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Isolation of Seed Borne Fungi Associated with Pigeon pea (*Cajanus cajan*, *Linn*.) Seeds

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Abstract: ***Using ISTA techniques, the seed borne fungi of Pigeon pea (Cajanus cajan Linn.) was studied. A total of 15 genera and 29 species of fungi were isolated, and been recorded from seeds of Pigeon pea. The blotter method agar plate method was found to be most suitable technique for detection of fungi in Peageon pea. There was the detection of Fusarium spp, Aureobasidium pullulans, Gleomatix sp., Gleosporium sp., Trichoderma sp. and Penicillium purpurogenum done by these two methods.***

Keywords: *Cajanus cajan, Linn*., Seed Mycoflora, Blotter Paper Method, Agar Plate Method, ISTA, etc

1. Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a perennial member of the family leguminosae. It is a multi-purpose species, is extensively used as food grain and green manure crop for soil fertility amelioration in local cropping systems. Other common names are **"Red gram, Congo pea, Gungo pea, Gunga pea, and No-eye pea"**. It is an important grain legume crop of rain-field agriculture in the tropics and subtropics. Compared with other grain legumes, pigeonpea ranks only sixth in area and production, but it is used in more diverse ways than others [5, 10, 11, 21]**.** Many fungal diseases (31), involving 45 pathogens, are known; the most serious is wilt disease (*Fusarium udum*), favored by soil temperatures of 17°–20°C. The fungus enters the plant through the roots and may persist in soilborne stubble for a long time. The only effective control measure is development of resistant cvs (e.g., 'C-ll,' 'C-36,' 'NP-15,' 'NP-38,' and 'T-17'). Rotation with tobacco and intercropping with sorghum is said to decrease the wilt problem. Other fungi include: *Cercospora* spp., *Colletotrichum cajanae, Corticium solani, Diploidia cajani, Leveillula taurica, Macrophomina phaseoli, Phaeolus manihotis, Phoma cajani, Phyllosticta cajani, Phytophthora* sp., *Rhizoctonia bataticola, Rosellinia* sp., *Sclerotium rolfsii,* and *Uredo cajani* (rust). So far, economic damages by these have been small or negligible, but rust is locally of some importance. Pigeon pea is also attacked by the bacterium *Xanthomonas cajani* and the sterility mosaic and yellow mosaic viruses [8]. Fungi reported from seeds of Peageon pea are *Alternaria sp*., *Aspergillus sp., Colletotrichum lagenarium, Coleophoma empetri, Fusarium equiseti, Macrophomina phaseolina, Myrothecium roridum, Rhizoctonia solani, Rhizopus sp.,* and *Sclerotium rolfsii [14, 15, 16, 17]*. The present study focused on isolation of seed borne fungi of Peageon pea using Blotter paper and Agar plate method.

2. Materials and Methods

Two hundred seeds from each lot (250 g) were collected from seed market were selected for isolations by using standard blotter paper and agar plate method [12]. From the samples, twenty seeds were placed on three layers of moistened blotter paper in a 9 cm diameter petridish without any pretreatment, while for internal seed-borne fungi, seeds pretreated with 0.1% HgCl2. Same procedure was repeated in case of agar plate method. All the petridishes were incubated at 22 $+1^0$C for eight days. After eight days, these plates were examined directly under stereoscopic microscope. The surface sterilized seeds were nicked with a pointed needle to find out the actual location of mycoflora associated with different seed components. The fungal growth appearing on seeds was identified with the help of colony color, sporulation type and shape of spores, up to species level with the help of compound microscope and relevant literature [1, 4,9,20, 22]

3. Result and Discussion

Using blotter paper method and agar plate method, total 15 genera and 32 species were isolated from 10 samples of Pigeon pea collected from Seed Market.

Of the fungi isolated 32 viz., *Alternaria alternata, A.citri, Aureobasidium pullulans, Aspergillus flavus, A. fumigatous, A. niger, A. tamarii, A. terreus, A. wentii, Cladosporium cladosporoidae, C. oxysporum, C. sphaerospermum, Drechslera australiensis, Fusarium moniliformae, F. solani, F. oxysporum, Nigrospora oryzae Penicillium purpreogenum, P.notatum, Rhizoctonia solani, Rhizopus suinus, Scytalidium sp., Stemphyllium sarciniformii, Trichoderma atroviride, T. flavofuscum, T. harzianum, and T. polysporum* were reported on seeds of pigeon pea. External infestation was high on both the blotter paper and agar plate method respectively, which was reduced in case of pre treatment of seeds with HgCl2, **(Table I, Plate no.1)**. Similar results were also obtained by [7], who concluded that by chlorine treatment the infestation of saprophytic fungi could be reduced. Seed surface disinfection with HgCl2 usually suppresses the growth of saprophytic and other superficial fast growing fungi [3]. Only *F. oxysporum* and *F.solani* were recorded from embryo of seeds at low frequency **(Table II)**.

Aspergillus sp., Cladosporium sp., Fusarium oxysporum, Fusarium solani and Rhizopus sp., were most frequent in pigeon pea seeds. *Rhizopus sp., were consistently isolated from seeds of pigeon pea. Gliomastix, Gleosporium,*

Scytalidium and Stemphyllium sarciniformii were found associated with some discolored and ungerminated seeds and also with seeds having abnormal seedlings.

The standard blotter method yielded maximum number of fungi. Such similar results have been observed from the detection of seed borne fungi in rice [13], Cotton [3], Cajanus [6] and Sunflower [7]. [2] reported that blotter paper method was found most suitable for detection of most infectious fungi of cucurbits.

High percentage of above mentioned potential pathogens having both internal and external mode of infestation. For the production of healthy and certified quality seeds, seed health certification programme has to be followed and seed must be tested and treated with suitable seed dressing fungicides.

Plate no: 1: External and Internal Seed Mycoflora of *Cajanus cajan*, Linn.

Untreated Seeds

Treated Seeds ($Hgcl_2$ solution)

Table 1: Occurrence of fungi on Pigeon pea seeds using Blotter Paper Method

Fungi	Blotter Paper Method					
	Control Set			Treated Set		
	Mean	St.D	Variance	Mean	St.D	Variance
Alternaria alternata	13.30 ± 7.37	16.75	279.22	17.74 ± 7.52	14.05	226.417
Alternaria citri	8.97± 0.0	-	-	8.25 ± 0.0	-	-
Aspergillus flavus	15.0 ± 10.0	14.14	200.00	-	-	-
Aspergillus fumigates	23.28± 7.9	20.46	500.65	22.60 ± 7.83	22.15	490.751
Aspergillus niger	13.08± 6.64	15.25	254.76	8.75 ±3.75	8.49	72.125
Aspergillus niger	4.75± 0.0	-	-	0.75 ± 0.0	-	-
Aspergillus tamari	8.25± 0.0	-	-	2.0 ± 0.0	-	-
Aspergillus terreus	12.36± 2.84	7.54	56.83	7.57 ± 2.36	6.25	39.09
Aspergillus wentii	4.75± 0.0	-	-	1.25 ± 0.0	-	-
Aureobasidium pullulas	4.25 ± 0.0	-	-	1.8 ± 0.0	-	-
Cladosporium cladosporoidae	16.78± 0.0	-	-	2.75 ± 0.0	-	-
C. oxysporum	14.12 ± 8.68	27.26	452.43	9.58 ± 6.28	16.84	318.392
C.sphaerospermum	8.45 ± 1.67	2.89	8.39	4.50 ± 1.68	3.37	11.417
D.australiensis	6.28 ± 0.0	-	-	2.75 ± 0.0	-	-
Fusarium equisetii	2.0 ± 1.3	2.38	5.68	1.25 ± 0.0	-	-
F. moniliforme	3.5 ± 0.25	0.43	0.188	5.0 ± 0.72	1.25	1.563
F. oxysporum	13.24 ± 0.0	-	-	8.58 ± 4.77	8.27	68.396
F.solani	5.25 ± 1.6	2.12	4.500	4.85 ± 1.06	3.27	5.644
Gleosporium sp.	15.8 ± 8.75	17.52	306.932	13.68 ± 8.05	16.09	259.141
Gliomastix sp.	-	-	-	4.33 ± 1.01	1.75	36.125
Nigrospora oryzae	7.0 ± 4.25	8.48	72.00	2.25 ± 0.0	-	-
Peniclillium notatum	3.25 ± 0.0	-	-	-	-	-
P.purpurogenum	3.87 ± 1.87	-	-	2.0 ± 0.0	-	-
Penicillium sp.	7.0 ± 6.0	-	-	2.5 ± 0.50	0.71	0.50
Rhizoctonia solani	3.25 ± 0.0	2.65	1.875	2.25 ± 0.0	-	-
Rhizopus suinus	7.0 ± 6.0	8.48	72.000	13.47 ± 4.62		213.492
Scytalidium sp.	37.4 ± 16.38	36.6	1341.00	4.73 ± 3.07		28.563
Stemphyllium sarciniformii	10.76 ± 7.51	10.61	112.50	2.07 ± 0.98	1.70	2.896
Trichoderma atroviride	11.23 ± 0.0	-	-	-	-	-
T. harzianum	1.76 ± 0.0	-	-	-	-	-
T .flavofuscum	7.5 ± 4.25	6.01	36.125	3.86 ± 1.87	2.65	7.031
T. polysporum	1.75 ± 0.0	-	-	-	-	-

Table 2: Occurrence of fungi on Pigeon pea seeds using Agar Plate Method

Fungi	Agar Plate Method					
	Control Set			Treated Set		
	Mean	St.D	Variance	Mean	St.D	Variance
Alternaria alternata	16. 10 ± 6. 37	10.95	120.021	15.07 ± 5.32	9.22	85.136
Alternaria citri	4.0± 3.23	4.54	21.124	3.13 ± 1.86	2.64	7.031
Aspergillus flavus	1.5 ± 0.0	-	-	2.0 ± 0.0	-	-
Aspergillus fumigates	19.10 ± 6.91	19.56	382.182	19.0 ± 7.68	21.70	472.00
Aspergillus niger	9.05 ± 3.70	8.29	68.835	9.10 ± 3.59	7.19	51.76
Aspergillus niger	3.5± 0.5	-	-	3.26 ± 0.0	-	-
Aspergillus tamari	4.73 ± 0.0	-	-	7.43 ± 2.05	-	-
Aspergillus terreus	9.80 ± 1. 84	5.03	25.268	0.5 ± 0.0	5.43	29.451
Aspergillus wentii	0.5± 0.0	-	-	5.0 ± 0.0	-	-
Aureobasidium pullulas	3. 35 ± 2.86	4.06	16.531	2.76 ± 0.0	-	-
Cladosporium cladosporoidae	16.78± 0.0	-	-	12.70 ± 9.10	-	-
C. oxysporum	15.42 ± 10.50	23.61	557.394	3.82 ± 0.88	20.34	414.418
C.sphaerospermum	5.55 ± 1.87	3.76	14.182	-	1.51	2.271
D.australiensis	-	-	-	2.75 ± 0.0	-	-
Fusarium equisetii	1.24 ± 0.0	-	-	3.0 ± 0.25	-	-
F. moniliforme	2.51 ± 0.35	0.66	0.437	8.66 ± 4.22	0.35	0.125
F. oxysporum	5.57 ± 4.22	7.34	53.895	7.48 ± 1.47	7.31	53.521
F.solani	4.21 ± 1.33	2.92	8.544	18.31 ± 9.68	3.24	10.571
Gleosporium sp.	14.31 ± 7.15	14.30	204.599	5.15 ± 1.77	19.36	375.474
Gliomastix sp.	2.00 ± 0.58	1.01	1.021	3.37 ± 2.87	3.95	15.675
Nigrospora oryzae	4.50 ± 2.51	3.52	12.500	8.21 ± 2.79	4.00	16.531
Peniclillium notatum	7.25 ± 2.43	6.43	41.425	7.75 ± 3.22	7.40	54.780
P.purpurogenum	6.18 ± 2.47	4.95	24.556	3.81 ± 0.74	6.44	41.583
Penicillium sp.	3.62 ± 1.37	1.95	3.780	3.81 ± 1.72	1.07	1.125
Rhizoctonia solani	4.05 ± 2.26	3.93	15.437	-	3.44	11.891
Rhizopus suinus	-	-	-	2.83 ± 1.72	-	-
Scytalidium sp.	4.58 ± 3.71	6.43	41.395	8.81 ± 3.98	2.98	8.896
Stemphyllium sarciniformii	15.46 ± 6.24	12.47	155.901	1.91 ± 0.44	7.96	63.432
Trichoderma atroviride	7.68 ± 4.81	9.64	92.891	1.91 ± 0.44	0.763	0.582
T. harzianum	3.41 ± 1.96	3.40	11.582	5.75 ± 2.29	3.968	15.750
T .flavofuscum	7.5 ± 4.25	6.01	36.125	3.86 ± 1.87	2.65	7.031
T. polysporum	1.75 ± 0.0	-	-	-	-	-

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