

Bacterial wilt (*Ralstonia solanacearum*) of Irish potatoes: Incidence and pathogen diversity in Kenya

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Irish potato (*Solanum tuberosum* L.) ranks second as the most important food crop after maize in Kenya. Bacterial wilt (*Ralstonia solanacearum*) is the second most important biotic constraint to potato production after late blight. There is not enough information on its incidence, prevalence and pathogen diversity in Western Kenya. A survey was conducted in three districts of Western Kenya during the long rainy season, July 2008. A questionnaire was administered to potato farmers to assess bacterial wilt status and its management practices. Selective sampling of infected potato tubers was carried out during the survey and subsequently pathogen isolation from these tubers done. The diversity of *R. solanacearum* isolates was then evaluated using biochemical and pathogenicity tests. Survey data was analysed using descriptive statistics, frequencies and correlation. Pathogenicity results were subjected to analysis of variance test while means were tested for similarity using cluster analysis. Bacterial wilt incidence was highest in Bungoma West (6.9%) and lowest in Bungoma North (1.3%) with a prevalence of 70% and 50% respectively. A significant and negative correlation ($P \leq 0.05$, $r = -0.878$) existed between bacterial wilt incidence and altitude. Management of the disease was by use of chemicals (19%), crop rotation (3%), roguing (6%), choice of season (3%), while 69% of farmers did not apply any control. The *R. solanacearum* isolates of Western Kenya belonged to race 3 biovar 2A. Isolates did not differ significantly ($P = 0.187$) in their aggressiveness. However, using cluster analysis two main aggressiveness groups were noted.

Key words: Bacterial wilt, incidence, *R. solanacearum*, isolates.

Introduction

Irish potato is an important food and cash crop in Kenya playing a major role in national food security and nutrition, poverty alleviation and income generation. There are approximately 25,000 to 30,000 hectares of Irish potatoes grown annually in Kenya. Potato production in Kenya is gaining popularity due to growth in population and a diversification of crops in growing areas with favourable climatic conditions where maize has been the main crop (MoA., 1998). The crop is grown in the Kenya highlands at altitudes of between 1200 to 2800 meters above sea level. The pests and diseases attacking potato include bacterial wilt (*Ralstonia solanacearum*) which is the second most important biotic constraint to potato production after late blight (Barton *et al.*, 1997). Bacterial wilt is a devastating disease to Irish potatoes and affects other economically

important hosts such as tomato, banana and tobacco (Hayward, 1991). The disease occurs throughout Central and Southern Africa, and is a serious production constraint in Uganda, Ethiopia, Kenya, Madagascar, Rwanda, Burundi, Nigeria and Cameroon. In these areas bacterial wilt constitutes a serious obstacle to the production of many solanaceous plants besides potato. High prevalences of bacterial wilt in Irish potato have been reported in Kenya; 78.9% in North Rift (Kwambai T.K., unpublished) and 71% in Central Province (Ateka *et al.*, 2001). This indicates that bacterial wilt has steadily spread to most potato growing areas in the country. However there is inadequate information on the occurrence of bacterial wilt of Irish potatoes in Western Kenya.

Several resistant cultivars of potato, as well as other crops are available but the race and strain diversity of the pathogen make it difficult to utilize these resistant cultivars in different countries and regions (Hartman and Elphinstone, 1994). Knowledge of the existence of variability in pathogen population is therefore important for plant breeding and the consequent crop improvement programme. There are also speculations that race 3 biovar N2 could have been introduced to Kenya from Uganda (Nyangeri J.B., personal communication). Due to these inadequacies a study was initiated with the aim of determining the incidence of bacterial wilt and the diversity of *R. solanacearum* of Irish potato in Western-Kenya.

Materials and Methods

Field survey

A field survey was conducted in July 2008 in selected potato growing regions of Western Kenya to determine the incidence and prevalence of bacterial wilt of Irish potato in the region. The diversity of *R. solanacearum* pathogenic to Irish potatoes in the region was also determined. The survey covered important potato growing areas of Mt Elgon, Bungoma North and Bungoma West Districts of western Kenya.

Bacterial wilt incidence and prevalence

Potato fields with crops at the flowering stage were chosen randomly. A total of thirty potato fields in each district were selected at intervals of 5-10 Km. Each farmer was asked a set of questions intended to describe potato management practices and their effect on bacterial wilt disease.

To determine bacterial wilt field incidences, plots were split into four portions representing the whole plot. Within the split portions, ten rows of ten plants were selected at random and plants showing bacterial wilt symptoms counted. Bacterial wilt incidence was based on the total number of plants showing wilt symptoms expressed as a percentage of the total number of plants observed. Spot altitudes of each field visited were taken using a Geographical Positioning System (GPS).

Bacterial wilt disease prevalence was hence calculated as a percentage of the total number of fields found infected over the total number of fields visited.

Diversity of R. solanacearum infecting potato in Western Kenya

Sampling of affected potato tubers was done selectively in the fields visited for laboratory isolation and determination of *R. solanacearum* biovars present in the study site. Selective sampling of tuber samples from bacterial wilt infected plants was done in thirty of the farms visited. *R. solanacearum* isolation from the diseased samples were hence done using Kelman's tetrazolium agar (Kelman, 1954). Biovar determination was subsequently performed on the isolates as described by French *et al.*, (1995). Isolates testing positive to biovar 2 were then differentiated into biovar N2 or 2A on the basis of their ability to oxidize D (-) ribose and L (-) tryptophan (Hayward, 1994).

Pathogenicity tests of R. solanacearum isolates of Western Kenya

Pathogenicity test was performed on the thirty isolates according to OEPP/EPPO (2004) procedures using the susceptible tomato cultivar "money maker" (Williamson *et al.*, 2002). *R. solanacearum* isolates were cultured in Kelman's tetrazolium chloride agar at 30°C for 48 hours then transferred to nutrient agar and incubated at 30°C for 48 hours. Inoculum was prepared by suspending the bacteria in sterile de-ionised water and adjusting to a concentration with absorbance of 590 nm (approximately 10^6 bacteria cells ml⁻¹).

Test plants were grown in sterile soil: sand mixture in the ratio of 3:1 contained in plastic pots (10 cm diameter and 10 cm deep) with drainage holes punched. Plants were watered with sterile water until one day to the day of inoculation. Inoculation was performed using the soil soak inoculation method (Jill *et al.*, 2004) using plants at the third true leaf stage. Inoculation entailed pipetting 10 ml of prepared inoculums into indentation made in the root zone of unwounded plants. Control plants were inoculated with sterile water.

Treatments were arranged in a completely randomized design with three replicates. The plants were then grown in a polythene screen house at temperature range of 13°C to 32°C. Test plants were then observed daily up to two weeks after inoculation for typical bacterial wilt symptoms. Plants showing these symptoms were observed daily until complete and were considered completely wilted when 50% of the plant were wilted.

Re-isolation of the bacteria from stem sections taken from wilting plants was subsequently performed by use of Kelman's tetrazolium medium.

Data analysis

Data was analysed using descriptive statistics, frequencies and correlation. Pathogenicity test results were subjected to analysis of variance test while means were compared for similarity through cluster analysis using GenStat Discovery Edition (GenStat, 2003).

Results

Bacterial wilt incidence in Western Kenya highlands

Potato production in Western Kenya is predominantly practiced by small scale farmers owning farms of 5 acres and below. The popular potato varieties grown in the region were Alga (65%), Nyayo (10%) and Asante 10%. Other varieties grown in the area were Diseree (5%),

Tigoni (2.5%) and Rebica (2.5%). Bacterial wilt was encountered from the altitude ranges of 1600 to 2399 metres above sea level (masl) (Figure 1). The disease was not encountered at altitudes above 2400 masl. A significant and negative correlation ($r = -0.878$, $P \leq 0.05$), was observed between bacterial wilt incidence and altitude with bacterial wilt incidences generally reducing at higher altitudes (Fig. 1).

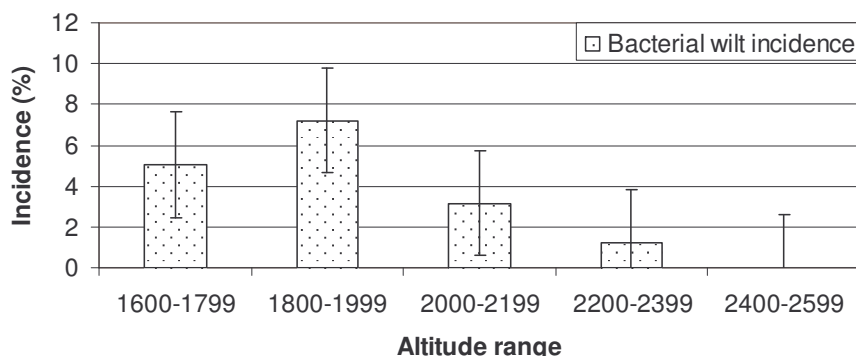


Fig. 1. Influence of altitude on bacterial wilt incidence in Western Kenya. Error bars represent standard error

Bacterial wilt incidence and prevalence in Western Kenya

Potato fields in the three Districts visited were found infested with bacterial wilt. Bungoma West District had the highest bacterial wilt incidence and prevalence of 6.9% and 70% respectively. Mt Elgon District was intermediate with an incidence and prevalence of 3.6% and 68% respectively. The lowest bacterial wilt incidence and prevalence was recorded in Bungoma North District with 1.3% and 50% respectively. Overall, there was a mean bacterial wilt incidence of 3.9% and a prevalence rate of 62.7% in the three Districts.

Bacterial wilt importance and its control in Western Kenya

Bacterial wilt was ranked second to late blight as the disease of greatest importance limiting potato production with 23% prominence. However 59% of the respondents admitted that the disease reduces potato yields and quality whenever it occurs. Most of the respondents (72%) could recognize bacterial wilt symptoms in potato fields.

Bacterial wilt control methods used in Western Kenya were as follows; 19% controlled the disease by spraying with fungicides or insecticides or a combination of both which they referred to us “sumu” (poison). However the same farmers complained that the use of these chemicals was ineffective in the control of bacterial wilt. A minority of the farmers controlled the disease using crop rotation (3%), roguing (6%) and planting during the short rains (3%) while a majority of the respondents (69%) did not control the disease at all. Rotation crops used by these farmers were cabbage, kale, onions, maize, carrots and common beans.

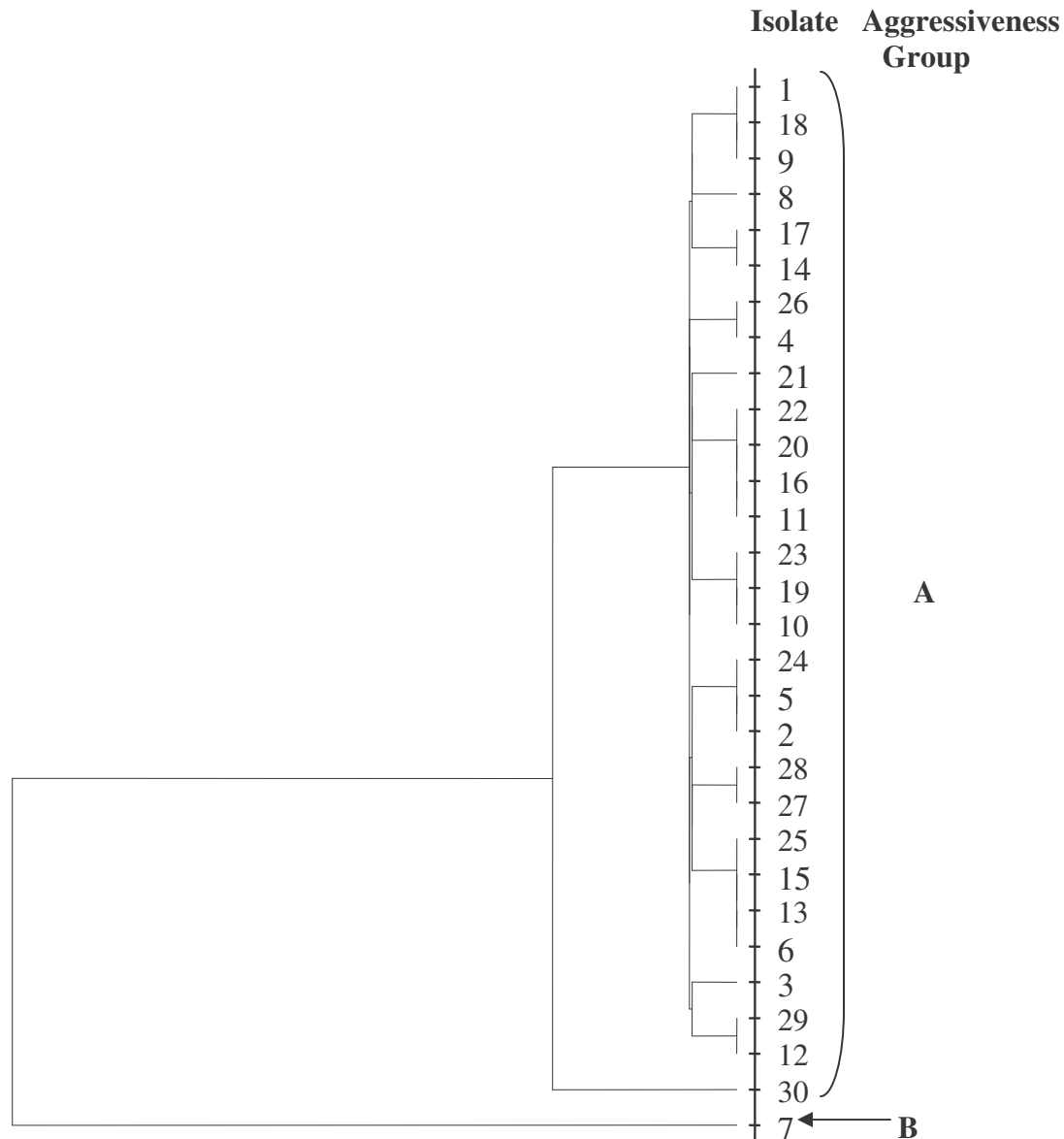


Fig. 2. Dendrogram constructed using the nearest neighbour method showing the correlation between the mean days taken by the 30 isolates from inoculation to onset of wilting on test plants. This gives an indication of aggressiveness.

Diversity of R. solanacearum in Western-Kenya

All the 30 *R. solanacearum* isolates infecting potato in Western Kenya belonged to biovar 2A. These isolates were highly pathogenic to tomato variety “money maker”. Between 5 and 11 days after inoculation test tomato plants showed typical bacterial wilt symptoms. All the inoculated test plants were dead 14 days after inoculation. The isolates’ aggressiveness was not significantly different ($P = 0.187$) as indicated by the time the isolates’ took to show bacterial wilt symptoms on test plants. However when means were compared using cluster analysis, differences in aggressiveness was noted. Two major aggressiveness groups denoted A and B were noted (Fig. 2). Within aggressiveness group A several subgroups can be noted (Fig. 2).

Bacterial colonies re-isolated from wilting tomato plants confirmed the causative agent of the wilting to be *R. solanacearum*.

Discussion

Bacterial wilt of potato was found in western Kenya at altitude range of between 1600 to 2399 masl. Disease incidence correlated negatively with altitude thus confirming reports by Nyangeri *et al.*, (1984) that the disease is more prevalent at lower altitudes. At higher altitudes, the temperatures are low and therefore not conducive to pathogen survival and spread. Pathogen virulence is also reduced at low temperatures. Although *R. solanacearum* race 3 biovar 2A, the only pathogenic race and biovar of potato in Western Kenya is adapted to low temperatures, its survival in very cold temperatures is reduced, (Van Elsas *et al.*, 2000). *R. solanacearum* race 3 biovar 2 population densities decline significantly at between 15 and 20°C and are severely reduced at 4°C (Van Elsas *et al.*, 2000).

Bacterial wilt of potato presence in Western Kenya confirms reports by Michieka (1993) that *R. solanacearum* has spread to most potato growing areas of Kenya. However the incidence and prevalence rate were low compared to what was recorded in the North Rift of Kenya (Kwambai T.K., unpublished) and in Central Kenya (Ateka *et al.*, 2001). Variation in bacterial wilt incidence and prevalence was observed in the three districts which can be attributed to differences in temperature, rainfall, potato cultivar grown in the region and cropping systems (Nyangeri *et al.*, 1984).

Farmers in Western Kenya rated bacterial wilt as the second most important disease affecting potato production after late blight in this region. Similar observations have been encountered throughout Eastern Africa (Adipala, *et al.*, (2000); Ateka *et al.* (2001) and Kwambai T.K., unpublished). Similarly this disease is threatening to establish itself as a menace of great importance in the temperate regions of Europe (Van Elsas, *et al.*, 2000).

Potato strains of *R. solanacearum* can belong to biovars 1, 2A, N2, 3 and 4 (Denny and Hayward, 2001). *R. solanacearum* race 3 biovar 2A was found to be the sole causative agent of potato bacterial wilt in Western Kenya. The identification of biovar 2A was based on biovar tests (French *et al.*, 1995) and tomato inoculation of the pathogen to determine its pathogenicity (OEPP/EPPO, 2004). These *R. solanacearum* isolates were found to be pathogenic to tomato variety “money maker” and agrees with the findings of Williamson *et al.* (2002).

Contrary to expectation, race 3 biovar N2 of *R. solanacearum* was not found on potatoes in Western Kenya. Previous reports in Kenya had indicated that biovar 2A was predominant over biovar N2 whose presence had in the past been reported only once in Nyeri by Smith *et al.* (1995). *R. solanacearum* biovar N2 isolates have predominantly been isolated from South America, with a few exceptions (Swanepoel, 1988). Presence of biovar N2 in the neighbouring Uganda had also been speculated (Nyangeri, J.B. personal communication). The absence of biovar N2 in the Western Kenya *R. solanacearum* isolates is encouraging since a universal control measure of bacterial wilt of potato can be easily formulated for the region.

The *R. solanacearum* isolates of Western Kenya varied in their aggressiveness to tomato variety “money maker” but did not vary in their metabolic activity. Previously Smith *et al.* (1995) had indicated that race 3 biovar 2A of *R. solanacearum* populations of Kenya were phenotypically homogenous and their genetic diversity was low. This could explain their indifference in metabolic activity. Another reason that could account for the low diversity among *R. solanacearum* potato strains may be the lack of selection pressure from potato genotypes and

soil environment on *R. solanacearum* to evolve new biovars as observed in ginger by Kumar *et al.* (2004). Further it has been suggested that the host may be able to induce metabolic diversity within *R. solanacearum* (Elbaz *et al.*, 2003). Three potato varieties in Western Kenya composed 85% of all the potato varieties grown in the region. Probably the low variability in potato genotypes in Western Kenya could be the reason for the low metabolic diversity of *R. solanacearum* in the region. However the small difference in aggressiveness of the *R. solanacearum* isolates of western Kenya could not be explained.

Conclusion

Bacterial wilt of potato was found to be a disease of great concern in Western Kenya as depicted by the high prevalence but relatively low incidence in the region.

Biovar 2A of *R. solanacearum* was the only biovar found to affect potatoes in Western Kenya. Variation in biochemical reaction was not observed in these *R. solanacearum* isolates but variation in aggressiveness was noted. *R. solanacearum* biovar N2 was not encountered in this region, although it has been reported in Uganda.

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