop of streptomycin was added in each plate for the suppression of bacterial growth. Similarly, second lot of 100 seeds was incubated on blotters (ISTA, 1976) for the growth of fungal colonies. The third lot of seeds was dissected individually and aseptically into component parts (seed coat, cotyledons and embryo) and the components parts were placed on PDA medium for the growth of fungal colonies. The fourth lot of seeds was kept in plastic vials@ 10 seeds in a vial plugged with cotton wool. The vials were kept at-20C for 24 hrs. The seed samples were then put on PDA medium and incubated at 25°C (±1°C) for 5-7 days under an alternating cycle of 12 h darkness for the growth of seed borne fungi into discernable fungal colonies. The somatic and reproductive structures of fungi forming different types of colonies were studied in detail under microscope. The characteristic features of the isolated seed borne fungi were tallied with the descriptions given in the authoritative literature for identification. (Raper and Thom, 1949; Thom and Raper, 1945; Gilman, 1957; Ellis, 1971; Hawksworth ***et al.***, 1983; Booth, 1977). The total number of fungal species isolated by means of 4 different incubation methods was calculated on percent basis to find out the difference in nature and number of fungi arising out between treated with disinfectant and non-treated seeds and the difference between the four incubation methods.

Results and Discussion

A total number of 39 species of fungi belonging to 15 genera was isolated from the 6-month old seeds of soybean William 82 by means of four incubation methods (Table 1). The highest number of fungal species was obtained without surface disinfection of seeds as compared to the kind and number of fungi on treatment with Sodium hypochlorite (NaOCl). Surface sterilized seeds yielded lesser number of fungi than from seeds without sterilization. The highest number of fungal species was obtained on Potato Dextrose Agar (PDA) medium with or without treatment

followed up in number of species on blotter and gradually diminishing in number on seed components and was least on deep freezing of seeds (-20C).

Table 1: Occurrence (%) of fungi on seeds of Soybean var. William 82 following Incubation methods on PDA (Potato Dextrose Agar), BT (Blotter), CP (Component Part) and DF (Deep Freezing)

	Seeds without Treatment				Seeds with Treatment			
Name of Fungi	PDA	BT	CP	DF	PDA	BT	CP	DF
Altemaria alternata	4.2	3.1	2.8	1.8	1.7	1.5	1.7	1.5
A. brassicae	2.3	2.4	-	1.2	1.3	-	1. 0	-
A. longipus	2.4	1.9	1.7	1.5	1.2	1.3	-	0.8
A. tenuissima	2.2	2.3	2.1	-	1.4	-	1.2	-
A. longissima	2.4	2.5	2.2	2.1	-	1.2	-	0.6
Aspergillus niger	3.1	-	2.9	1.8	1.5	-	0.8	0.7
A. candidus	2.5	2.1	2.3	-	-	1.7	-	-
A. fumigatus	2.6	2.3	2.5	1.8	1.2	-	1.1	-
A. terreus	2.1	2. 0	-	1.5	-	1.2	0.8	0.6
A. flavus	2.3	2.1	2.2	1.4	1.2	-	-	-
Botryoplodia theobromae	2.7	2.5	2.6	1.7	-	1.2	1.3	-
Cladosporium cladosporoides	3.2	2.9	3.1	2.2	1.8	1.2	1.1	1.4
C. herbarum	4.5	3.4	2.6	1.7	-	-	-	-
Chaetomium globossum	2.5	1.6	1.4	-	1.4	1.3	0.7	0.8
Colletotrichum dematium	2.3	2.2	2.4	1.5	-	1.2	0.8	-
C. truncatum	2.4	2.3	-	1.7	1.5	-	-	0.5
Curvularia lunata	3.5	3.4	3.6	-	1.4	1.3	0.8	-
Diplodia sp	2.2	1.8	2.3	1.5	-	-	1.2	-
Drechslera australiansis	3.1	2.9	2.9	-	1. 0	0.9	-	-
D. hawaiiensis	2.3	-	-	1. 0	1.4	1.2	-	0.7
D. sorkiniana	2.6	2.2	2.4	1.4	-	-	-	-
D. specifera	2.7	-	2.5	2.1	1.6	1.4	1.3	0.9
D. biseptata	2.5	2.1	2.2	1.2	-	-	1.3	-
Fusarium moniliformae	2.8	2.6	2.7	2.1	1.7	1.7	-	0.7
F. solani	2.7	1.8	1.2	1.7	1.3	1.5	1.3	0.5
F. semitectum	2.3	2.4	2.3	-	1.5	-	-	-
F. equiseti	2.1	-	2.2	1.6	1. 0	1.3	-	0.5
F. oxysporum	3. 0	2.8	3.2	2.1	-	1.2	1.2	1.1
F. roseum	2.2	2.1	3.1	2.1	1.6	1.1	-	-
Macrophomina phaseolina	2.9	2.4	2.6	1.8	1. 0	1.3	1.1	1.2
Nigrospora species	2.1	2. 0	2.2	1.3	1.3	1.2	-	-
Phoma species	1.0	0.8	-	1. 0	-	-	0.7	0.4
Penicillium expansum	1.6	1.5	1.3	-	0.9	1.5	-	0.8
P. citrinum	2.1	1.7	2.3	2.4	-	-	0.9	-
P. species	4.8	3.2	3.3	2.8	1.2	1.2	-	0.4
Rhizopus nigricans	2.5	2.1	2.6	2.2	1.3	1.3	1. 0	-
R. stolonifer	2.6	2.1	2.6	-	-	-	-	0.6
Rhizoctonia rolfsii	2.9	2.2	-	-	1.3	1. 0	0.7	-
R. solani	2.3	2.2	2. 0	2.1	1.6	1.1	1.3	1.4

There is a long list of research works on seed borne microorganisms of soybean emanating from soybean growing countries of the world situated in diversified climatic conditions (Karmaker ***et al.***, 1982; Zad, 1982; Nik, 1983; Popoola and Kueshi, 1986; Hussain ***et al.***, 1989; Singh, 1991; Jordan ***et al.***, 1986; Garcia ***et al.***, 1993; El-Kady ***et al.***, 1993). In isolating fungi by the different isolation methods, a difference in kind and number of fungi have been observed. Michail, ***et al.*** (1981) found blotter method superior for detecting Cephalosporium, Fusarium and Myrothecium species whereas PDA medium was preferable for ***Macrophomina phaseolina*** causing charcoal root rot of soybean. Both PDA and blotter methods were found to be equally good for isolating other species of seed borne fungi of soybean. Khattak ***et al.*** (1993) studied the efficacy of various methods employed for the study of seed borne microflora of soybean. Agar plating yielded the highest total number of fungi out of the three employed methods. Hussain ***et al.*** (1989) isolated and identified 15 species of seed borne fungi from soybean seeds from North West frontier province of Pakistan. We isolated 39 fungi on PDA, 30 on blotter 25 on component parts & 16 by the deep freezing method from the south west of Pakistan. El-Kady ***et al.*** (1994) isolated 73 species belonging to 32 genera of fungi from 100 soybean samples collected from Egypt which were incubated at 28 and 45°C. Jordan ***et al.*** (1992) found environmental factors on the incidence of seed borne fungi of soybean in the north and south of Illinois as over riding factors in pre disposition of seeds to fungal infection regardless of soil types. Reduced plant vigor resulted in increased seed infection by Chaetomium, Cladosporium and Fusarium species. Low rainfall and high temperature in the early growing season of soybean caused seed infection with Macrophomina phaseolina.

A regional survey of literature on seed borne fungi of soybean revealed 12 species by Karmaker ***et al.*** (1980) from India, 9 species by Zad (1982) from Iran which are situated in the east & west of Pakistan respectively. Hussain ***et al.*** (1989) reported 15 species of fungi from NWFP, Pakistan and we found 39 species of fungi from southern region of Pakistan. A comparative study of the fungi isolated from the 3 countries situated in the same region show similarity in the nature of the seed borne fungi of soybean which is an indication of the possibility of evolving a common strategy towards the control of seed borne fungi of soybean.

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