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ELECTROLYTE LEAKAGE FROM PIGEONPEA (*CAJANUS CAJAN* (L.) MILLSP.) SEEDS INCITED BY SEED- BORNE MYCOFLORA

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ABSTRACT
Seed deterioration either due to mechanical damage caused by tissue colonization or production of different type of toxin by the associated seed borne mycoflora which leads to increased leakage of various substances from pigeonpea seeds during the imbibitions stage of seed germination. In this context, electrolyte leakage of seeds from three stored pigeonpea varieties incited by seed borne mycoflora was studied. The amount of electrolyte leakage in seed leachates was influenced by the associated fungal species and stored periods. Fungal treatment, regardless of the species, induced higher amount of electrolyte leakage from the seeds of three pigeonpea varieties tested as compared with the uninoculated control seeds. The maximum electrolyte leakages (7.00 ds m^{-1}) was induced by *Aspergillus flavus* in variety Rajeev Lochan, by *Aspergillus niger* and *Fusarium udum* in variety ICPL-87119 (6.89 ds m^{-1}) and by *A. flavus* in Farmer's variety (8.74 ds m^{-1}) after 60 days of storage. An increasing trend in Electrolyte leakage was also recorded when the storage period of fungal treated pigeonpea seeds increased from 0-60 days at an interval of 15 days. It is concluded that association of mycoflora with the seeds deteriorate the quality of seeds as electrolyte comes out of seeds.

INTRODUCTION

Good and healthy seeds are not only essential requirement but also an important component for any successful production programme. The fungi associated with crop seeds play an important role in seed deterioration during storage (Neergaard, 1977). The seed pathogen may adhere to the seed surface either in form of spores or mycelium or may be deep seated internally (Shakshi Singh *et al.*, 2014). Seed can be stored for a long time but unscientific and traditionally followed storage practices provide a free entry for storage fungi which subsequently cause bio-deterioration of seeds and storability of seed can be increased by seed treatment (Patil *et.al.*, 2014). These associated mycoflora reduced the nutritive values of seeds by producing toxin, which make seeds unfit for human consumption. It is hypothesized that seed deterioration either due to mechanical damage caused by tissue colonization or production of different type of toxin by the associated seed borne mycoflora which leads to increased leakage of various substances from pigeonpea seeds during the imbibitions stage of seed germination (Vidhyasekaran*et al.* 1986; Kohmoto and Otani, 1991). During the early stage of seed imbibitions the electrolyte release rate from the seed is very high which is related to the integrity of the cell membrane system (Bewley and Black, 1985). Measurement of electrolyte concentration in seed leachates in terms of electrical conductivity (EC) is one of the standard and rapid methods of seed health testing (Mathews and Powell, 1981). In this context, present study deals with effect of seed borne mycoflora on seed quality in terms of electrolyte leakage during storage.

MATERIALS AND METHODS

The fungal species selected for the study were *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus sp,* Mycelia sterilia, Mucor sp. and Penicillium sp. and *Fusarium udum*. These fungal species were the most frequently isolated species from the seeds of three pigeonpea varieties viz. Rajeev Lochan, ICPL-87119 and Farmer's variety by four incubation methods- standard blotter, agar plate, 2,4-D and roll towel method (ISTA, 1976). For study on effect of seed borne mycoflora on electrolyte leakage method of Singh et al. (2001) was followed. Accordingly, one hundred gram of pigeonpea seeds were surface sterilized with 0.5% NaOCl solution followed by three washing with sterile distilled water. These seeds were then inoculated separately with 10 ml spore suspension of associated mycoflora which were isolated earlier from pigeonpea seeds. Surface sterilized seeds treated with 10 ml of sterile distilled water served as control. The inoculated seeds were stored in polythene bags at 28 ± 1°C. Electrical conductivity of seeds leachates was measured periodically at 0, 15, 30, 45 and 60 days of storage. Two hundred seeds from each treatment and control were separately soaked in 100 ml distilled water for 24 hours. Amount of electrolyte leakage as Electrical conductivity (EC) of the seed leachates was measured by using a systronic direct reading conductivity meter. Four replications for each treatment were maintained.

RESULTS AND DISCUSSION

The amount of electrolyte leakage as revealed by EC reading of seed leachates was influenced by the type of associated

Table 1: Effect of seed mycoflora on electrical conductivity (ds m^{-1}) of seed leachates of different pigeonpea varieties

Mycoflora	Pigeonpea varieties/storage periods (days) Rajeev Lochan						ICPL-87119						Farmer's variety					
	0	15	30	45	60	Mean	0	15	30	45	60	Mean	0	15	30	45	60	Mean
Aspregillus flavus	1.62	1.96	3.00	6.10	7.00	3.93	1.73	2.20	3.42	5.31	6.54	3.84	1.97	2.56	3.96	6.44	8.74	4.73
Aspregillus niger	1.62	1.91	2.83	5.70	6.80	3.77	1.73	1.98	3.37	5.72	6.89	3.93	1.97	2.34	3.68	6.21	8.61	4.56
Aspregillus fumigatus	1.62	1.84	2.71	4.50	5.60	3.25	1.73	1.87	3.28	4.90	5.21	3.39	1.97	2.20	3.69	6.70	7.62	4.43
*Aspregillus*sp.	1.62	1.87	2.78	4.30	5.72	3.25	1.73	1.96	3.34	4.74	5.32	3.41	1.97	2.29	3.32	6.22	7.90	4.32
Mucor sp.	1.62	1.72	2.28	3.72	4.80	2.82	1.73	1.85	3.12	4.41	4.90	3.13	1.97	2.18	3.63	6.02	7.06	4.17
Penicillium sp.	1.62	1.86	2.80	5.00	6.53	3.56	1.73	1.94	3.28	4.80	5.30	3.41	1.97	2.40	4.30	6.24	7.38	4.48
Fusarium udum	1.62	1.93	2.76	5.21	5.90	3.48	1.73	2.00	3.40	6.10	6.42	3.93	1.97	2.38	3.85	6.70	6.92	4.36
Mycelia sterilia	1.62	1.80	2.92	5.30	5.70	3.46	1.73	1.86	3.14	4.53	5.81	3.41	1.97	2.14	3.52	5.83	6.50	3.99
Control	1.62	1.74	2.07	2.90	3.66	2.39	1.73	1.82	2.64	3.90	4.87	2.99	1.97	2.23	3.18	4.37	5.18	3.38
Mean	1.62	1.84	2.68	4.74	5.74		1.73	1.94	3.22	4.93	5.69		1.97	2.30	3.68	6.08	7.32	

*Electrical conductivity (ds m^{-1})

fungal species, type of seed variety and storage periods (Table 1). Fungal treatment, regardless of the species, induced higher amount of electrolyte leakage from the seeds of pigeonpea varieties (Rajeev Lochan, ICPL-87119 and Farmer's variety) tested as compared with the uninoculated control seeds. However, different fungal species induced differential electrolyte leakage on different pigeonpea varieties.The result showed that seeds of Rajeev Lochan variety inoculated with *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus sp,* Mycelia sterilia*, Mucor*sp.,*Penicillium*sp. and *Fusarium udum*and stored for 60 d showed higher ECof seed leachates (5.74 ds m^{-1}) as compared to 0 periods of inoculation (1.62 ds m^{-1}). Seed inoculated with *Aspergillus flavus* showed higher mean EC of seed leachates (3.93 ds m^{-1}) followed by *Aspergillus niger* (3.77 ds m^{-1}) as compared to control (2.39 ds m^{-1}). Seed inoculated with *Aspergillus flavus*showed a higher EC of seed leachates (7.0 ds m^{-1}) followed by *Aspergillus niger* (6.80 ds m^{-1}) and least EC of seed leachates except control was recorded in seed inoculated with *Mucor* sp. (4.80 ds m^{-1}) followed by *Aspergillus fumigatus* (5.6 ds m^{-1})after 60 d storage.

In variety ICPL-87119, seed stored for 60 dshowed higher EC of seed leachates (5.69 ds m^{-1}) as compared to 0 periods of inoculation (1.73 ds m^{-1}). Seed inoculated with *Aspergillus niger*and *Fusarium udum* showed higher mean EC of seed leachates (3.93 ds m^{-1}) closely followed by *Aspergillus flavus* (3.84 ds m^{-1}) as compared to control (2.99 ds m^{-1}).

The stored seeds of Farmer's variety showed highest EC of seed leachates in all periods of incubation as compared to seeds of above varieties (Rajeev Lochan and ICPL-87119). Stored seeds of Farmer's variety after 60 d storage, showed higher EC of seed leachates (7.32 ds m^{-1}) as compared to 0 periods of inoculation (1.97 ds m^{-1}). Seed inoculated with *Aspergillus flavus*showed higher mean EC of seed leachates (4.73 ds m^{-1}) closely followed by *Aspergillus niger* (4.56 ds m^{-1}) as compared to control (3.38 ds m^{-1}). The least EC of seed leachates except control was recorded in seed inoculated with Mycelia sterilia (3.99 ds m^{-1}) followed by *Mucor* sp.(4.17ds m^{-1}).

The variation in amount of electrolyte leakage may be due to the susceptibility of seeds of pigeonpea varieties to a particular fungal species. Thefinding of Davidson *et al*. (1994) in maize and wheat seeds, Singh *et al*. (2001) found in rice seed and Rawal *et al*. (2010) reported in funnel seeds supports the finding of present study. The fungal species increases the permeability might be due to production of toxins in stored seeds. Different mechanism are involved in seed deterioration by associated fungi, of which fungal enzymes and toxin (Vidhyasekaran, 1977) and physical damage due to tissue invasion are important factors. The phytotoxic metabolites are also released by dormant fungal structure like spore (Fraser, 1991). The fungal toxins generally disrupt permeability of cell plasma membranes (Wheeler and Hanchey, 1986; Vidhyasekaran *et al*., 1986). All these factors will contributes to enhanced leakage of electrolyte from the seed. Gradual increase in EC of seed leachates with longer storage period was observed in both fungal treated and control seed. Seed aging also results in the gradual loss of semi-permeability of membranes (Dighe, 1993). The electrolyte test is based on the finding that the seed with low vigour are found be more permeable leading to an increase in leakage of electrolytes during imbibitions stage. This study concludes that seed deterioration in longer storage period is due to altered cell membrane permeability incited toxins producing mycoflora associated with seeds.

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