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Implication of variation in aggressiveness of *Phytophthora infestans* (Mont.) *de Bary* isolates found in Kenya to potato production

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Abstract. A survey was carried out to determine the incidence and severity of late blight in four major growing potato districts. *Phytophthora infestans* isolates were found to have varying levels of aggressiveness as indicated by their reaction on tubers and detached leaves. The variability of ten different isolates of *P. infestans* was evaluated by their ability to cause disease on both detached leaf and tubers of different potato genotypes. Isolate 023 had the largest lesion diameter of 35.67mm on leaves of Kerr's Pink variety and this was significantly ($P \leq 0.05$) higher than all the other lesion diameters recorded on other isolates. Isolate 032, with a lesion diameter of 13.33mm, was the only isolate regarded as virulent on the leaves of variety Kenya Sifa. There were no isolates regarded as virulent on variety Kenya Rutuku. On tuber rot, isolate 008 was the most virulent with a diameter of 16.04mm. Isolate 035 was considered least virulent with a mean diameter of 10.04mm. Isolate 008 was the most virulent isolate with a diameter of 16.04mm. Cultivation of some popular varieties like Kerr's Pink has become impossible, as the variety is highly susceptible to the disease due to the emergence of more aggressive strains. These developments also imply that the late blight management has to be broadened to include tubers reactions to *P. infestans* in order to avail farmers varieties that meet their requirements.

Introduction

Potato is a major food crop in Kenya with an annual production of 500,000 tonnes on acreage of 106,000 ha (Ng'ang'a *et al*., 2001). Late blight of potato caused by *Phytophthora infestans* is the most important single factor influencing potato production in Kenya and causes significant yield losses (Olanya *et al*., 2001). For the last sixty years, the search for potato varieties with high yields, resistance to late blight, good agronomic traits and acceptable post harvest qualities was of high priority in the potato programme (Lung'aho *et al.*, 1997). Numerous potato varieties have gone through the breeding programme and have been released to the farmers based on their tolerance to late blight. However, despite cultivation of varieties with resistance to late blight, the disease continues to devastate the crop. At times when the rainfall is high coupled with cool temperatures, the disease can be extremely high even on the most tolerant varieties leading to complete loss of the crop.

The prevalence of late blight epidemics in Kenya has prompted efforts to study the structure of this fungus. The characterization of the fungus has revealed that it is of the mating type A1 (US-I-clonal lineage) based on restriction fragment length polymorphism (RFLP) (Vega-Sanchez *et al.*, 2000). Varying sensitivity to metalaxyl has also been reported

(Olanya *et al.*, 2001). Similarly, different abilities to produce oospores, which are an important character in evolution of diverse strains of the fungus, have also been reported (Hohl, 1998). The population changes have resulted in reduction of crop yields and harvested crop losses due to tuber rots. These events have prompted the efforts to carry out this study to determine the variability of aggressiveness of *P. infestans* isolates from major potato growing areas. In addition, information of the responses of potato cultivars to the *P. infestans* could determine the potential impact of a new pathogen population on potato production.

Materials and Methods

Survey for late blight incidence and severity. Four major potato growing districts were visited in August-September 2001 in order to determine the incidence and severity of late blight. Incidence was scored as the general number of plants showing symptoms in a field while severity was scored as the percentage total foliage affected by blight.

Sample collection and laboratory isolation of *Phytophthora infestans*. Samples of potato leaves infected with late blight pathogen were collected from major potato growing areas of Kenya. This was done in Nyandarua and Nakuru districts on 6-8th August 2001, a total of 54 samples were obtained. Meru area was covered in September 2001, with 20 samples being collected. Potato leaf samples infected with late blight were obtained in fields 5-10 km apart. The samples were put in polythene bags, carefully labeled and placed in a cooler box to maintain freshness and to keep the pathogen alive. The samples were transported to the Plant Pathology Laboratory at the National Research Laboratory (NARL) for isolation of the pathogen.

Laboratory isolation. Isolation was done using the procedures described by Forbes (1997). Infected leaf sections were washed with tap water to remove soil particles, surface sterilized in alcohol for five minutes and then with 5% calcium hypochlorite for 5 minutes and finally rinsed with four changes of sterile distilled water.

Meanwhile potato tubers were washed and dried to remove excess water and flamed in alcohol. Using a sterile knife, sections of approximately 2 mm were obtained from the tubers. The potato slices were placed in sterile Petri-dishes lined with sterile moistened blotter. The leaf sections were slotted under the potato slice and the set up left for a period of 4-7 days to allow the fungus to grow through the potato slice. Once there was substantial mycelial growth, mycelia from the surface were aseptically transferred to selective V8 media (Forbes, 1997). The plates were incubated at 15-20º C for 10 days. Isolations were repeated until pure cultures were obtained. A total of 40 isolates were obtained.

Storage of cultures. Molten selective V8 medium was prepared and poured in universal bottles placed at an angle to form slants. Each pure isolate was allowed to grow to cover the surface of the slant after which the cork was tightened and stored at 4ºC. Little mycelia were obtained periodically for sub culturing as required.

Variation in aggressiveness of *P. infestans* as indicated by their abilities to cause pathogenicity on detached leaves. Pathogenicity tests were carried out in 4-6 weeks time when the host plants were ready for inoculation. Five potato varieties, namely Tigoni, Kenya Sifa, Kenya Mavuno, Dutch Robyjn and Kerr's Pink with varying sensitivity to late blight were used. Leaves of the test plants were detached and washed with sterile distilled water for about 20 minutes and blotted with paper towels to remove excess moisture. The leaf bases were covered with cotton wool to avoid desiccation.

The leaves were placed upside down on 9cm plastic Petri dishes containing moistened filter paper. Sporangial suspensions obtained from actively growing cultures were adjusted

to 10,000 sporangial/ml. Three drops of 10µl of the sporangial suspension were applied to the mid-rib. The experimental design was complete random design (CRD) with each treatment replicated four times. Each experiment was repeated three times. Incubations were done at 17-20ºC for 7 days. The diameters of lesions were measured. An isolate was regarded as being virulent if the lesion diameters were more than 10mm. The data were pooled and subjected to analysis of variance using the SAS Programme (SAS, 1997).

Variation in aggressiveness of *P. infestans* as indicated by their abilities to cause tuber rot. Tuber rot studies using isolates collected across Kenya during the year 2001 were also performed. The method of inoculation was adopted from Peters and Platt (1999). The tubers used in the month of December 2001 had been harvested during the month of October in the same year. The tubers were stored at room temperature (18-23ºC). The isolates had been previously subcultured on V8 and stored at 4ºC. The experimental design was complete random design (CRD) with each treatment replicated three times. Each experiment was repeated three times. The isolates were allowed to grow for 10 days at 15ºC on V8. Sterile distilled water was added to cultures and the mycelial growth scrapped slowly using a glass rod. Inoculum was diluted to concentration of 10,000 sporangia/ml using a haemacytometer. The inoculum was allowed to stand at 10ºC for 1hour to encourage release of zoospores prior to use in tuber inoculations. Tubers of different varieties (Tigoni, Asante, Dutch Robin, Kerr's Pink, Kenya Sifa and Kenya Mavuno) were used in this experiment. The tubers had been harvested a month prior to inoculation. The medium sized tubers (2 inches in diameter) were washed gently to remove adhering soil and punctured at the bud end using a cork borer. The potatoes were dipped in a suspension of individual strains of *P. infestans* for 3 minutes. The tubers were incubated in darkness at 15ºC for 21 days in clear plastic paper bags on which holes had been punctured to allow air circulation. After three weeks, the tubers were cut longitudinally and the length of tuber necrosis measured. The data were pooled and subjected to analysis of variance using the SAS Programme (SAS, 1997).

Results

Incidence and severity of late blight. Results of disease levels are shown in Table 1. The incidences and severity ranged between 10 - 85.5% and 5 - 91%, respectively. Nakuru district had the highest disease pressure while Meru had the lowest.

Studies on variation in aggressiveness of *P. infestans* on detached leaves. Results of aggressiveness of isolates on detached leaves are shown in Table 2. Different isolates expressed varying degree of aggressiveness on different cultivars. With the exception of isolates 028 and 029, the rest were very aggressive on variety Kerr's Pink. Isolate 023 had the largest lesion diameter of 35.67mm on Kerr's Pink and was significantly ($P \leq 0.05$) higher than all the lesion diameters recorded caused by other isolates. Isolate 032 with a lesion diameter of 13.33mm was the only virulent isolate on variety Rutuku. There were no virulent isolates on variety KP 90131.10. Isolates 023, 028, 034, 035 and 047 were virulent on Tigoni. Overall, isolate 023 caused the largest mean lesion diameter of 19.20mm, while 029 exhibited the least virulence with a mean diameter of 3.13mm.

Table 1: Incidence and severity of late blight in July 2001.

	Incidence (%)	Severity (%)
Kiambu	65	48
Nyandarua	65	54
Nakuru	85.5	815
Meru	-	5
Mean	66.3	46.8

Table 2: Lesion diameter (mm) on detached leaves different potato genotypes caused by varying isolates of *P. infestans.*

Isolate	Kerr's Pink	K. Sifa	K. Mavuno	Tigoni	D. Robyjn	Mean
008	11.00 d	1.00 d	6.67 ab	5.67 ef	6.00 d	6.07 c
023	35.67 a	9.00 b	5.67 b	10.33 cd	35.33 a	19.20 a
028	5.67 e	1.67 d	1.33 d	11.67 c	3.67 ef	4.80 d
029	3.67 e	3.67 d	1.00 d	4.67 ef	2.67 ef	3.13 d
032	10.33 d	13.33 a	8.67 a	7.00 e	11.33 c	10.14 b
034	24.00 bc	2.67 d	3.33 c	20.67 a	17.33 b	13.40 b
035	25.00 b	2.33 d	7.67 a	17.33 b	11.33 c	12.73 b
036	10.33 d	8.33 b	2.00 d	5.00 ef	7.67 d	6.68 c
047	26.67 b	8.33 b	2.00 d	13.67 c	24.00 a	14.93 b
Mean	19.93	5.59	4.26	10.67	13.15	
LSD	2.67					
CV %	20					

Greater than 10.00 mm are regarded as being virulent.

Table 3: Lesion diameter (mm) on tubers of different potato genotypes caused by varying isolates of *P. infestans.*

Isolate	Kerr's Pink	K. Sifa	K.Mavuno	Tigoni	D. Robyjn	Asante	Mean
008	18.5a	16.75a	20.00a	17.25ab	15.75a	8.00c	16.04ab
014	12.00b	13.25bc	15.50b	14.00bc	15.75a	13.00ab	13.92bc
023	16.75a	15.00ab	15.25b	14.25bc	14.75a	14.25a	15.04ab
029	15.50a	17.00a	20.00a	17.00ab	9.25b	15.75a	17.75a
032	14.25bc	14.50ab	14.25b	20.25a	12.00ab	12.00b	15.54ab
034	11.50c	12.25bc	11.50bc	17.50ab	10.25b	15.75a	13.13bc
035	10.00d	10.00cd	13.75b	9.25d	11.00b	6.25c	10.04c
036	15.00b	13.50bc	16.25b	13.75bc	15.00a	15.50a	14.83ab
047	15.50a	11.75c	14.25b	15.75b	11.00b	9.25b	12.92bc
Control	0.88e	0.88e	0.88cd	0.88e	0.88c	0.88d	0.88
Mean	12.99	12.49	13.01	13.99	10.68	11.01	
LSD	3.5						
CV %	46.1						

Means in the same column with same letter not significantly different.

Variation in aggressiveness of *P. infestans* on tubers. Results on tuber effect on aggressiveness of isolates is shown in Table 3. Isolate 029 was the most virulent isolate with a diameter of 17.75mm. Isolate 035 with a mean diameter of 10.04mm was the least virulent.

Discussion and conclusion

The history of late blight in Kenya dates as early as the turn of the previous century where the pathogen appears to have been introduced with the introduction of potato crop. The disease incidences and severity

Table 4: Relationship between reaction of isolates on leaves and on tubers.

	Correlation factor	(P≤0.05)
Leaf lesion vs Tuber rot	0.0034	Ns

The correlation value between pathogenicity on leaves and tuber rot was positive but not significant at (P≤0.05) (Table 4).

ranged between 10-90% and 5-85.5%, respectively. Nakuru district had the highest disease pressure while Meru had the lowest. The disease development is greatly influenced by the prevailing weather conditions. Nakuru district was characterized by high rainfall and low temperature at the period this study was carried out. In Meru at this time of the study the weather conditions were unfavourable for blight development explaining the low disease pressure.

The *P. infestans* isolates were found to vary in aggressiveness as indicated by their reaction on tubers and detached leaves. This variation can be explained by the differences in the genetic make up of the isolates. The variation in aggressiveness of *P. infestans* could result from oospore production that has been reported in mating and selfing tests in culture (Hohl, 1998; Mukalazi *et al*., 2001, Kedera *et al.*, 2002). In selfing, an isolate can act as a male or female during heterothallic hybridization. Extraneous factors have been reported to induce oospore production in culture. Grooves and Ristaino (2000) reported high oospore numbers in cultures amended with fungicides. Cultures amended with Ridomil 2E and Ridomil Gold 2E caused particularly high numbers of oospores. In Kenya, Dithane M45 and Ridomil are the most widely used fungicides in potato. The use of fungicides could probably have caused the emergence of more aggressive *P. infestans* strains compounding the management of late blight.

Management of late blight has been addressed by the use of late blight tolerant varieties (Landeo, 1996). While these are likely to contribute significantly to integrated control of this disease, it is important to note that the stability of their resistance is dependent on the aggressiveness of the *P. infestans i*solates. The Kenyan populations of *P. infestans* have evolved rapidly since the introduction of potato cultivars with more late blight tolerance genes. The US-1 genotype of *P. infestans* presently in Kenya is evolving to produce strains that are more aggressive with high insensitivity to metalaxyl creating new challenges in late blight control.

Pathogenicity tests and infection of tubers by different *P. infestans* isolates revealed significant differences in cultivar response. This observation is in agreement with reports by (Lapwood, 1965). Aggressive isolates are reported to have a lower latent period and a high sporulation index thus enhancing fitness for survival (Peters and Platt, 1999). Several isolates were virulent on varieties Tigoni and Asante implying that these two varieties are succumbing to late blight due to the emergence of aggressive biotypes. The varieties were released in the year 1997 based on the high level of foliar resistance to blight, high yields and superior processing qualities (Walingo *et al*., 2002). Across cultivars, Tigoni although with high foliar resistance had the highest value of tuber rot. The variety is late maturing and therefore does not meet farmers early demand for food. The farmers being commercially oriented would like to capitalize on high prices before the glut period and therefore the variety is harvested immaturely (Walingo *et al*., 2002). It is therefore prone to mechanical damage in which the pathogen can enter and initiate rotting during storage as well as in transportation to long distance markets. Most farmers use the forked jembe and the ox-drawn ploughs to uproot the mature tubers (Ng'ang'a, personal communication) resulting in mechanical injuries to the tubers that further facilitate the penetration of *P. infestans.* The emergence of these highly aggressive isolates may significantly increase tuber losses in storage.

The variety Kerr's Pink and Dutch Robyjn exhibited high levels of susceptibility to all *P. infestans* isolates confirming reports by other workers (Lung'aho *et al.*, 1997). These varieties in contrast revealed high tuber resistance to late blight. Tuber resistance can be attributed to both physical and chemical means. Schoer (1980), while working with tuber resistance in different potato cultivars, reported that synthesis of a suberin wall around the inoculated portion of the tuber inhibits further proliferation of the mycelia. For biochemical reactions, phenolics, glyalkaloids and phytoalexins are produced in higher quantities in varieties with high tuber resistance. These varieties can therefore be stored for considerable periods even with the emergence of virulent strains of *P. infestans*. They are popular with processors for making into crisps as they have high dry matter content and can be stored for long periods (Walingo *et al*., 1997; Ng'ang'a *et al*., 2001). The varieties also tuberize early therefore could escape from build up of inoculum later in the growing season. In addition, being early maturing, the varieties are able to offer food to the farmers as well as capture high prices before the market is flooded. In times of low rainfall, farmers are able to obtain reasonable yields as the varieties are able to utilize limited quantities of water. The emergence of virulent strains also means that the farmers will increase the dosages of fungicides or reduce the spray intervals. Both of these are likely to have detrimental health and environment effects. Furthermore, the increased cost involved in potato production will also impact negatively on the gross margins. The population shifts to more aggressive pathotypes of *P. infestans* with increased insensitivity to metalaxyl has compounded late blight management strategies previously developed.

Between the two newly released varieties, Kenya Sifa and Kenya Mavuno, only one isolate (032) was virulent on Kenya Sifa. These cultivars were released based on high level of foliar resistance to late blight, good yields and superior processing qualities. The variety Kenya Sifa also has high tuber resistance to blight. The lack of correlation between foliar and tuber resistance imply that cultivars with high foliar resistance may not necessarily have high resistance in the tubers. These findings were in agreement with those reported by various workers (Plat and Tai, 1998; Stewart *et al*., 1994). The renewed efforts to breed for late blight resistance against emerging virulent strains have unfortunately focused on foliage resistance (Sikka, 2001; Lung'aho, 1997) and underestimated the role of tuber resistance (Platt and Thai, 1998).

Recommendations

The pathogen population shifts to more aggressive pathotypes of *P. infestans* has compounded late blight management strategies previously developed. The tuber is the marketable portion of the potato and emergence of more aggressive strains of *P. infestans* that in addition infect tubers which imply that losses will extend to ware and seed potato stores. It is of paramount importance that in search of cultivars with high resistance, both foliar and tuber responses towards *P. infestans* are considered.

The natural migration of the US-8 (A2) lineage is expected to reach the African region in the near future and strategies to keep out these aggressive strains should be put in place. Continuous studies of the population changes of *P. infestans* should be done to monitor the presence of A2 mating type. Authorities monitoring importation of plant materials should prohibit the importation of potato tubers from regions where the A2 mating type is predominant in order to safeguard the potato industry in Kenya.

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