Research Application Summary

Maize streak virus (MSV) diversity in Uganda and the assessment of gene silencing as a tool for development of resistance to MSV

Owor, B.E.[1], Thompson, J.A.[1] & Shepherd, D.M.[1]
[1]Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch 7701, South Africa
Corresponding author: betty.owor@gmail.com

Abstract

Through an extensive survey conducted in all maize-growing regions in Uganda as well as the application of gene silencing to develop a novel disease control option for maize streak disease, the diversity of Maize streak virus (MSV) in different regions in the country as well as a new disease resistance source were established. Sequence data indicated that all Ugandan MSV isolates belong to the maize-infecting MSV-A1 subtype that is predominant in many sampled areas in East Africa and that both inter- and intra-subtype recombination is common in Ugandan MSVs. Using a transient assay, it was established that MSV replication was abolished or greatly inhibited by a hairpin of a 678-bp portion of an intronless MSV REP gene. The rep hairpin construct also significantly reduced replication of three southern African isolates as well as Ugandan isolates identified in this study.

Key words: Gene silencing, maize, Maize streak virus, Uganda

Résumé

Grâce à une vaste enquête menée dans toutes les régions de culture du maïs en Ouganda ainsi que l'application de l'inactivation génique pour développer une option nouvelle de contrôle de la maladie pour la maladie de la striure du maïs, la diversité des virus de la striure du maïs (MSV) dans différentes régions du pays ainsi que une nouvelle source de résistance à la maladie ont été établies. Les données séquentielles ont indiqué que tous les isolats ougandais de MSV appartiennent au sous-type du MSV-A1 infectant le maïs qui est prédominant dans de nombreux secteurs échantillonnés en Afrique de l'Est et que la recombinaison des sous-types inter et intra est commune dans le virus de la striure de maïs ougandais. En utilisant une analyse transitoire, il a été établi que la réplication du MSV a été abolie ou fortement inhibée par une épingle à cheveux d'une portion de 678-bp d'un gène de REP MSV sans intron. La complexité de l''épingle à cheveux a également réduit de façon significative

la réplication de trois isolats de l'Afrique australe ainsi que les isolats de l'Ouganda ont été identifiés dans cette étude.

Mots clés: Inactivation génique, maïs, virus de la striure du maïs, Ouganda

Background

Maize streak virus is the causal agent of maize streak disease (MSD) that contributes significantly to low maize yields in Africa, thereby threatening food security of sub-Saharan Africa's poorest people. In Uganda, MSD has been identified as one of the most important constraints to maize production. In order to have a better understanding of the disease in that country, this research set out to establish MSD levels in farmers' fields; develop a new sampling and virus isolation method; assess the diversity of MSVs throughout Uganda; and, through the cloning of sampled virus genomes, to determine the genetic characteristics of different isolates. In addition, this study also included an assessment of RNA silencing as a resistance strategy against MSV.

Literature Summary

Maize (*Zea mays* L.) evolved from wild genotypes through modification by selective breeding in Central America and was introduced to the African continent by Portuguese traders in the 16th Century. It has subsequently become the most important cereal crop in sub-Saharan Africa, accounting for about 40% of cereal production on the African continent (FAOSTAT, 2008). Despite the maize crop producing the highest food yield per hectare in comparison to other cereals, African maize yields are the lowest in the world mainly owing to amongst other things, biotic and abiotic factors which contribute to maximum yields not being realised.

In Uganda, three major diseases responsible for yield losses in the country are leafblight, maize streak disease (MSD) and gray leaf spot (GLS) with GLS identified as the most important, followed by MSD (Bigirwa *et al.*, 2001). Caused by MSV (a species in the family *Geminivirdae*), MSD is capable of causing total crop losses in susceptible maize varieties, particularly if infected early. MSD, first described by Fuller over a century ago in South Africa (Fuller, 1901), occurs throughout sub-Saharan Africa and its neighbouring islands (Bock 1974; Rose 1978).

Study Description

An extensive survey was conducted in the main maize growing areas in Uganda in the main growing season to assess the diversity and distribution of MSV in the surveyed areas. During

the survey, diseased leaf samples were collected and an assessment of a new sampling and room-temperature storage technique using Whatman-based FTA Classic Card technology was also tested. To investigate the efficacy of an RNA silencing mechanism in hindering MSV replication, the replication associated protein gene (REP) of MSV was targeted using inverted repeats (hairpin) of sequences homologous to the target gene. A transient assay system was used to establish if MSV replication was abolished by the inverted repeat.

From the sampled leaf tissue, DNA was extracted, cloned and sequenced and sequence data compared with existing sequences in the data base. Furthermore, sequence data was analysed for recombination using recombination detection program (RDP3). Black Mexican sweetcorn suspension cells were co-inoculated with MSV and the inverted repeat construct, cells grown for three days and DNA extracted to assay for virus replication via PCR as part of the gene silencing assay.

Research Application

All sequenced Ugandan MSV isolates were found to belong to the maize-infecting MSV-A1 subtype that is predominant in many sampled areas in East Africa. Furthermore, both inter- and intra-subtype recombination were found to be common in Ugandan MSVs, with 52 of the 68 sequenced isolates being recombinant (Owor *et al*., 2007a). Based on detected recombination patterns, the Ugandan MSV sequences were classified into eight haplotypes named MSV-A1UgI to MSV-A1UgVIII with one haplotype designated MSV-A1UgIII accounting for more than 60% of all sampled MSV infections in Uganda. This recombinant virus is widely distributed throughout the sampled areas in the country (Fig. 1) and causes severe MSD symptoms. An assessment of a new sampling and room-temperature storage technique demonstrated the ease with which a Whatman-based FTA Classic Card technology could be used for large-scale sampling of MSV-infected plants (Owor *et al*., 2007b). This reduces the effort normally required to sample geminiviruses, which generally involves collection and transportation of leaf samples, storage of leaves at -80^{0}C, and DNA extraction. MSV replication was greatly inhibited by an inverted repeat of MSV REP gene (Fig. 2) (Owor *et al*., 2011). Furthermore, the rep hairpin construct significantly reduced replication of three southern African isolates: MSV-MatA and MSV-Gat; a Reunion isolate: MSVReu, which differs from the cognate MSV-Kom by 4.62%, and three Ugandan isolates identified and cloned in this study. Establishment of the genetic

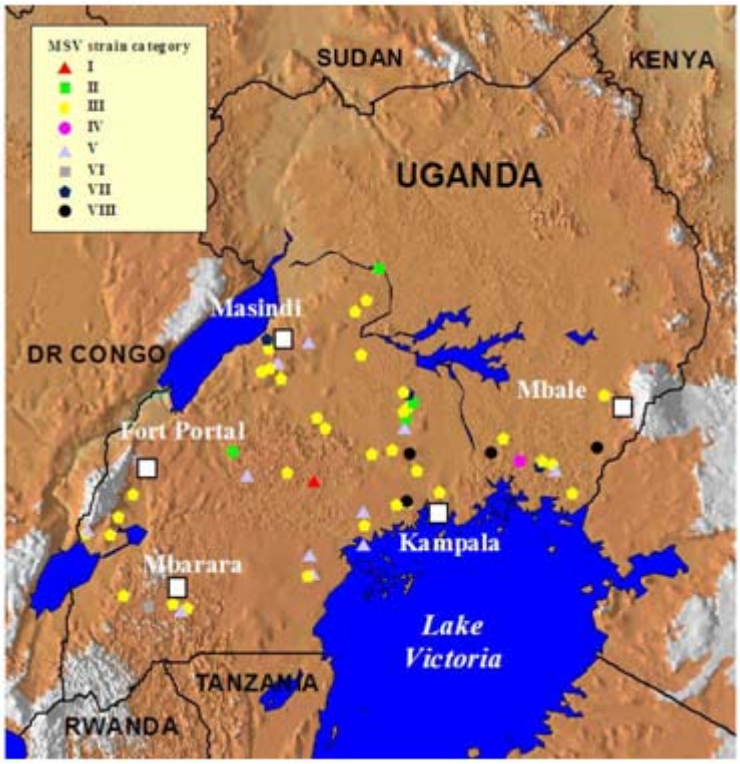

Figure 1. Distribution of eight identified haplotypes, MSV-A1UgI through to MSVA1UgVIII in surveyed areas in Uganda.

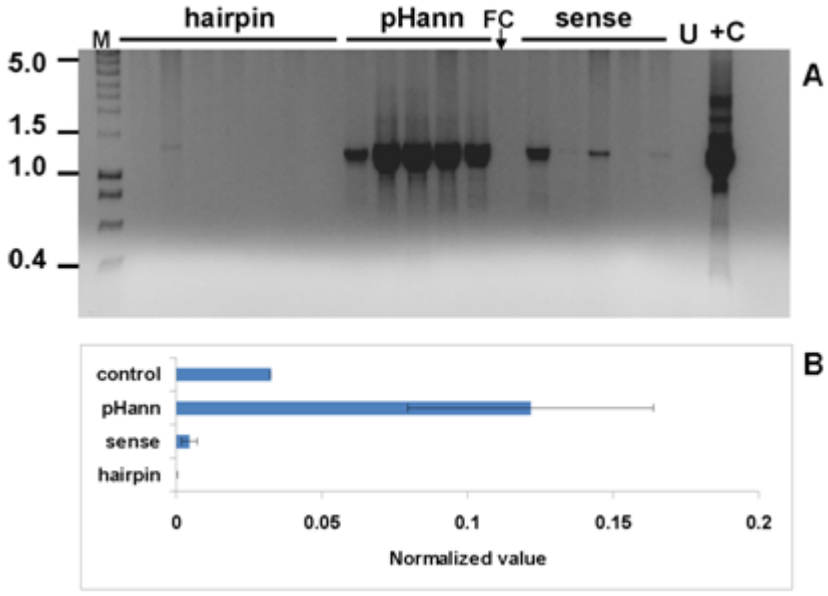

Figure 2. Transient assays showing A: Replicative-specific PCR of BMS cells cobombarded with hairpin pKom602 and B: real-time PCR testing the effect of the hairpin and sense rep on MSV replication. U: Unbombarded control and PCR positive control ("+C"); pHann: empty vector; FC: frozen control. Molecular marker sizes are given in kilo base pairs.

diversity of MSV in Uganda and the fact that the rep hairpin significantly reduces replication of the most diverse maize-infecting MSV-A isolates currently identified, highlights its potential in protecting transgenic maize plants throughout sub-Saharan Africa from MSD.

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