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FACTORS AFFECTING SEEDLING BLIGHT OF HYBRID MAIZE (*Zea mays* L.) VAR. 'SAMPATH' AND ITS PARENTAL LINES (CML 348, CML 20) CAUSED BY *Penicillium* sp.

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ABSTRACT

In Sri Lanka, seedling blight and retardation of seedling growth is a serious and a commonly occurring disease in the local hybrid maize (*Zea mays* L.) var. 'Sampath', and its male (CML 348) female (CML 20) parents. In this study *Penicillium* sp. was isolated from infected maize seeds and seedlings. The rate of transmission from seed to seedling infection is higher than that of transmission from seed to germinating seeds. *Penicillium* sp. has been recovered from all seed components, especially from the embryo and the pericarp. Disease severity was significantly affected by initial seed moisture content. In general, the disease incidence increased with the increasing initial seed moisture content. The results also revealed that the inoculum concentration is a significant factor influencing disease severity, and its effect depends on the susceptibility of the maize variety to the disease.

KEYWORDS: Initial moisture content, Inoculum concentration, Local maize hybrid, *Penicillium* sp., Seedling blight

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop in the dry zone of Sri Lanka, and the crop has a good potential due to its low cost of production and multipurpose use. The large-scale cultivation of maize in Sri Lanka started in about 1983 and gradually increased with the introduction of high yielding exotic hybrid maize varieties. Efforts have been made by the breeders to develop high yielding local maize hybrids to reduce importation of exotic hybrid seeds and the first hybrid maize variety 'Sampath' was released in 2004. This variety has shown distinct superiority to other hybrid maize varieties owing to its wider adaptability to different agroclimatic conditions and ability to withstand drought. Despite the advantages of the crop, the full potential of the crop is far from being exploited and the yield levels are lower than the exotic hybrids due to several biotic and abiotic constraints. Among the biotic factors, susceptibility to diseases is a major constraint (Karunarathne, 2009). Seedling blight and retardation of seedling growth was first observed in the male parent (CML 348) of local hybrid maize variety 'Sampath' during the *Yala* season 2010. Few months later, the F_1 hybrid and the female parent (CML 20) also showed the same disease symptoms. Isolation of pathogens from the diseased seedlings predominantly yielded a species of *Penicillium*. The first report of *Penicillium* as a causal agent of seedling blight of maize was in late 1920's (Johann, 1928). Penicillium seedling blight was considered as a potentially destructive seed borne disease in many parts of the maize growing countries (Baird *et al.*, 1994, Basak and Lee, 2002, Meiri *et al.*, 1988). Seed borne diseases cause enormous losses in the maize crop both in storage as well as in the field. Systemic transmission of fungi from seeds to seedling is well documented (Johann and Holbert, 1931, Somda *et al.*, 2008). Due the seriousness and common occurrence of seedling blight of local hybrid maize variety 'Sampath', and its parental lines, this study was undertaken to identify the seed borne fungi, its mode of transmission from seed to seedlings and seed factors that affects the disease severity.

MATERIALS AND METHODS

The maize seeds used throughout this study were 'Sampath' (F_1), and the male parent (CML 348) and female parent (CML 20) of the hybrid.

Isolation of pathogen

The seed lots that resulted in diseased maize seedlings in the field were sampled and examined for disease symptoms. The infected seeds were used for isolation of the causal organism by deep freezer blotter method (Limonard, 1968). Four hundred infected maize seeds were placed at the rate of 10 seeds per Petri plate on moistened blotters. Thereafter, the Petri plates were first incubated at 30°C for 24 h under alternate cycles of 12 h NUV light and darkness. For the next 24 h, the plates were incubated at -10°C in dark and then kept back under original conditions for the following five days. After eight days of incubation, the seeds were examined individually under stereo-binocular microscope.

The fungus growing in the seed was sub-cultured on Potato Dextrose Agar medium for identification. The identification of fungus was done based on the spore morphology and colony characters of the fungus. These cultures were further purified by the single spore isolation technique, and the pure cultures were maintained on Potato Dextrose Agar slants.

Disease transmission studies

The seed infection percentage was determined by testing seed health one day prior to the start of the transmission studies. Under *in vitro* conditions, the test tube seedling symptom test (Khare, 1996) was used. Test tube slants were prepared by pouring 6 ml of 2.0% water agar and sterilized in autoclave for 10 min and 15 psi pressure at 121°C. Fifty naturally infected seeds of the hybrid and parental lines placed in test tube at a rate of one seed per test tube. The test tubes with the seeds were incubated in the laboratory at room temperature (25 – 32°C). The mouth of the test tubes was securely plugged with cotton wool and the test tubes were placed on the wooden racks. The germinating seeds and seedlings in the test tube were examined for the presence of visible symptoms such as seed rot, death of the seedlings, yellowing of leaves. The symptoms produced on the germinating seeds and seedlings by the pathogen were confirmed by examining the seed under stereo – binocular microscope. The apparently healthy seeds were placed in test tubes with 2% water agar, which served as control. The percentage of seeds infected was calculated.

Location of *Penicillium* pathogen on seed

For determining the preferred site of sporulation, observations were made to locate the part of the seed from which the fungus was sporulating, according to the component plating technique (Maden *et al.*, 1975). The naturally infected maize seed sample of 'CML 348' was used for the study. Twenty five seeds of test line were washed four times with tap water, surface sterilized in 1% sodium hypochlorite for two min, washed with sterilezed water, soaked in sterilized water for 2 h and were dissected aseptically using a steril needle and forceps. The separated seed parts *viz.*, pericarp, endosperm and embryo were plated immediately before drying, on water agar plates. The plates were incubated at 25°C for five days and thereafter the seed components were examined under the stereo-binocular microscope for the presence of the pathogen in different seed parts.

Effect of initial seed moisture content on disease development

Freshly harvested seeds produced at Field Crops Research and Development Institute (FCRDI), Mahailluppallama, Sri Lanka were sun dried for different durations to obtain the required initial seed moisture content. Seed moisture content was determined using a moisture meter, which was pre-calibrated. One kg each of the seeds of CML 348 a different moisture contents were stored separately in polyethylene bags and were maintained at room temperature for a period of three months. Samples of the stored were drawn thereafter and evaluated for the incidence of seedling blight and germination.

Effect of inoculum concentration on disease severity

Inoculum suspensions of $1x10^5$, $1x10^4$, $1x10^3$, $1x10^2$, 1×10^1 conidia ml^{-1} of the *Penicillium* sp. were separately applied to 200 grams of CML 348, CML 20 and 'Sampath' (F_1) seed lots. Seeds were inoculated by spraying them with the 20ml conidia suspension using a hand sprayer. The inoculated seeds were evaluated for disease incidence after 5 days of inoculation using test tube seedling method as described earlier.

Statistical analysis

All data were submitted to the Analysis of Variance (ANOVA). Analysis of percentage data were based on arcsin transformed percentage values. Mean values were compared using the Duncan New Multiple Range test (DNMRT). Statistical analysis was supported by SAS (Statistical analysis system SAS Institute, INT; CARY, MC.).

RESULTS AND DISCUSSION

Disease symptoms, isolation and identification of pathogen

The characteristics of seedling blight were longitudinal yellow streaks and chlorosis on leaves, which became apparent at the 3-5 leaf stage. Some infected seedlings wilted due to weakened root system, while others continued to grow, but these seedlings were stunted when compared with asymptomatic seedlings (Figure 1). When the affected plants were uprooted, masses of blue green conidia were observed on the seed (Figure 2). A species of *Penicillium* was isolated from all symptomatic seedlings. Infection of stored grain resulted in blue-green discoloration of the embryo (Figure 3).

Microscopic examinations of the fungus revealed the septate, hyaline hyphae. Conidia are borne on long conidiophores that were typically branched in a broomlike manner. Conidia are single celled, round, resembling glass beads, green in colour and formed in chains at the tips of conidiophores.

The pathogen was identified as *Penicillium* sp. and the identity was further confirmed using the description of Commonwealth Mycological Institution (Tarr, 1962). *Penicillium* sp. has been reported to invade maize seeds causing seed rot and seedling blight (Sweets and Wright, 2008; White 1999).

Transmission of *Penicillium* sp.

Results of transmission from seed to germinating seeds and seedlings of three maize varieties as determined by test tube seedling test are presented in the Table 1. *Penicillium* spp. was found to be transmitted to the germinating seeds causing pre-emergence and post-emergence death. The rate of transmission from seed to seedling infection was higher than that of seed to germinating seeds. The highest percentage of seed borne infection, pre-emergence death or seed rot, seedling infection and total disease development were recorded in CML 348. The parental line 'CML 20' and the hybrid 'Sampath' produced few diseased seedlings. This could be due to that 'CML 20' and 'Sampath' had a low percentage of seed infection by *Penicillium* sp. compared with 'CML 348'. Hence, 'CML 20' and 'Sampath' appeared to be moderately resistant to the disease with less than 10% seed infestation. More than 30% of the seeds of 'CML 348' were infected and rated as a susceptible line to seedling blight. The 'CML 348' has exposed sheath coverage on kernels than that of 'Sampath' and CML 20. Varieties with poor sheath coverage (exposed kernels) are known to be more prone to infection (CIMMYT, 2004).

Penicillium was found responsible for the maize seedling blight. Sweet and Wright (2008) reported that *Penicillium* sp. cause severe seedling blight infestation in maize. Meiri *et al.* (1988) identified *Penicillium* sp. as a seed borne pathogen in maize. Under favourable environmental conditions, *Penicillium* sp. can infect the growing plants and can serve as a source of inoculum for the field grown crop. Thus, the infected seeds should not be preserved as propagules. A significant linear relationship (R^2 = 0.93) was found between seedling blight and seed infection (Figure 4). Meiri *et al.* (1988) reported of a positive correlation between seedling blight in the field and seed infestation by *Penicillium* sp. Seed germination ranged from 80 - 85% among the varieties having different infection level. There were no significant effects of fungal infection on germination of seeds at any infection level probably due to the high vigour of the maize seeds tested. These results are in agreement with that of Meiri and Solel (1990) who did not find a relationship between infection by *Penicillium* sp. and the germination of maize seeds.

Table 1. Transmission of *Penicillium* sp. from maize seeds to germinating seeds and seedlings as determined by test tube seedling test.

Variety	*% of seed borne infection*	*Germination %*	*% seed rot and seedling blight*				*Total disease development*
			Seed rot	*Rate of transmission*	*Seedling blight*	*Rate of transmission*	
CML 348	35 a*	90a	3	8.5	28 a	80	31 a
CML 20	6 b	90a	0	0	1 b	16	1 b
Sampath F1	10 b	95a	1	10	3 b	30	4 b
CV%	7.12	5.32	4.12		9.33		6.14

* Within each column, means followed by the same letter are not significantly different at p=0.05.

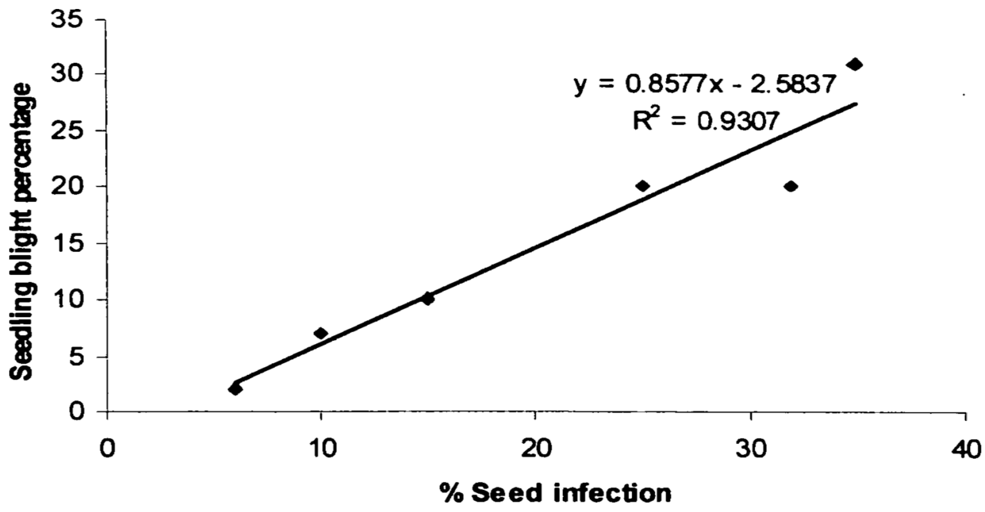

Figure 4. Changes in seedling blight incidence with per cent seed infection

Location of *Penicillium* pathogen on seed

Penicillium sp. was recovered from all seed components (Table 2). However, the embryo showed the highest percentage of infection, followed by the pericarp. The least infection was observed in the endosperm. Earlier work reported that sporulation of *Penicillium* sp. is confined to pericarp, embryo and scutellum (Meiri and Solel, 1990). The results suggested that the embryo of the viable maize seed is the most susceptible to infection by *Penicillium* sp. The embryo and scutellum of maize seeds has a high protein content (Bewley *et al., 2006*) and this may possibly enhance the sporulation of *Penicillium* sp.

Table 2. Percentage of recovery of *Penicillium* sp. from whole seeds and different components of naturally infected maize seeds

Component part	*Disease incidence*
Endosperm	13 c*
Pericarp	16 b
Embryo	36 a
CV %	9.26

* Within each column, means followed by the same letter are not significantly different at p=0.05

Effect of initial seed moisture content on disease development

The effect of initial seed moisture content on seedling blight and germination is shown in Table 3. The disease severity was significantly ($p < 0.05$) affected by the initial seed moisture content. In general, disease incidence increased with the increasing initial seed moisture content. The disease incidence was the lowest at initial seed moisture content of 9 and 11%, but there were no significant difference ($p>0.05$) in the germination percentage among different treatments. Hence, maize seeds with less than 13% initial moisture content should be used for storage. Other studies have also proven that high seed moisture content was strongly associated with the infection from seedborne pathogens. As shown by Mettananda *et al.* (2001), maize seeds should be stored in moisture proof containers such as polythene after drying to a minimum of 12% moisture. Karunarathne (2009) reported that more than 16% seed moisture content in maize was favourable for infection by storage pathogens.

Table 3. Effect of initial moisture content on germination and seedling blight

Initial seed Moisture content (%)	*Germination %*	*Seedling blight percentage*
9	82a	5 e*
11	81 a	5 e
13	88a	11.66 d
15	86 a	26.66 c
17	85 a	53.33 b
19	80 a	73.33 a
21	79a	78.33 a
CV %		11.23

* Within each column means followed by the same letter are not significantly different at $p=0.05$

Effect of inoculum concentration on disease severity

Disease severity increased with the increasing inoculum concentration from 10^1 to 10^3 conidia mi^{-1}. In highly susceptible 'CML 348', the disease severity was greater than 10% for all inoculum concentrations, and there was no significant increase ($p>0,05$) in the severity with the increases in inoculum concentration. In 'CML 20' and 'Sampath' (F_1), the disease severity depended greatly on the inoculum dose (Figure 5). The results revealed that the inoculum concentration is a significant factor influencing disease severity, and its effects depend on the susceptibility of the maize variety to the disease. Changes in disease susceptibility with inoculum dose have been reported by Meiri and Solel (1990). Results of the effect of inoculum dose is of greater importance in screening maize varieties for disease resistance to *Penicillium* seedling blight.

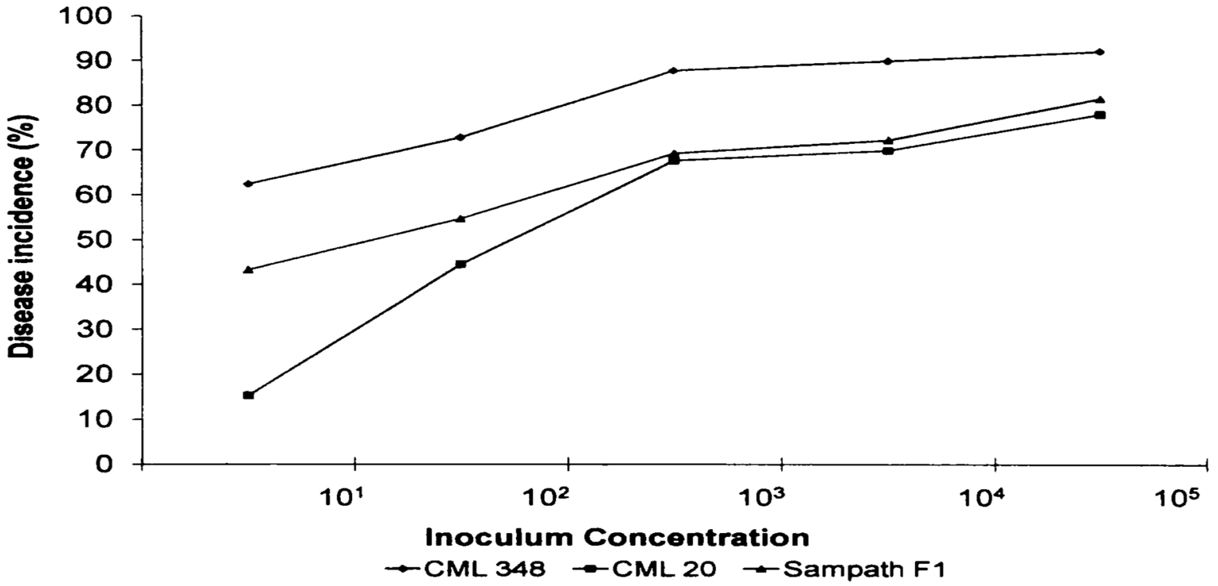

Fig 5. Effect of inoculum concentration on infection of three maize varieties by *Penicillium* sp.

CONCLUSIONS

A new disease of 'Sampath' and its male (CML 348) and Female (CML 20) parents has been identified as seedling blight caused by Penicillium sp. The pathogen had no effect on seed germination but resulting poor seedling emergence and blighting after emergence. The embryo of the living seed was the most susceptible to infection by *Penicillium* sp. The disease incidence increased with increasing initial seed moisture content, and seeds with <13% moisture content should be used for storage. Response to inoculum doses could be used for screening of maize varieties for disease resistance to penicillium seedling blight

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REFERENCES

Baird, R.E., C. Nankam, P. Moghaddam and J.K. Pataky. 1994. Evaluation of seed treatment on Shrunken – 2 Sweet corn. Plant Disease. 78:817 – 821.

Basak, A. B. and M. W. Lee. 2002. Prevalence and transmission of seed-borne fungi of maize grown in a farm of Korea. Mycobiology. 30 (1): 47 – 50.

Bewley, J.D., M. Black and P. Harmer. 2006. Cereals *In:* The Encyclopidia of seeds; scienec, technology and uses. Wallingford, CABI, UK. Pp 65-70.

CIMMYT. 2004. Maize disease. A guide for field identification, Mexico D.F., CIMMYT.

Johann, H. 1928. *Penicillium* injury to corn seedlings. Phytopathology. 18:239- 242.

Johann, H. and J. J. Holbert. 1931. Further studies of *Penicillium* injury to corn. Journal Agricultural Research. 43:757 -790.

Karunarathne, K.M. 2009. Maize Cultivation. Asliya Printers, Kurunegala. 91p.

Khare, M.N. 1996. Methods to test seeds for associated fungi. Indian Phytopathology. 49:319 – 328.

Limonard, T. 1968. Ecological aspects of seed health testing. Proceedings of the International Seed Testing Association. 33:1.

Maden, S., D. Singh, S.B. Mathur, and P. Neergard. 1975. Detection and location of seed borne inoculum of *Ascochyta rabei* and its transmission in chickpea. Seed Science and Technology. 3: 667-671.

Meiri, A., Z. Solel, R. Tamari and R. Cohen. 1988. Seedborne *Penicillium* in sweet corn; its damage and control. Phytoparasitica. 16: 87.

Meiri, H.A. and Z. Solel. 1990. Factors affecting seedling blight of sweet corn caused by seedborne *Penicillium oxalicum*. Plant Disease. 74: 36 – 39.

Mettananda, K.A., S.L. Weerasena and Y. Liyanage. 2001. Effect of storage environment, packing material and seed moisture content on storability of maize (*Zea mays* L.) seeds. Annals of the Sri Lanka Department of Agriculture. 3:131-142.

Somda, I., J. Sanou and P. Sanon. 2008. Seed – borne infection of farmer – saved maize seeds by pathogenic fungi and their transmission to seedlings. Plant Pathology Journal. 7(1): 98 – 103.

Sweets, L.E. and S. Wright 2008. Corn plant protection programme. In Integrated pest management. University of Missouri. Pp91

Tarr, S.A.J. 1962. Diseases of sorghum sudan grass and broom corn, Kew, Surrey: The Commonwealth Mycological Institute. 380 p.

White, D.G. 1999. Compendium of Corn Diseases. St. Paul, Minnesota: The American Phytopathological Society. Pp. 44.