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Susceptibility of sweetpotato germplasm to rhizopus soft rot caused by *Rhizopus stolonifer* and *Rhizopus oryzae* in Kenya

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Abstract

Susceptibility of fifteen local and introduced sweetpotato germplasm to Rhizopus soft rot disease caused by *Rhizopus stolonifer* and *Rhizopus oryzae* was tested in 1999 and 2000. Roots were surface sterilised, wounded and inoculated with 9 mm diameter agar plugs removed from the edge of actively growing two-day old cultures of the test pathogen. Infection was evaluated by measuring the diameter and depth of the internal lesion developing around the inoculation wound under room temperature for 48 hours. The germplasm exhibited wide variability in susceptibility to infection. The cultivars *Maria Angola, Santa Amaro*, SPK 013, KEMB 10 and *Marooko* consistently developed smaller lesions compared to other cultivars, and were considered more resistant to Rhizopus soft rot disease. The cultivars *Naveto, Tainung* 64, KEMB 23 and *Yanshu* 1 consistently developed larger lesions compared with other cultivars and were considered more susceptible. Variability in susceptibility expressed by the germplasm shows the possibility of using host resistance as a means of controlling Rhizopus soft rot disease in sweetpotato root tubers.

Key words: Kenya, *Ipomea batatas*, post harvest diseases, Rhizopus soft rot-disease

Introduction

Rhizopus soft rot is the most widespread and destructive postharvest disease of sweetpotato (*Ipomea batatas* (L.) Lam. Worldwide (Clark and Moyer, 1988). It is caused by the fungi *Rhizopus stolonifer* (Syn. R. *nigricus* and *R. tritici*) (Snowdon, 1990; Clark, 1992). The two pathogens are soil and air borne with harvested sweetpotato roots usually contaminated with fungal spores (Snowdon, 1990). However, disease development requires wounds for penetration and establishment of the fungus (Clark, 1992; Clark and Moyer, 1988). Consequently, infection usually begins from one or both ends of the root, which are susceptible to injury, or it may begin at other wounded sites (Clark and Moyer, 1988; Snowdon, 1990). Affected roots rapidly turn soft and moist, and where the skin has raptured, a copious coarse white mould with sporangia develops (Snowdon, 1990; Clark, 1992). The colour of the affected root tissue may not change significantly, but a strong alcoholic odour is produced (Clark and Moyer, 1988). Infection and decay are influenced by the prevailing environmental conditions (Clark and Moyer, 1988). The ideal temperature ranges from 22°C to 30°C and relative humidity from 75 to 85% (Clark and Moyer, 1988; Snowdon, 1990). Under these conditions in a susceptible cultivar, an infected storage root of moderate size could be decayed entirely within three to five days (Spalding, 1969; Holmes and Stange, 2002). The rapid destruction of host tissue is caused by maceration by toxic enzymes produced by the invading fungus (Spalding, 1969).

Host resistance, as a tool in disease management has been recognised for a long time and has recently gained additional impetus and importance because of a growing need for consumer safety (Clark *et al.*, 1992; Agrios, 1997). Sweetpotato genotypes are known to vary widely in their resistance to

postharvest diseases, but neither the mechanism of resistance nor the correlation between resistance to various fungal diseases is known (Clark, 1992). Germplasm evaluations conducted in the United States of America (Harter and Weimer, 1921; Clark and Hoy, 1994) and in Tanzania (Muhanna *et al.*, 2001) have shown that the level of resistance to Rhizopus soft rot vary widely in sweetpotato genotypes. Similar results have also been obtained in preliminary germplasm evaluation in Kenya (Kihurani, 1997). The aim of this study was to evaluate local and introduced sweetpotato germplasm for resistance to Rhizopus soft rot caused by *R. stolonifer* and *R. oryzae* in Kenya.

Materials and methods

Single-spore isolates of *R. stolonifer* and *R. oryzae* were obtained from naturally infected sweetpotato storage roots. The diseased root samples were obtained from main sweetpotato growing areas around the lake Victoria basin in western Kenya and at the Kibirigwi irrigation scheme in central Province. Relative virulence of ten single spore isolates of each test pathogen was determined by inoculating health storage roots of the sweetpotato cultivar KSP 20. The most virulent isolate of each test pathogen was selected and preserved in sterile soil according to Smith and Onions (1983) and subsequently used to inoculate healthy storage roots of the test sweetpotato germplasm.

Fifteen test germplasm comprising of eight important local, and seven introduced sweetpotato cultivars were used in the study (Table 1). These varieties were selected on the basis of their relative importance in the sub-Saharan region for human consumption, animal feed and income generation (Carey *et al.*, 1999). They were grown on 30 m long ridges spaced at 80 cm, in an experimental plot at the National Agricultural Research Laboratories (NARL) about 6 km west of Nairobi City. Planting materials comprised 25 cm long apical-end vine cuttings. They were obtained from the International Potato Centre (CIP) sweetpotato germplasm conservation plot at the field station of the University of Nairobi, Kabete Campus and planted at a spacing of 30 cm between hills.

The experimental site has deep well drained friable clay soil (Nitosols) (Siderius, 1976). It is 1740 m above sea level and experiences bimodal precipitation with a main rainy season from mid-March to May and a secondary one from Mid-October to December (Siderius and Muchena, 1977). No fertiliser or manure was applied to the plants during the growing season, and the plot was kept weed-free by regular hand weeding. Harvesting was done at 22 weeks after planting using a hand hoe, and care was taken to minimise mechanical damage to the roots during harvesting and handling.

Harvested storage roots of uniform size were selected and washed in tap water to remove adhering soil, and surface sterilised with alcohol (96% ethanol). Each root was injured at the median by creating a shallow wound of about nine-mm in diameter and six mm in depth. A sterile nine-mm cork borer was used to cut agar plugs from the margin of an actively growing two-day-old potato dextrose agar cultures of the test pathogen. The agar plugs were removed and placed onto the wounds with the mycelium side facing down. Control roots were also inoculated using similar, but sterile, agar plugs. The inoculated roots were placed in polyethylene bags (autoclavable sun-transparent Sigma cell culture 44.0 x 20.5 cm with 24 mm 0.02 micron filter disc) and incubated at room temperature for 48 hours.

The experiment was first conducted in 1999 and repeated in 2000. In each experiment susceptibility of the sweetpotato germplasm to infection by isolates of *R. stolonifer* and *R. oryzae* was tested. In the first trial, roots were inoculated on 10th November 1999, using 12 stored roots per entry, and in the second test, roots were inoculated on 17th May 2000 using 15 freshly harvested roots per entry. The experiments were arranged following a randomised complete block design with three replicates.

Disease development was assessed by cutting each inoculated root longitudinally through the inoculation wound and measuring diameter and depth of the developing internal lesion. Mean internal lesion dimension was used as a measure of lesion size and was obtained by computing the average mean lesion diameter and depth according to Duarte and Clark (1993). The data were analysed by

analysis of variance (ANOVA) using Statgraphics plus 3.1 software and cultivar means compared by Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Results

The interaction between cultivar and pathogen was not significant in the first test, but it was significant ($P<0.05$) in the second. In addition, all the cultivars were susceptible to infection by both *R. stolonifer* and *R. oryzae*. In both trials, lesion size on the inoculated storage roots differed significantly ($P<0.05$) among the cultivars and between the pathogens (Table 1). The cultivars *Maria Angola, Santa Amaro*, SPK 013, KEMB 10 and *Marooko* developed smaller lesions compared with the other cultivars, while the cultivars *Naveto, Tainung* 64, KEMB 23, and *Yanshu* 1 developed larger lesions compared to the other cultivars in both tests. Lesion size in the cultivars KEMB 36, SPK 004, KSP 20, *Jayalo, Mugade* and KSP 11 differed between the two trials. All the cultivars developed larger lesions with both pathogens in the first trial (1999) compared to the second (2000) (Table 1).

Lesion sizes were larger with *R. stolonifer* compared with *R. oryzae* in the first trial (1999). They ranged from 37.43 mm to 58.43mm compared to 9.39 mm to 37.33mm with *R. oryzae*. In the second trial (2000), with the exception of *Yanshu* 1, lesions sizes were larger with *R. oryzae* infection compared to *R. stolonifer* in six cultivars, KSP 20, KEMB 10, *Mugade*, SPK 004, SPK 013 and *Tainung* 64. In addition, lesions sizes did not differ between the pathogens in the remaining eight cultivars.

Discussion

The fact that interaction between cultivar and pathogen was significant in one trial and not in the other showed that the cultivars reacted to the pathogens in a similar manner in one trial and differently in

Table 1. Mean internal lesion dimensions (mm) on storage root cultivars inoculated with Rhizopus stolonifer and Rhizopus oryae in 1999 (trial 1) and 2000 trial 2).

Cultivar	CIP No. or local Name	Origin	*Rhizopus stolonifer*		*Rhizoipus oryzae*	
			1999	2000	1999	2000
Maria Angola	420008	Peru	37.43a	7.00a	12.30ab	08.56a
KEMB36	*Muibai*	Local	39.02a	9.81a	24.70cd	12.56ab
SPK004	*Kakamega* 4	Local	40.67ab	7.23a	23.04bcd	17.60abc
Santa Amaro	400011	Brazil	40.67ab	9.08a	16.00abc	11.17ab
SPK013	None	Local	43.44abc	6.33a	09.39a	11.44ab
KSP20	440170	IITA	44.80abcd	7.30a	25.60cde	16.60ab
KEMB10	440169	Local	45.80abcd	7.0a	23.53bcd	16.00ab
Marooko	*Marooko*	Local	46.50abcd	8.00a	17.08abc	12.21ab
Jayalo	*Jayalo*	Local	49.13bcde	6.33a	23.50bcd	11.43ab
Mugade	440163	Rwanda	49.37bcde	10.17a	22.00bcd	30.87c
Tainang 64	440189	Taiwan	50.47bcde	11.70cde	32.00de	24.23bc
KSP11	None	Local	51.70cde	8.60a	26.67cde	13.22ab
KEMB23	*Gikanda*	Local	54.63de	19.11b	27.04cde	24.42bc
Naveto	440131	P.N.Guinea	58.29e	11.17a	36.75e	18.04abc
Yanshu 1	440024	China	58.43e	32.94c	37.33e	20.25abc
Mean			47.36	10.79	28.80	16.57
			1999		**2000**	
LSD ($P\leq0.05$) for comparing cultivar means			9.12		8.25	
LSD ($P\leq0.05$) for comparing pathogen means			6.45		5.83	
LSD ($P\leq0.05$) for cultivar X pathogen interaction			Not significant		11.66	

other test. This inconsistency in the interaction is an indication that cultivar susceptibility to infection was influenced by other factors besides presence of the pathogens.

Cultivar response to infection was consistent in the majority of cultivars tested, and this was an indication of stability in resistance or susceptibility to Rhizopus soft rot disease. The cultivars *Maria Angola, Santa Amaro*, SPK 013, KEMB 10 and *Marooko* exhibited stable resistance to infection, while the cultivars *Naveto, Tainung* 64, KEMB 23, and *Yanshu* 1 exhibited stable susceptibility. The cultivars KEMB 36, SPK 004, KSP 20, *Jayalo, Mugade* and KSP 11 exhibited unstable susceptibility/ resistance. Similar findings have been reported in Tanzanian sweetpotato germplasm and the phenomenon was attributed to the influence of the prevailing storage and crop growth conditions (Muhana *et al.,* 2001). The reason for the observed disease reactions was not investigated in the present study.

The results also showed that all cultivars were more susceptible to infection by both pathogens in the first trial (1999) compared to the second (2000), and this was attributed to differences in the prevailing temperature during incubation. During the first trial the ambient temperature ranged from 22°C to 28°C and in the second trialranged from 20°C to 25°C. The higher temperature during the first test provided a more favourable environment for pathogen activity, and this may have enhanced infection. The influence of the prevailing environmental conditions on infection and decay of sweetpotato roots has also been reported by Clark and Moyer (1988) and Wills *et al.* (1998).

Although in the first trial, all the cultivars developed larger lesions, suggesting greater susceptibility to *R. stolonifer* compared with *R. oryzae,* this trend was repeated in the second trial. Six of the cultivars, KSP 20, KEMB 10, *Mugade*, SPK 004, SPK 013 and *Tainung* 64, developed larger lesions and therefore showed greater susceptibility to *R. oryzae*. In addition, the other eight cultivars did not show any difference in susceptibility to either *R. stolonifer* or *R. oryzae*. Although Clark and Hoy (1994) reported that sweetpotato genotypes are generally more susceptible to *R. stolonifer* than to *R. oryzae,* the results of this study showed that the tested sweetpotato germplasm varied in susceptibility to either of the two *Rhizopus* species. Cultivar variability in susceptibility to *R. stolonifer* and *R. oryzae* exhibited by the tested sweetpotato germplasm shows that it is possible to pursue host resistance as a means of controlling Rhizopus soft rot in some sweetpotato cultivars. While this may be possible in cultivars exhibiting consistent response to infection, it may not work in cultivars that fail to show consistency in their response to infection. There is therefore need to regulate the storage environment for sweetpotato roots since cultivar susceptibility to infection is influenced by the prevailing environmental conditions.

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