REACTION OF SWEETPOTATO GENOTYPES TO *Botryodiplodia theobromae* Pat. and *Macrophomina phaseolina* Tassi

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ABSTRACT

Sweetpotato genotypes were screened for resistance to *Botryodiplodia theobromae* Pat. and *Macrophomina phaseolina* Tassi, the two major postharvest pathogens found infecting sweetpotato roots during storage in the Philippines. The effect of different stages of maturity of sweetpotato plants on host resistance and the nature of postharvest disease development were also investigated, Assessment of 437 genotypes for resistance to *B. theobromae* infection showed that 0.7% and 15% were highly resistant and resistant, respectively. Out of 434 genotypes evaluated for resistance to *M. phaseolina* only 0.2% and 6% were noted to be highly resistant and resistant, respectively. Majority of the genotypes screened against both pathogens were found to be moderately susceptible to susceptible. Roots taken from 3 months old plants were more resistant to infection by either *B. theobromae* or *M. phaseolina* than those taken from 4 and 5 months old plants. The resistant, moderately resistant, and susceptible lesion types were categorized based on visual and histological examinations. *Ipomeamarone* was detected in inoculated resistant, moderately resistant and moderately susceptible sweetpotato roots but not in susceptible roots. No visible formulation of *ipomeamarone* was noted in healthy uninoculated roots.

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KEY WORDS: Sweetpotato. *Ipomoea batatas.* Postharvest pathogen. *Botryodiplodia theobromae. Macrophomina phaseolina* Resistance.

INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is one of the most important root crops in the world. Today, with improved production technology such as the use of high yielding cultivars and appropriate fertilizers and pesticides, more and more sweetpotato roots and their by-products reach an increasing number of households. Along the food line, however, *i.e.*, from the producer to the consumer, a chain of events happen which could adversely affect the quality and quantity of the sweetpotato roots. One of these events, which is practically a major factor contributing to losses in sweetpotato after harvest, is the rapid deterioration of roots due to microorganisms that infect the roots during storage. Some of the most important diseases infect roots within a few hours after digging.

To help conserve the supply of good quality sweetpotato roots various approaches are employed to control postharvest diseases of sweetpotato. Among these are the use of broad spectrum fungicides, such as thiabendazole and benomyl (Ogundana, 1971; Martin, 1971; COPR, 1978), and curing (Avinze *et al.*, 1975). Majority of the marginal root crop farmers, however, consider these control measures impractical and uneconomical so that the use of host resistance becomes a better alternative. This study was, therefore, conducted to identify genotypes of sweetpotato that are resistant to one or both of the major postharvest pathogens. Furthermore, the effect of different stages of maturity of sweetpotato on host resistance and the nature of postharvest disease development were investigated.

MATERIALS AND METHODS

Preparation of test materials

Sweetpotato roots of different genotypes were obtained from the Philippine Root Crop Research and Training Center's germplasm collection. The roots were washed to remove adhering soil and plant debris, air dried, and then, screened for resistance against tuber rot caused by *B. theobromae* and charcoal rot caused by *M. phaseolina*. Screening was done by batch of entries using sweetpotato roots with the same age of maturity, with susceptible and resistant checks maintained in every batch.

Isolation of pathogen and preparation of inoculum

Isolation of fungi by tissue plating described by Tuite (1969) was followed. Sections of tissues taken from advancing edges of rot lesions were surface sterilized with 70% ethyl alcohol for 3 - 5 minutes, blotted dry with sterile filter paper, and aseptically transferred to acidified potato dextrose agar (PDA). Bits of mycelial growth typical of the pathogen arising from the plated tissues were transferred to PDA slants for pure culture. These served as stock culture of inoculum and were maintained until required for screening. Three to four-day old replated cultures of *B. theobromae* and *M. phaseolina* obtained from the pure culture stock served as inoculum source upon screening.

Inoculation and disease rating

Roots harvested 4 months after planting were washed, air dried, then, surface sterilized with 70% ethyl alcohol. From the roots previously blotted dry, holes (6 mm diameter and 8 - 10 mm deep) were made at the midsection using a sterile 6 mm diameter cork borer. A disc of the mycelium cut with the same cork borer from the edge of young fungal colonies growing on PDA was placed into each hole with the mycelium facing upward. The holes were sealed with masking tape. The inoculated roots (five samples for each genotype) were incubated in the laboratory under ambient condition (25 - 32°C).

Disease response was rated 9 days after inoculation based on the internal lesion area using the disease rating scale developed by Palomar *et al.* (1980) and Jenkins (1981) but with modification as follows:

Disease Score	Description
1	*Highly Resistant.* No infection.
3	*Resistant.* Limited rotting extending at most from 1 - 10 mm from the inoculation site; well developed periderm maybe observed bordering the lesions.
5	*Moderately Resistant.* Rotting extends from 11 - 15 mm from inoculation site; developed periderm bordering the lesions.

7 *Moderately Susceptible.* Lesions extend from 16 - 20 mm from the inoculation site.

9 *Susceptible.* Rotting extends more than 21 mm from inoculation site; characterized by rapid decay; complete rotting of roots may occur earlier than 9 days.

Nature of disease development

Representative genotypes showing resistant (V2 - 42 and V15 - 70) and susceptible (VSP 4 and PRS 4) reactions were utilized. The roots were inoculated with *B. theobromae* and *M. phaseolina* and disease development was observed visually and histopathologically at 0 hr, 6 hr, 1 day, 2 days until 9 days after inoculation.

Extraction of ipomeamarone from sweetpotato tissues

The extraction method was a modification of that of Kojima and Uritani (1976). Sweetpotato roots showing resistant, moderately resistant, moderately susceptible and susceptible reaction to *M. phaseolina* and *B. theobromae* 9 days after inoculation were sliced into 2 mm thick blocks. Then, 1 gram each of infected tissues of resistant, moderately resistant, moderately susceptible and susceptible roots were homogenized with chloroform and methanol (1:1%) mixture. The suspension was filtered and the filtrate (20 ml) was mixed with 15 ml water. After vigorous shaking the mixture was set aside until the chloroform layer separated from methanol water layer.

Silica gel thin-layer chromatography

The chloroform soluble fraction from the infected tissue extract was applied to a silica gel thin-layer plate by micropipette and was developed using a solvent composed of n-hexane and ethyl acetate, (8:2 v/v). After development, the location of *ipomeamarone* was detected by spraying Ehrlich's reagent.

RESULTS AND DISCUSSION

Host resistance

Out of 437 genotypes screened 0.7, 15 and 24% showed highly resistant, resistant and moderately resistant reactions to *B. theobromae* infection. The rest exhibited moderately susceptible to susceptible reactions (Table 1). On the other hand, out of 434 genotypes evaluated for resistance to *M. phaseolina*, 0.2% was noted to be highly resistant while 6 and 13% were resistant and moderately resistant, respectively. Majority of the genotypes screened for *M. phaseolina* resistance showed moderate susceptible to susceptible disease responses. Resistance (*i.e.*, highly resistant to resistant) to both *B. theobromae* and *M. phaseolina* were observed in some sweetpotato genotypes like V2 - 42, V16 - 42, V21 - 345, V22 - 56, V22 - 99, V24 - 37, J - 8, J - 19, PRS 886, PRS 906 and Kinabakab.

Table 1. Response of the different sweetpotato genotypes to *B. theobromae* and *M. phaseolina* at 4 months after planting.

Total No. of Genotypes Screened	Pathogen	Disease Response (%)[1]				
		HR	R	MR	MS	S
437	*B. theobromae*	0.7	15	24	30	30
434	*M. phaseolina*	0.2	6	13	28	52
433	*B. theobromae* and *M. phaseolina*	0.2	2	6	10	22

[1]HR = Highly Resistant, R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible, S = Susceptible.

Effect of stages of maturity on resistance

Genotypes of sweetpotato roots harvested at 3 months after planting and inoculated with *B. theobromae* and *M. phaseolina* showed significantly lowe, infection than those harvested at 4 and 5 months (Table 2). At 4 and 5 months from planting most of the genotypes were susceptible to the attack

Table 2. Average reaction of sweetpotato roots infected with *B. theobromae* and *M. phaseolina* at different stages of maturity. Disease rating was taken 9 days after inoculation.

Stage of maturity (mo)	Pathogen[1]	
	B. theobromae	*M. phaseolina*
3	2.10a	3.20a
4	3.83b	4.04b
5	3.90b	4.40b

[1]In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

by *B. theobromae* pathogen (Table 3). Only V21 - 255 remained resistant at all ages tested. With *M. phaseolina* infection, most of the genotypes that exhibited resistance at 3 months old became moderately susceptible to susceptible at 4 and 5 months old (Table 4). Similar results were obtained by Sardsud (1979) who explained that the presence of more toxic substances or low level of carbohydrate content in younger tissues do not favor fungal growth. Delayed harvesting may decrease enzyme activity and formation of toxic substances and hence increased infection. Sardsud's (1979) report corroborated the findings of Grainger (1968) who stated that if the host has an adequately high exploitable level of carbohydrate a fungal or bacterial pathogen can easily attack it. But if the Cp (weight of total carbohydrate in the whole plant)/Rs (carbohydrate free, residual dry weight of the shoot) of the host is sufficiently low, pathogen cannot attack it. This contention is well illustrated by considering the changing degree of proneness to disease of potato plants at different stages of development. Young plants and those approaching maturity are regarded as subject to severe attacks by *Phytophthora infestans,* but Grainger (1969) reported that there is a period in mid-growth when the fungus does not infect or attacks only very slightly even when inoculum is present and external condition is suitable for infection. He attributed such disease reaction to physiological factors operating within the host and which changes during the period of growth of the individual plant.

Table 3. Reaction of the roots of different sweetpotato genotypes harvested at different stages of maturity to *B. theobromae* infection.

Genotypes	Disease rating at different stages of maturity[1]		
	3 MAP[2]	4 MAP	5 MAP
V21 -255	2	2	2
V11 -206	2	2	3
PRS 121	2	3	3
V10 - 16	2	3	4
V14 - 64	2	3	4
V26 - 85	2	3	4
V36 - 46	2	3	4
V11 -256	2	4	4
V12 -123	2	4	4
V11 -398	2	4	5
V14 - 38	2	5	3
V5 - 26	2	5	4
G145 - R4	2	5	4
V23 - 86	2	5	5
V22 -263	2	5	5
PRS 12	2	5	5
PRS 113	2	5	5
V15 - 20	2	5	2
V24 - 37	3	3	2
V26 -301	3	4	4
PRS 127	3	3	4
V11 -194	3	3	5
V21 -345	3	4	4

[1]Average of five replications. Disease rating: 1 = highly resistant, no infection; 2 = resistant, rotting extends from 1-10 mm from inoculation site; 3 = moderately resistant, 11-15 mm; 4 = moderately susceptible, 16-20 mm; 5 = susceptible, greater than 20 mm.

[2]Months after planting.

Table 4. Reaction of the roots of different sweetpotato genotypes harvested at different stages of maturity to *M. phaseolina* infection.

Genotypes	Disease rating at different stages of maturity[1]		
	3 MAP[2]	4 MAP	5 MAP
V10 - 16	2	4	5
V15 - 20	2	4	3
PRS 121	2	4	4
V21 - 345	3	4	3
V14 - 64	3	3	4
V24 - 37	3	3	4
PRS 125	3	3	4
V11 - 194	3	3	5
V11 - 206	3	3	5
V5 - 26	3	5	5
V11 - 398	3	5	5
V26 - 301	3	5	5
V12 - 123	5	4	4
V26 - 85	5	4	5

[1]Average of five replications. Disease rating: 1 = highly resistant, no infection; 2 = resistant, rotting extends from 1 - 10 mm from inoculation site; 3 = moderately resistant, 11 - 15 mm; 4 = moderately susceptible, 16 - 20 mm; 5 = susceptible, greater than 20 mm.

[2]Months after planting.

Nature of disease development

Three types of lesions were observed in sweetpotato roots inoculated with *B. theobromae* and *M. phaseolina* depending upon their reaction to the pathogen. These were found to be similar to those reported earlier by Sardsud (1979), Jenkins (1981) and Dalisay *et al.* (1978).

The first type of lesion observed in susceptible roots was characterized by rapid rotting of tissues in which lesion extended to a distance of approximately 70 mm from the inoculation point in 3 days. The infected roots were totally damaged in 4 to 6 days and the affected tissues became light brown in color, soft and water soaked. Histological observation showed

the invasion of the fungi either intercellularly or intracellularly with the mycelia of the pathogens spreading faster and causing the tissues to become disintegrated. Mycelia were always present on the infected tissues. No sign of dark-colored cells was observed ahead of mycelia.

The second type of lesion as exhibited by moderately resistant roots was distinguished by the slow increase in internal diameter of lesions. The infected tissues turned brown and delineated from the healthy tissues by a smooth margin. In some roots, the diameter of the lesion continued to increase slowly over a certain period of time and eventually the roots became totally infected. In other roots, however, no further increase in lesion areas was noted. Histological examination revealed that fungal mycelia penetrated slowly and cells collapsed ahead of them. The penetrated cell walls were observed to be suberized as it was stained with red safranin-fast green combination.

The reaction observed in resistant roots characterized the third type of lesion. This type of lesion exhibited an apparent necrosis of the cells and formation of wound periderm that restricted infection at the point of inoculation. The infected tissues turned white and delimited by a narrow brown zone with irregular margins. When infected tissues were examined under the microscope it was noted that the pathogens penetrated slowly and intercellularly through the cork cells reaching to only a few millimeters into host tissues. Several layers of cortical cells close to the periderm collapsed ahead of mycelial penetration. This implies that the fungi could produce extracellular enzymes and/or toxic substances sufficient enough to diffuse ahead of mycelia. Mycelia were found restricted only at the cork layer. This might be due to the effect of antifungal substances produced by the healthy cells adjacent to the collapsed cells in response to the stimulation of the enzyme or toxic substances secreted by the fungus (Norkrans, 1963; Stahman *et al.*, 1966; Akasawa and Uritani, 1961).

Role of ipomeamarone in sweetpotato resistance

The presence of furanoterpene in healthy and inoculated sweetpotato roots were determined using thin layer chromatography (TLC). Results showed the presence of *ipomeamarone* in inoculated resistant, moderately resistant and moderately susceptible sweetpotato roots but none in susceptible roots. In healthy uninoculated roots, no visible formation of *ipomeamarone* was noted.

The findings of some investigators strongly support this observation. For instance, Kojima and Uritani (1976) reported that furanoterpenoid compounds are not contained in the fresh tissue of sweetpotato but is produced in the tissue in response to infection after one day period. In addition, Martin *et al.* (1978) revealed that resistance to *B. theobromae* is correlated with *ipomeamarone* accumulation. *Ipomeamarone*, a major component of furanoterpenoid substances is a kind of phytoalexin and may have a fungistatic activity in the infected tissues (Uritani, 1965). Hence, it is possible that the accumulation or absence of furanoterpenoid substances may be responsible for the different reactions or types of lesion distinguished in this investigation.

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