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Effect of Host Resistance on Foliar Late Blight Severity, Disease Development and Progress on Selected Irish Potato Varieties in Kenya

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Authors' contributions

This work was carried out in collaboration among all the authors. Author MOM designed the study, wrote the protocol, gathered the initial data and performed preliminary data analysis. Authors JN and JKM being supervisors, managed the literature searches, anchored the field study, interpreted the data and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Late Blight Disease, caused by the fungal pathogen, Phytophthora infestans, is a major constraint of Irish potato production in Kenya. The disease can destroy a crop, causing up to 100% yield loss. Small scale holder farmers in Kisii County continuously grow Irish potato that is susceptible to P. infestans, which require a number of fungicide sprays. This study was formulated out of the realization that Irish potato plays a major role in food security and contributes to poverty alleviation. Also, the commonly used protectant fungicides for late blight disease are expensive, hazardous and are not effective against P. infestans.

Aim: To evaluate the effect of host resistance to foliar late blight severity, disease development and progress on selected potato varieties under field and glasshouse conditions in Kisii County, Kenya.

___ **Study Design:** Nine potato varieties (Tigoni, Meru red, Kenya mpya, Sherekea, Shangi, Purple gold, Asante, Mang'ere and Nyayo) were grown in the field and glasshouse following a randomized

complete block design.

Place and Duration of the Study: This research was carried out in Kisii County, Kenya between January 2015 and September 2015.

Methodology: Late blight infected Irish potato leaves were collected from the field. Field symptoms of late blight were used to identify the diseased leaves. In the laboratory isolation and identification was done using culture and microscopic techniques. Phytophthora infestans inoculum was prepared using pure culture, standardized to $1x10^7$ sporangia/ml concentration using haemocytometer. Pathogenicity test was done to confirm pathogenicity of the pathogen. Ten millimetres of the inoculum was sprayed on each healthy potato plant. Disease development and progress was monitored, and the area under disease progress curve was used to score the severity of late blight progress on various potato varieties. The relative area under disease progress curve was also used to compare data from different experimental plots. Data collected was analysed by analysis of variance using GENSTAT directive Version 12.0. Treatment means were separated using significant difference test at P<.001.

Results: Irish potato varieties from various experimental plots showed varied reaction to 1x10⁷ sporangia/ml concentration P. infestans inoculum in both the field and glasshouse. Late blight severity differed significantly among various Irish potato varieties. The results showed that Sherekea had better tolerance of 7.0 (0.003) in the glasshouse and 468.0 (0.223) in the field to late blight. Mang'ere showed high susceptibility of 630.0 (0.3) in the glasshouse and 2093.0 (0.997) in the field to late blight. The major finding was that Sherekea can yield despite being infected by late blight disease.

Conclusion: Late blight disease remains a serious threat to Irish potato production causing significant yield and economic losses to farmers. It is suggested that growing Sherekea variety could significantly reduce late blight yield losses.

Keywords: Potato variety; P. infestans; host resistant; severity; Kisii County; Kenya.

1. INTRODUCTION

The Late Blight (LB) disease is caused by a fungus, Phytophthora infestans. Irish potato Blight is one of the most devastating plant diseases. The disease destroyed Irish potato (Solanum tuberosum L) crop and led to mass starvation. In the great Irish famine of 1845 to 1847, up to one million people died and similar number of people emigrated to the rest of Europe and United States of America. It was not until 1861 that Antony de Barry, who was considered the father of plant pathology, that the question of the cause of blight was finally settled [1]. Irish potato was introduced to East Africa by British farmers in the 1880s. It was first introduced in Kenya by Irish missionaries, for whom this was the staple food hence the name "Irish potato" [2]. In Kenya, Irish potato had been grown for more than half a century before blight was first observed and reported in 1941 [3]. Irish potato can easily be grown and provides nutritious food faster on less land than any other food crop. Potato plays a major role in food security and contributes to poverty alleviation through income generation and employment creation. It is mainly grown by smallholder farmers as a cash crop [3,4]. The major constraint of potato production is the late blight

disease [4,5]. Planting potato varieties that are susceptible to P. infestans has contributed to yield losses. Late blight is one of the diseases that can destroy a crop causing up to 100% yield loss [6]. Phytophthora infestans pathogen belongs to the kingdom chromista and has different biochemical pathways from the true fungi. Therefore, many fungicides are not effective against this pathogen. Also, the humid and wet conditions under which P. Infestans thrives makes it difficult for the pathogen to be controlled by protectant fungicides [7]. The diversity of P. infestans in Kisii County has been aggravated by presence of alternative hosts including tomatoes (Lycopersicon esculentum), peppers (Capsicum spp), black night shade (Solanum nigrum), a number of weeds such as Sodom apple (Solanum incanum) and small land holdings, which has led to impracticable crop rotations [8]. The prevailing weather conditions favours P. infestans spores germination and development on foliage. During humid weather, whole plants may be attacked by P. infestans and killed in few days [6,9]. Due to the variability and emergence of new strains of P. infestans, there is regular resistance breakdown among commonly grown potato varieties, hence the need to develop potato varieties that are more resistant [1,10]. Late blight of potato is a serious

production constraint in the major potato growing regions of Kenya. Except for the two varieties (Tigoni and Asante) released in 1998, most of the other varieties including farmers' types are susceptible to LB [6]. Lack of quality seeds of potato varieties, which are resistant to P. infestans, is a major problem adversely affecting the expansion of potato production in Kenya [3,4,11]. Although fungicides are available to control the disease, they are expensive, environmentally unfriendly, and pose serious health hazards to smallholder farmers, who grow most of the crops in Kenya [4]. The cultivation of resistant varieties of potato reduces the amount of fungicide sprays used to control late blight hence minimising environmental pollution and chronic health problems. Cultivation of potato varieties tolerant to P. infestans is the most sustainable approach for the management of late blight and for tackling food insecurity [12]. This study was formulated out of the realization that late blight is rampart in Kisii County, and the many methods available for controlling the disease are not effective because of the susceptible Irish potato varieties grown. It is suggested that, use of tolerant varieties could significantly reduce late blight yield loss problem and this would enable farmers to get better yields. To reduce the information technology gap about potato varieties, there is need to identify varieties with high level of tolerance to late blight.

2. MATERIALS AND METHODS

Kisii County has red volcanic soils rich in organic matter which supports growth of potatoes. It exhibits a highland equatorial climate with a bimodal rainfall pattern with an average annual rainfall of 1500 mm, with long rains between February and June, and short rains between September and November. The months of January and July are relatively dry. The area has maximum day time temperature range of 21° -30 \degree C and minimum temperature range of 15 \degree C -20℃ [13]. Late blight diseased plant materials were collected from infected potato grown plots in the County. The P. infestans were isolated, identified, pure culture was prepared, and pathogenicity tests done on potato test plants. The P. infestans inoculum was inoculated in the experimental plots in the field and glasshouse.

2.1 Laboratory Experiments

2.1.1 Isolation

The late blight infected Irish potato leaves from the field were washed under running tap water. They were surface sterilised by dipping in 70% ethyl alcohol for 30 seconds and then gently blot dried on paper towel for quick removal of alcohol. Petri-dishes were sterilised in an autoclave at 121°C for 20 minutes, cooled to 50-60°C and then dried in the lamina flow cabinet for 30 minutes. 117 gms of potato dextrose agar was added into 3 litres of sterile distilled water. It was autoclaved at 121° for 15 minutes. The potato dextrose agar was cooled to $50-60\degree$ before pouring into the petri-dishes. This yielded hundred petri-dishes. Thirty millimetres of sterile potato dextrose agar were added to each dry petri-dish and solidified in the lamina flow cabinet for 2 hours. Small fresh and recently infected leaf pieces from the edge of actively growing lesion were cut off using a sterilised scalpel and five cut pieces were transferred to each petri-dish containing solidified potato dextrose agar. They were then incubated at 18°C in a germination chamber for 14 days. Sporulation was examined and P. infestans spores were isolated and identified [7,14].

2.1.2 Preparation of pure culture

The identified P. infestans spores were isolated and transferred to a fresh sterile potato dextrose agar. It was then incubated at 18°C in a germination chamber for 14 days, sporulation was examined, and P. infestans spores were isolated for preparation of inoculum [7,14,15].

2.1.3 Identification

The infected leaves in the laboratory were identified based on the symptoms of late blight [16]. Sporulating P. infestans in the petri-dishes were also identified based on branching of the sporangiophores on which the sporangia were borne. The characteristics of the mycelium, and whether or not the culture produced spores assisted in identification. The mycelium and spores were isolated, mounted on a microscope slide and were observed [7,15].

2.1.4 Preparation of inoculum

The sporulating petri-dishes were flooded with 10 ml distilled sterile water and spores were harvested [7]. Serial dilutions were made until a concentration of the inoculum of $1x10⁷$ sporangia/ml in a fresh pure culture was obtained. The inoculum was standardized using a haemocytometer, and it was inoculated to the field and glasshouse experimental plots.

2.1.5 Pathogenicity tests

The P. infestans inoculum prepared with $1x10⁷$ sporangia/ml concentration, and the inoculum was inoculated into healthy Irish potato test plants using hand pump sprays [7]. The Irish potato plants were observed for symptoms on the $7th$ day after inoculation [16]. The P. infestans were isolated from the inoculated potato test plants, cultured and microscopically identified. Two and half litres of inoculum with $1x10⁷$ sporangia/ml concentration was prepared. The standardized $1x10^7$ sporangia/ml P. infestans inoculum was inoculated to the field and glasshouse experimental plots.

2.2 Glasshouse Experiments

The soil which was used in the glasshouse was sterilised in a steam steriliser at a temperature of 121°C before planting in the glasshouse. The 10 kg cooled soil was placed in the pots measuring 22 cm in diameter and 24 cm in height. Diamonium phosphate (DAP 18:46:0) fertilizer was applied at the rate of 10 gm per hole and was mixed thoroughly with the soil. There were nine Irish potato varieties (Tigoni, Meru red, Kenya mpya, Sherekea, Shangi, Purple gold, Asante, Mang'ere and Nyayo) which were under investigation. For each variety three plants were inoculated, and this was replicated three times. For each variety one control plant was not inoculated. The 10 ml^{-1} volume inoculum of $1x10⁷$ sporangia/ml concentration prepared was sprayed into each health potato plant using hand pump sprayer in the experimental plots on the $20th$ day after crop emergence, at this time 8 leaves had fully expanded. Inoculated plants were regularly observed at 3-5 days interval. A total of 81 leaves per replication were evaluated for late blight disease severity in the glasshouse experimental plants. At the onset of late blight symptoms, disease severity in all the inoculated plots was quantified weekly using the area under disease progress curve (AUDPC).

2.3 Field Experiments

The field was selected for establishing Irish potato varieties. Primary cultivation was carried out deeply to remove all couch grass weeds. Harrowing was done and medium tilth was obtained. The experimental plots were laid out in a randomized complete block design with nine treatments in three replications. It was demarcated into small plots measuring 1.5 m long by 0.6 m wide for each treatment. The distance between the rows (ridges) was 75 cm, and seed tubers within each row were planted 30 cm apart. Planting holes were made at a depth of 10 cm. DAP fertilizer was applied at the rate of 10 gm per hole and was mixed thoroughly with the soil. Potato seeds which were obtained from Kenya Agriculture and Livestock Research Organization (KALRO) Tigoni and Agricultural Development Corporation (ADC) Molo, and locally available seeds were used for planting at KALRO-Kisii field site. The sprouted potato seeds, measuring approximately 3-6 cm in diameter, were arranged with rose-end facing upwards and the heel-end downwards. Each planting hole was well covered with light soil. Guard rows were established round the experimental plots. Two hundred and fourthy three seed tubers were established in both inoculated and uninoculated Irish potato plants. The uninoculated plants were sprayed with dithane fungicides while the inoculated plants were unsprayed. Weeding and earthing up were done regularly during the growth period of the crop following recommended practices [17]. Uninoculated experimental plots were also established using a randomized complete block design in three replications and nine treatments. A total of 243 leaves per replication were used to evaluate late blight severity in the field experimental plots. The pure P. infestans culture isolated was diluted to $1x10^7$ sporangia/ml concentration. This inoculum was inoculated into healthy potato test plants using hand pump sprays. The potato plants were observed for the symptoms [16] on the $7th$ day after inoculation.

2.4 Assessment of Late Blight Development and Progress

Reaction of the late blight to potato varieties was assessed visually as percentage infected leaf area and calculated using trapezoidal integration of area under disease progress curve, and records were made [18,19]. Symptoms of late blight were observed regularly. Three potato leaves from each plant which showed late blight symptom were tagged to measure LB expansion. Leaves at the base of the stem from each Irish potato plant were not evaluated for late blight severity. The percentage of infected leaf area was estimated visually as soon as the symptoms were noticed and were recorded after every seven days because the LB progressed faster. Recording of infection measurement was stopped when there was no more lesion expansion, or when susceptible varieties were near total destruction.

Since susceptible varieties cannot get more infected, the infection of more resistant varieties will tend to catch up. The first two infection percentages recorded were added. The result was divided by two and an average or mid-value of the two readings was obtained. The average or mid-value was multiplied by the time intervals of every seven days. The result was recorded in units of percentage days and the value was the area of a trapezoid. The area under disease progress curve was calculated using the following equation;

$$
\begin{array}{l} N\text{-}1 \\ \sum \left[Y_i+Y_{i+1}/2\right] \left[X_{i+1}\text{-}X_i\right] \left[18\right] \\ i\text{=}1 \end{array}
$$

Where;

- $Y_{i}=$ % of blighted leaf area on the ith observation.
- X_i = the date of observation of lesions in days after spraying with inoculum suspension on foliage.
- N= the number of disease severity readings.

Step one to four was repeated for the second and third infection readings which were taken. Their result was the area of the second trapezoid. Steps one to four were repeated for the third and fourth infection readings until the trapezoid areas for all the readings were calculated. All the trapezoids were added and the AUDPC was calculated and records made [18,19]. The relative AUDPC [rAUDPC] was calculated by dividing the AUDPC by N, where N^1 = (The total number of days between the first and the last evaluation) multiplied by 100. rAUDPC = AUDPC of each variety/ ((Total number of days of last evaluation-Total number of days of first evaluation)* 100) [20]. Data collected were analysed using analysis of variance (ANOVA) directive GENSTAT version 12.0.

3. RESULTS AND DISCUSSION

Phytophthora infestans produced sporangia on the surface of potato dextrose agar. The sporangia of P.infestans appeared at the tips of the sporangiophores and appeared lemon shaped under microscopic examination. The 18℃ temperature in a germination chamber favoured P. infestans growth. Pathogenicity test which was done on healthy test plants showed brown/black lesions on the Irish potato test plant leaves and stems and this confirmed the presence of P. infestans. These results are in conformity with the findings of [16] who reported about late blight symptoms and [7] who reported about pathogenicity test. The LB lesions were small at first but soon coalesced only in a few days after the first lesions were observed (Fig. 1a, 1b, 1c, 1d and 1e).

Phytophthora infestans inoculum of $1x10^7$ sporangia/ml concentration reacted differently on various varieties in the experimental plots (Fig. 2 and Fig. 3). There was a rapid sporulation on potato leaves typically within 3-5 days after infection. This resulted in a rapid build-up of P. infestans on various potato varieties in the field as compared to glasshouse. The sporangia in the field travelled reasonable distances on wind currents, and this resulted to faster late blight progression (Fig. 2). Late blight progress in the glasshouse was generally slower as compared to the field (Fig. 2 and Fig. 3). The symptoms on the Mang'ere and Nyayo progressed faster as compared to symptoms development on other varieties in the field and glasshouse (Fig. 2 and Fig. 3).

An evaluation of 100% of late blight diseased foliage for all evaluation dates would have a value of 1.0. All values of rAUDPC were expressed as a ratio of this value [20]. Potatoes with lower AUDPC and rAUDPC indicated low infection levels during the evaluation periods, they corresponded to more resistant varieties. The varieties which were grown in the glasshouse experimental pots showed varied late blight severity (Table 1). Higher late blight severity value (630.0, 0.3) of Mang'ere variety indicated high susceptibility to the pathogen. The lower late blight severity value (7.0, 0.003) of Sherekea indicated high tolerance to the pathogen (Table 1). These results were in conformity with the findings of [16,18,19] who reported about AUDPC and rAUDPC. Late blight infection in the glasshouse was significantly lower. The infection among neighbouring plants was reduced due to controlled movement of wind currents, which can spread sporangia within the glasshouse. Other contributing factors which reduced late blight infection in the glasshouse included: controlled temperature, relative humidity, irrigation and sterilised soil.

Late blight severity in the glasshouse differed significantly (F $_{(8, 16)} = 106.15$, P<0.001) among the varieties (Table 2). Sherekea (7.0, 0.003), purple gold (14.3, 0.007) and Kenya mpya (17.5, 0.008) were the resistance varieties in the

glasshouse. Mang'ere (630, 0.3) and Nyayo (402.8, 0.19) were the susceptible varieties (Table 1). There was no visible late blight symptom present in the field before inoculation. At the time of inoculation, $20th$ day after crop emergence the wet humid conditions were favourable for late blight disease, and this allowed the late blight to spread rapidly. On the 7th day after inoculation, three Irish potato varieties showed symptoms of late blight disease (Mang'ere, Nyayo and Shangi) in both the field and glasshouse. On the $14th$ day after inoculation all the nine varieties showed symptoms of late blight. On the $28th$ day after inoculation, Mang'ere had all the leaflets killed by the P. infestans (100% loss of leaves) (Fig. 3). Late blight impacted negatively on Irish potato growth through destruction of foliage and consequently reduced their photosynthetic capacity. Photosynthetic capacity reduced faster on Mang'ere(1e), Nyayo(1b) and Meru red(1c) as compared to other varieties such as Kenya mpya, Sherekea and Purple gold (Figs. 1a-1e and 3). Higher late blight severity (2093, 0.997) of Mang'ere indicated high susceptibility to the

pathogen in the field. The lower late blight severity (467.8, 0.223) of Sherekea indicated high tolerance to the pathogen in the field (Table 1). These results are in agreement with the findings of [16,18,19] who reported about late blight severity in various Irish potato varieties.

The field severity of late blight was significantly different (F $_{(8, 16)} = 74.44$, P< 0.001) among the Irish potato varieties (Table 3). Sherekea, Purple gold and Kenya mpya were among the resistant varieties in the field. Mang'ere and Nyayo were among the susceptible varieties in the field (Table 3). The Irish Potato varieties identified had different reactions to P. infestans (Table 1). These results are in agreement with [21] who reported that all farmers' Irish potato types are susceptible to late blight disease. These results are in conformity with [22] who reported that utilisation of late blight resistant Irish potato varieties are considered as a sustainable long term solution to yield reductions. The field severity of late blight was significant different $(F_{(8, 16)} = 74.44, P < 0.001)$ among the Irish potato varieties (Table 3).

Fig. 2. Bars representing standard error of means of glasshouse foliar late blight severity and progression on selected Irish potato varieties

S. no.	Variety		Glasshouse		Field
		AUDPC	rAUDPC	AUDPC	rAUDPC
	Mang'ere	630 ^s	0.3°	2093 ^s	0.997 ^s
$\mathbf{2}$	Nyayo	402.8^s	0.19^{s}	1800 ^s	0.857^s
3.	Meru red	73.5 ^{mr}	0.035^{mr}	1479 ^{mr}	0.704^{mr}
4.	Shangi	35.3 ^{mr}	0.016^{mr}	1342 ^{mr}	0.639 ^{mr}
5.	Asante	28 ^{mr}	0.013^{mr}	1030 ^{mr}	0.490 ^{mr}
6.	Tigoni	24^{mr}	0.011^{mr}	953 ^{mr}	0.454^{mr}
7.	Kenya mpya	17.5°	0.008 ^r	730 ^r	0.348 ^r
8.	Purple gold	14.3°	0.007 ^r	651 ^r	0.31 ^r
9.	Sherekea	⇁	0.003 ^r	468^r	0.223 ^r

Table 1. Late blight disease severity in the glasshouse and field

Varieties were significant different at p<0.001

Key: $s=$ Susceptible varieties, $r=$ Resistant varieties and $mr =$ Moderate resistant varieties

Table 2. ANOVA test for late blight severity in the glasshouse

S.no.	Source of variation	d.f.	S.S.	m.s.		
. .	Rep		7414	3707	2.61	
<u>.</u>	Variety		1206777	150847	106.15	<.001
J.	Residual	16	22737	1421		
	Total	26	1236927			

Table 3. ANOVA test for field severity of late blight

Fig. 3. Bars representing standard error of means of field foliar late blight severity and progression on selected Irish potato varieties

Sherekea, Kenya mpya, Purple gold, Nyayo and Mang'ere were significantly different in the field inoculated experiments (Tables 1 and 3). Sherekea, Purple gold and Kenya mpya were among the resistant varieties and Mang'ere was highly susceptible variety in the field (Table 1).

Late blight disease severity in the glasshouse was significantly different $(F_{(8, 16)} = 106.15,$ p<0.001) (Table 2). Sherekea, Purple gold and Kenya mpya were among the resistant varieties and Mang'ere was the most susceptible variety in the glasshouse at KALRO Kisii (Table 1). These results are in agreements with the findings of [23] who reported about low level of late blight disease in various Irish potato varieties could because both have relatively high levels of horizontal resistance to the disease as compared to other varieties. These results are in conformity with [23] who reported that high tolerance levels may be attributed to their percentage from population A which contains major resistant (R) genes.

4. CONCLUSION

In Kenya late blight disease remains a serious threat to Irish potato production causing yield and economic losses to farmers and therefore the use of resistant varieties could reduce late blight problem significantly. Late blight was significantly different among the varieties assessed in various experimental plots. There was high severity of late blight disease on local Irish potato varieties such as Mang'ere and Nyayo as compared to resistant Irish potato varieties such as Sherekea and Kenya mpya. Therefore, farmers can effectively utilize Sherekea and Kenya mpya Irish potato varieties for their host-resistance and maximise their yields by reducing the use of fungicides inputs.

5. RECOMMENDATION

The farmers are recommended to grow Kenya mpya and Sherekea because they were identified as resistant varieties as compared to other varieties which were grown in the field and glasshouse conditions. Growing the two varieties could reduce yield loss problem and this would enable the farmers to get better yields. Further research is recommended to determine the effect of late blight disease on yield on selected Irish potato varieties.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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