

Potential application of natural variability of *Arabidopsis thaliana* accessions in genomics of *Xanthomonas campestris* pv *musacearum*

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Abstract

The banana *Xanthomonas* wilt epidemic in the Great Lake region of Africa has destroyed banana and is affecting livelihoods of thousands of people who depend on the crop. Currently, there are no banana varieties resistant to the disease. Genetic engineering is the most feasible approach to introduce resistance to the disease in banana. This however requires a good understanding of the underlying genomics of the bacterium. In this study, the capacity of *Arabidopsis thaliana* to study *Xanthomonas* strain diversity was evaluated. Two isolates of *Xanthomonas campestris* (Xcm2251, Xcm4383) and one isolate of *Xanthomonas vasicola* (Xvv206) were infiltrated into four *A. thaliana* genotypes. There was variable response by *A. thaliana* accessions towards the three bacterial strains. The effect of *Xanthomonas* strains was also significant. It is concluded that *A. thaliana* can be used for host pathogen interaction studies of Xcm and Xvv.

Key words: Banana, Great lakes region, host-pathogen interaction

Résumé

L'épidémie de fanage *Xanthomonas* de la banane dans la région des Grands Lacs de l'Afrique a détruit la banane et affecte les moyens de subsistance de milliers de personnes qui dépendent de la culture. Actuellement, il n'existe pas de variétés de bananes qui résistent à cette maladie. Le génie génétique est l'approche la plus réaliste pour l'introduction de la résistance à la maladie de la banane. Cela exige toutefois une bonne compréhension de la génomique sous-jacente de la bactérie. Dans cette étude, la capacité d'*Arabidopsis thaliana* pour étudier la diversité des souches de *Xanthomonas* a été évaluée. Deux isolats de *Xanthomonas campestris* (Xcm2251, Xcm4383) et un isolat de *Xanthomonas vasicola* (Xvv206) ont été infiltrés en 4 génotypes de *A. thaliana*. Il y avait une réponse variable par les adhésions de *A. thaliana* vers les trois souches bactériennes. L'effet des souches de *Xanthomonas* était

également significatif. Il est conclu que *A. thaliana* peut être utilisé pour des études hôte d'interaction des agents pathogènes de Xcm et Xvv.

Mots clés: Banane, région des Grands lacs, interaction hôte-pathogène

Background

Food security in the Great Lakes region of Africa is highly threatened due to banana destruction as a result of the rapid spread of the banana Xanthomonas wilt (BXW) (*Xanthomonas campestris* pv. *musacearum*) (Xcm) (Tushemereirwe *et al.*, 2006). *Xanthomonas vasculorum* pv. *vasicola* (Xvv) is closely related to Xcm and is pathogenic to maize and sugar cane but causes a hypersensitive reaction in *Musa* spp. The Xcm mechanism of host destruction is based on Type three secretions of effectors (TTSS) into the *Musa* host but is not well defined for *Musa* hosts. Management of BXW using resistant varieties is not possible as there are no resistant varieties to this disease yet cultural control mechanisms are labour intensive (Kubiriba *et al.*, 2012). Some current efforts to develop BXW resistant varieties is through genetic engineering. Among these is Tripathi *et al.* (2010), where genes from sweet pepper were introduced into banana. However, there is need for thorough understanding of the underlying genomics of the bacterium for such efforts to be effective.

Genetic variation among wild-type populations of plants is an important source of information about biological traits. The information potential of these natural accessions from *Arabidopsis* accessions has long been recognised and is increasingly exploited to uncover genetic loci controlling biological traits (Van Poecke *et al.*, 2007).

The interaction between a plant and a pathogen can have different outcomes, ranging from the plant not being a host to full susceptibility or to resistance. The identification and characterisation of contrasting situations is the first step to understanding the biological and genetic basis for the outcome (Gassman, 2005). Therefore this study aimed at determining the response of four *Arabidopsis thaliana* accessions to two strains of Xcm and one of Xvv.

Literature Summary

Arabidopsis is diploid and has a small genome of 125 Mbp, distributed in five pairs of chromosomes whose complete sequence is known. There are genetic and physical maps of all

five chromosomes and thus *Arabidopsis* is easily transformed. Studies of gene function have greatly benefited from the development of knockout populations in *Arabidopsis*. Recently, a number of laboratories have turned their attention to exploring the genetic variability found in *Arabidopsis* germplasm (Gassmann, 2005).

Different research groups have applied studies of *Arabidopsis* resistance to *Xanthomonas* (Godard *et al.*, 2000). Each new study of the interaction between *Arabidopsis* and a different isolate of a pathogen reveals new elements of host resistance and susceptibility. It is envisaged that the next breakthroughs in understanding the plant-pathogen interaction will come from pathosystems where both the plant and the pathogen have their genomes completely sequenced. The genomes of Xcm4383 and Xvv206 were previously sequenced and more recently more strains of Xcm and Xvv have been sequenced (Studholme *et al.*, 2010 and 2011).

Study Description

Four accessions of *Arabidopsis thaliana* namely Columbia-0 (Col-0) a wild type ecotype, Columbia-glabrous (Col-5), Landsberg erecta (Ler) and Fab-1 which contains increased levels of saturated fatty acids were used in the pathogenicity study. *Xanthomonas* strains Xcm2251 (Ethiopia, 1967), Xcm4383 (Uganda, 2005) and Xvv206 (R. South Africa, 2005) received from NCPPB, York, UK were infiltrated into 3 leaves of 4 plants of 8 week old *Arabidopsis thaliana* accession for each bacteria strain (0.0002) using a 1ml needleless syringe. Plants were then maintained in a growth room for 7 days and observed for symptoms 3 and 7 days after infiltration.

Macro-symptoms of bacterial infection were visually observed and recorded using a digital camera. The digital images were further analysed to quantify differences in the amount of green colouration in the inoculated plants using ImageJ (Rasband, 2011). Quantitative data were extracted and used to categorise the response of the accessions to the different bacteria. Two leaves were randomly used for performing bacterial population counts based on culture growth on KingsB after 3 days. These experiments were repeated twice.

Research Application

All the four *A. thaliana* accessions showed variable response to infiltration by the three bacterial strains 7 dai. Accessions Col-5 and Ler had low necrotic area as compared to Col-0 and Fab1 accessions (Plate1) when infiltrated with Xvv206. There



Plate 1. *Arabidopsis thaliana* accessions inoculated with Xcm4383 7DAI.

were no significant differences in the experiments for level of green and full coloration of test plants. However, the effect of Xanthomonas strains was significant with intensity of Xcm4383 being quite different from Xcm2251 or Xvv206 for all the accessions. The incidence score for Ler (resistant) was very different from Col0, Col5 and Fab1 for the three Xanthomonas strains.

Recommendations

The study has demonstrated that different *Arabidopsis thaliana* accessions respond variably to Xcm and Xvv inoculation. This variable response is an indication of the inherent genetic response of particular accessions to the advance of bacterial virulence factors. Arabidopsis accessions have been used extensively in the study of molecular plant host-microbe interactions including Xcc and Xoo (Carmo *et al.*, 2007). Therefore we conclude that using Col0 and Ler can be used for host pathogen interaction studies of Xcm and Xvv.

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