INT. J. BIOL. BIOTECH., 2 (3): 711-713, 2005.

SEEDBORNE MYCOFLORA OF SOYBEAN

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ABSTRACT

A total number of 20 species of fungi belonging to 12 genera were isolated from soybean seed collected from different localities of Pakistan by using blotter, agar plate and deep freezing methods as recommended by ISTA (International Seed Testing Association). Where 12 fungal species are new reports from Pakistan. Of these methods blotter method yielded highest number of fungi as compared to agar plate and deep freezing methods.

Key words: Seedborne mycoflora, Soybean, ISTA technique

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.). is an important oil seed crop. Seed is an important source of protein in the human diet (Sastri, 1956).In Pakistan soybean cultivated on an area of 1320 hectares and the production is about 1898 Kg/hectare (Anon, 2003). Soybean seeds contains 20% oil which is composed of 80% unsaturated fatty acid and 7-8% linolenic acid. A number of fungal species are found to be associated with soybean , seed which includes *Alternaria* spp., *Aspergillus* spp., *Dreschlera* spp., *Macrophomina phaseolina, Penicillium* spp., *Fusarium* spp., (Hussain *et al*,. 1989) Ayaydin *et al.* (1984) reported the presence of *Fusarium* spp., *Alternaria* spp., *Sclerotium rolfsii, Pythium* spp. and *Rhizoctonia* spp., at the seedling stage of soybean whereas, *Aspergillus* spp., *M. phaseolina* were at the flowering stage. Due to deterioration of seeds by number of fungi, experiments were therefore carried out to study the seed borne mycoflora of soybean.

MATERIALS AND METHODS

Twelve seed samples of soybean were collected from different markets of Karachi(5), Sindh (95-1,95-2, 95-3 . 95-4) and Faisalabad (AGS-20, PR-142,MA –4085) .Using ISTA technique(Anon,1976), .400 seeds from each sample were tested with the standard blotter technique, untreated and seeds after treatment with 1% $Ca(OCl)_2$ were placed on three layers of moistened blotter @ 10 seeds per petridish . For agar plate method the treated and untreated seeds were plated on potato dextrose agar (PDA) @ 10 seeds per petri dish and the dishes were incubated at 24°C in alternating cycle of 12 h of light and darkness for 7 days . In deep freezing method the treated and untreated seeds were incubated for 1 day each at 24°C and 0°C in freezer followed by 5 days incubation at 24°c . Fungi growing on each seed were identified after reference to Ellis(1971), Domsch et *al.* (1980), Nelson *et al.* (1983), Raper and Fennel (1965). Data were subjected to Analysis of Variance (ANOVA) following the procedure as given by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

A total number of 20 species of fungi belonging to 12 genera viz., *Alternaria alternata* (Fries.) Keisser, *A tenuissima* Simmons & Crosier, **Aspergillus candidus* Link ex., **A. flavus* Link ex Gray, *A. niger* Van Tieghem, **A. parasiticus* Speare, **A. pencilloides* Spegazzini, **A. sulphureus* (Fres) Thom & Chruch, **A. ustus* (Bain) Thom & Chruch. **A. wentii* Wehmer, *Chaetomium globossum* Kunz ex Stend, *Cladosporium* sp.,. Berk & Curt, **Dreschlera demotiodea* (Bubak & Worblewski,) Subram & Jain, **Fusarium solani* (Mart.) Appel & Wellenw, *Macrophomina phaseolina* (Tassi) Goid, **Mucor* spp,. Mich. ex.st. **Paecilomyces* sp., (Bain) *Penicillium* sp,. Link ex Fr., **Rhizoctonia solani* Kuhn, **Rhizopus stolonifer* (Ecicuh ex Link.) Lind were isolated from soybean seeds collected from different localities of Pakistan . Of these 12 fungal species marked with an asterisk are new reports from Pakistan (Ghafoor and Khan, 1976; Ahmad *et al.*, 1992). Of the 12 samples tested only one seed sample was found to be infected with *R. solani* where 0.2% infection was observed in surface sterilized seeds (Table 1). About 50% samples of seeds were found to be infected by *A. alternata* with an infection range of 1.0-1, 4% in surface sterilized seeds and 0.6- 1.1% in nonsterilized seeds . All samples of seeds were infected with *A. flavus* and *A. niger*. Surface sterilization of seeds with 1% $Ca(OCl)_2$ significantly

reduced the incidence of *A. flavus* ($p<0.001$). *A. alternata* showed a significant increase ($p< 0.05$). Infection of *M. phaseolina* was recorded only from one seed sample (0.1%) in surface sterilized seeds and (0.08%) in nonsterilized seeds. Infection of *F. solani* ranges 0.8-2.1% in surface sterilized seed and 1-2% in nonsterilized seeds. Of the different methods used for isolation of seed borne fungi blotter method yielded highest number of fungi as compared to agar plate method and deep freezing methods ($p<0.001$). Such similar results were observed by Khan *et al.* (1988) on rice, Dawar and Ghaffar (1991) on sunflower and Rasheed *et al.* (2004) on groundnut. Jovicevic (1980) also reported that the filter paper method was most practical method for routine analysis of seeds.

Table 1. Seedborne mycoflora of soybean.

Name of fungi	Surface Sterilized			Non Sterilized		
	Agar plate	Blotter	Deep freezing	Agar plate	Blotter	Deep freezing
	SI % ± SD	SI % ± SD	SI % ± SD	SI % ± SD	SI % ± SD	SI % ± SD
Alternaria alternate	1.12 ± 2.13	1.41 ± 2.06	1.12 ± 2.52	0.66 ± 1.43	1.08 ± 3.32	2.75 ± 5.05
A. tenuissima	0.12 ± 1.72	0.45 ± 2.06	0.29 ± 2.18	0	0.16 ± 0.70	0.29 ± 1.06
Aspergillus candidus	0.37 ± 6.77	0.91 ± 2.07	0.66 ± 1.41	5.25 ± 4.62	0.58 ± 2.12	1.08 ± 3.27
A. flavus	37.20 ± 30.91	40.95 ± 26.03	38 ± 20.62	70.5 ± 20.02	44.12 ± 20.08	31.62± 13.22
A. niger	37.20 ± 30.91	28 ± 24.62	24.4 ± 19.12	50.20 ± 21.90	35.04 ± 21.05	23.95 ± 15.11
A. paraciticus	1.83 ± 0.0	0.08± 24.7	0	0	0.04 ± 0.0	0
A. penicilloides	0.12 ± 0.0	0	0	0	0	0
A. sulphureus	6.29 ± 11.42	2.87± 24.46	1.83 ± 1.78	7 ± 2 4.46	3.75 ± 5.03	1.62 ± 3.30
A. ustus	0.79 ± 10.0	0.25 ± 24.32	0.79 ± 1.47	0.25 ± 25.69	0.26± 1.15	0.41± 0.64
A. wentii	1.41± 5..51	0.66 ± 0.00	0.29± 1.06	1.58± 2.28	1.37± 3.77	0.83 ± 0.97
Chaetomium globosum	0	0.12± 0.00	0	0	0	0
Cladosporium sp.	0.04 ± 0.00	0	0	0	0	0
Drechslera dematioidea	0	0	0.08± 0.00	0	0	0.12± 0.35
Fusarium solani	0.87 ± 2.62	1.12± 2.46	2.12± 3.46	1.16± 1.50	1.5± 3.20	2.41±3.54
Macrophomina phaseolina	0.12 ± 0.00	0	0	0.08± 0.00	0	0
Mucor sp.	1.66 ± 12.72	0.83± 3.70	0.08± 0.00	0.29± 0.00	1.04± 0.00	0.4± 0.00
Paecilomyces sp.	0.20 ± 9.98	0	0	0	0	0
Penicillium sp.	0.20 ± 0.00	0.41±1.04	0.12± 0.00	0	0.54± 5.61	0.12± 0.35
Rhizoctonia solani	0.16 ± 0.00	0	0	0	0	0
Rhizopus stolonifer	1.66 ± 1.22	5.62 ± 9.22	2.5 ± 3.22	2.5± 3.22	8.29 ± 16.28	1.95± 8.18

SI % = Seed Infection %; SD = Standard deviation

Soybean varieties AGS-20 and 95-4 showed the incidence of pathogenic fungi viz., *Fusarium solani, M. phaseolina, R.solani* and *Alternaria* spp. Present results showed that surface sterilization of seed reduced the infection of *A. flavus* and *A. niger* and increase the incidence of pathogenic fungi. Such similar results were observed by Fakir *et al.*, (1976), Dawar and Ghaffar (1991), Rasheed *et al.*, (2004). Present results showed that saprophytic fungi like *A .flavus* and *A.niger* were predominant among the isolated fungi. Such similar reports have been made by Dawar and Ghaffar (1991) on sunflower seeds and Rasheed *et al.* (2004) on groundnut seeds. Christensen (1973) found that saprophytic species reduce the germination of seed and damage the seeds in storage. Goldblatt (1969) reported that storage fungus like *A. flavus* is an important mycotoxin producer which produces aflatoxin which is hepatocarcinogenic. Soybean is an important oil seed crop and a number of saprophytic and pathogenic fungi reduced the quality of seeds, therefore there is a need to reduce the infection of these fungi for obtaining high yield of crop.

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(Accepted for publication May 2005)