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Genetic Diversity of *Phytophthora infestans* (Mont.) de Bary in the Eastern and Western Highlands of Uganda

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With 2 figures

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Abstract

Eight isolates of *Phytophthora infestans* were recovered from late blight infected samples collected from the districts of Mbale and Mbarara in the Eastern and Western highlands of Uganda in 2001 and analysed using mitochondrial deoxyribonucleic acid (DNA) haplotype and Amplified Fragment Length Polymorphism (AFLP) markers. Polymerase chain reaction amplification with the P2 primer followed by digestion with *Msp*I yielded a three-fragment pattern characteristic of isolates belonging to the US-1 clonal lineage; the polymorphism was confirmed by DNA sequencing. AFLP analysis yielded 60 markers, analysis of which clustered the Ugandan isolates with reference to US-1 isolates (US930258 and US940501). These results suggest that the examined Ugandan isolates belong to the US-1 clonage lineage.

Introduction

Late blight was first reported in East Africa in 1941 (Nattrass, 1944) and has since continued to plague potato production; a number of approaches have been adopted to manage this disease, with host resistance being the most plausible under the subsistence nature of cultivation characteristic of Ugandan agriculture (Hakiza et al., 2000). Over the years a variety of cultivars have been periodically released to counter the effect of the disease (Hakiza et al., 2000) but of late there has been a general increase in severity of late blight in the potato growing areas in the Eastern and Western highlands of the country. Research has revealed high levels of fungicide resistance and hybridization within isolates from this region (Mukalazi et al., 2001), although genetic analysis has so far only highlighted their clonal nature (Vega-Sànchez et al., 2000; Olanya et al., 2001). Late blight epidemics have become more difficult to manage worldwide because of an increased genetic diversity of *Phytophthora infestans* (Fry and Goodwin, 1997). Management strategies and forecasting systems in areas with clonal populations therefore depend on an understanding of its population genetics because of the relationship of genotypes to epidemiologically important features such as pathogen response to environmental conditions, host specificity or adaptation, cultivar response and fungicide sensitivity. This research was initiated to explore the level of genetic diversity within the Ugandan *P. infestans* population using highly sensitive molecular markers, in an attempt to establish the possible linkages to phenotypic diversity previously reported (Mukalazi et al., 2001).

Materials and Methods

Samples of potato leaves infected with late blight were collected from the highlands of eastern and western Uganda during November 2000 and May 2001 and propagated through cv Victoria (CIP381381.20), selective V8 media (Hohl, 1998) and pea broth. Deoxyribonucleic acid (DNA) was then extracted (Qiagen Inc., Valencia CA (Californian State), USA) and quantitative (Qiagen Inc.) and qualitative analyses were carried out. Eight Ugandan isolates (Mbale, three and Mbarara, five) were compared with four from the United States (Chris Smart, Cornell University) and one from the Netherlands (Dr S. Kamoun), representing different clonal lineage of *P. infestans*. Haplotype and Amplified Fragment Length Polymorphism (AFLP) analysis with restriction enzymes *Eco*RI and *Mse*I and the primer pair *Eco*RI-AA/*Mse*I-CC (Perkin Elmer Applied Biosystems Inc., Wellesley MA (Massachussetts State), USA) were performed. Sequences were generated at the Molecular and Cellular Imaging Centre, Ohio State University and analysed using Sequencher^{TM}. Phylogenetic relationships were established

*Msp*I restriction site

Ugandan isolates	CAACAAAACTACTTGAAC**CCGG**AATAGACATATTTGCT
I-b haplotype	CAACAAAACTACTTGAAC**CCGG**AATAGACATATTTGCT
I-a haplotype	CAACAAAACTACTTGAAC**CTGG**AATAGACATATTTGCT
II-a haplotype	CAACAAAACTACTTGAAC**CTGG**AATAGACATATTTGCT
II-b haplotype	CAACAAAACTACTTGAAC**CTGG**AATAGACATATTTGCT

Fig. 1 Sequence alignment of P2-R2 amplicons of Ugandan *P. infestans* isolates and representative haplotypes

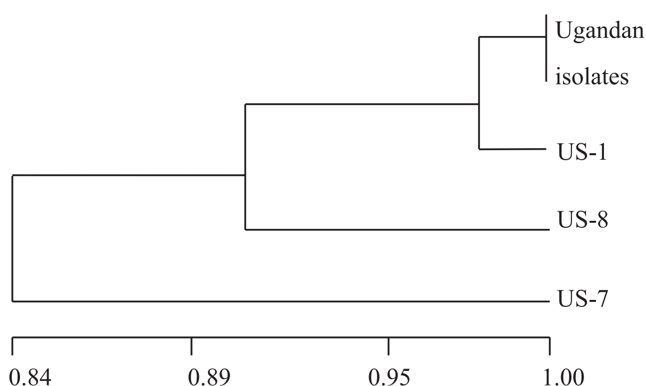

Fig. 2 Genetic similarity of selected Ugandan and US clonal lineage *P. infestans* isolates based on 43 AFLP markers. Isolates were from Mbarara District (UG7002, UG7010, UG7011, UG7012) and Mbale District (UG6013) and of lineage US-1 (US930258 & US940501), US-7 (US940330) and US-9 (US940480)

using Unweighted Pair Group with Mathematical Averaging in Genotyper®.

Results

Polymerase chain reaction amplification yielded 1070 bp size bands with the P2 primer, which on digestion with the restriction enzyme *Msp*I gave a I-b mitochondrial DNA haplotype pattern (Griffith and Shaw, 1998) identical with the Ugandan and reference US-1 isolates. Sequence analysis revealed the *Msp*I restriction sites (Fig. 1) identified by Ristaino et al. (2001) and AFLP analysis generated 60 markers that confirmed the identity between Ugandan and US-1 profiles (Fig. 2).

Discussion

The objective of this research was to investigate the level of genetic diversity in Ugandan populations of *P. infestans* in order to shed light on the possible causes of the recent surge in late blight severity. The markers used in this study revealed homology between Ugandan isolates and the US-1 lineage, often associated with the A1 mating type (Griffith and Shaw, 1998), confirming the findings of Vega-Sànchez et al. (2000) and Olanya et al. (2001). The sample size analysed was however too diminutive to reflect the true diversity situation countrywide and only elucidates the clonal nature of the isolates, necessitating a more structured survey to reveal the factual population structure of *P. infestans* in the country.

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