Journal of Virological Methods 198 (2014) 86–94

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

A bead-based suspension array for the multiplexed detection of begomoviruses and their whitefly vectors

S.L. van Brunschot[a,b], J.H.W. Bergervoet[c], D.E. Pagendam[d], M. de Weerdt[c], A.D.W. Geering[e], A. Drenth[e], R.A.A. van der Vlugt[c,*]

[a] Plant Biosecurity Cooperative Research Centre, LPO Box 5012, Bruce, ACT 2617, Australia
[b] School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, QLD 4072, Australia
[c] Plant Research International, Wageningen University and Research Centre, PO Box 16, 6700 AA Wageningen, The Netherlands
[d] CSIRO Mathematics, Informatics and Statistics, Ecosciences Precinct, Dutton Park, QLD 4102, Australia
[e] Centre for Plant Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, GPO Box 267, Brisbane, QLD 4001, Australia

Article history:
Received 22 August 2013
Received in revised form
13 December 2013
Accepted 18 December 2013
Available online 31 December 2013

Keywords:
Luminex
Diagnostic
Bemisia tabaci
Tomato yellow leaf curl virus
qPCR

A B S T R A C T

Bead-based suspension array systems enable simultaneous fluorescence-based identification of multiple nucleic acid targets in a single reaction. This study describes the development of a novel approach to plant virus and vector diagnostics, a multiplexed 7-plex array that comprises a hierarchical set of assays for the simultaneous detection of begomoviruses and *Bemisia tabaci*, from both plant and whitefly samples. The multiplexed array incorporates genus, species and strain-specific assays, offering a unique approach for identifying both known and unknown viruses and *B. tabaci* species. When tested against a large panel of sequence-characterized begomovirus and whitefly samples, the array was shown to be 100% specific to the homologous target. Additionally, the multiplexed array was highly sensitive, efficiently and concurrently determining both virus and whitefly identity from single viruliferous whitefly samples. The detection limit for one assay within the multiplexed array that specifically detects *Tomato yellow leaf curl virus*-Israel (TYLCV-IL) was quantified as 200 fg of TYLCV-IL DNA, directly equivalent to that of TYLCV-specific qPCR. Highly reproducible results were obtained over multiple tests. The flexible multiplexed array described in this study has great potential for use in plant quarantine, biosecurity and disease management programs worldwide.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Whitefly-transmitted begomoviruses (genus *Begomovirus*, family *Geminiviridae*) cause economically important diseases of vegetable, grain legume and fiber crops in tropical, subtropical and temperate regions of the world (Navas-Castillo et al., 2011; Varma and Malathi, 2003). Begomoviruses have either monopartite or bipartite circular single-stranded DNA (ssDNA) genomes that are encapsidated in twinned quasi-isometric virions. Most monopartite begomoviruses are also associated with auxiliary symptom-modulating subviral satellite molecules, termed betasatellites (DNA β) (Brown et al., 2012).

Tomato (*Solanum lycopersicum*) is a host of at least 68 species of begomovirus, the largest number described for any single crop (Brown et al., 2012). The most geographically widespread and damaging of these viruses is *Tomato yellow leaf curl virus* (TYLCV). TYLCV is an unusually invasive Old World monopartite begomovirus that has spread globally over past twenty years, and is now considered a significant constraint to tomato production worldwide (Navas-Castillo et al., 2011). Symptom expression characteristic of begomovirus infection in tomato is dependent on a variety of factors including the species of the virus, the host genetic background and the prevailing environmental conditions, and may include distorted plant growth and stunting, leaf curling, interveinal and marginal chlorosis, flower abortion and reduced fruiting.

Begomoviruses are transmitted persistently by the highly polyphagous whitefly *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae), an important agricultural pest with a global distribution (Cohen and Nitzany, 1966; De Barro et al., 2011). Recent research suggests that *B. tabaci* is a cryptic species complex composed of at least 28 morphologically indistinguishable species, commonly referred to as biotypes (De Barro et al., 2011; Dinsdale et al., 2010; Hu et al., 2011). Two species of the *B. tabaci* complex, namely *B. tabaci* Middle East Asia Minor 1 (commonly referred to as biotype B, hereon MEAM1) and *B. tabaci* Mediterranean

* Corresponding author. Tel.: +31 317 480675.
E-mail address: rene.vandervlugt@wur.nl (R.A.A. van der Vlugt).

http://dx.doi.org/10.1016/j.jviromet.2013.12.014
0166-0934/© 2014 Elsevier B.V. All rights reserved.

(commonly referred to as biotype Q, hereon MED) have spread well beyond their home ranges, and have displaced some indigenous *B. tabaci* species in regions that it has invaded (Brown et al., 1995; Liu et al., 2007). *B. tabaci* MED poses a significant biosecurity threat to many countries, due to its propensity to develop strong resistance to commonly used insecticides (Horowitz et al., 2005).

Over the past twenty years, the emergence and continued geographic spread of begomovirus diseases has been closely linked with the parallel movement and establishment of the invasive *B. tabaci* MEAM1 and MED species (De Barro et al., 2011; Navas-Castillo et al., 2011; Polston and Anderson, 1997). In turn, the global dispersal of these begomoviruses and whitefly vectors have been concomitantly linked with increased long distance movement of virus-infected or whitefly-infested plant material, including ornamentals (Cheek and Macdonald, 1994; Chu et al., 2006; Dalton, 2006; Polston and Anderson, 1997). The control of begomovirus and whitefly outbreaks is inherently difficult, expensive and often unsuccessful (Polston and Lapidot, 2007). Good quarantine measures are therefore needed to prevent pest and pathogen incursions, as well as active surveillance to provide early warnings of a breach of quarantine or a looming disease epidemic that could be controlled by early intervention. Efficient and reliable diagnostic strategies underpin the effectiveness of these programs (Rodoni, 2009).

As the different tomato-infecting begomovirus and *B. tabaci* species cannot be reliably distinguished using symptoms or morphological characters, DNA-based diagnostic methods are needed for definitive identification, of which several have been developed (Accotto et al., 2000; Brown, 2000; Davino et al., 2008; De Barro and Driver, 1997; Jones et al., 2008; Lefeuvre et al., 2007; Papayiannis et al., 2009, 2010). However, none of the currently available methods incorporate internal controls to alert the user of false negative results (Hoorfar et al., 2004), nor do they provide the capability to test simultaneously for different begomovirus and *B. tabaci* species. The accurate determination of both virus and vector identity is essential to inform decision-making processes so that appropriate management practices can be initiated to control outbreaks. For example, an outbreak of an exotic virus or *B. tabaci* species would be managed in a very different way and with a higher level of urgency compared to an endemic species. Significant improvements in the efficiency, throughput, sample turn-around time and overall cost of begomovirus and whitefly testing could be achieved by using multiplexed detection methods, enabling faster response to incursion events and improved biosecurity outcomes.

Liquid-phase suspension array technologies have the potential to overcome the multiplexing limitations of both conventional and real-time PCR, where typically no more than two or three assays can be combined. The Luminex MagPlex-TAG technology allows for the simultaneous detection of many targets (up to 150) in a single reaction, and has become a valuable tool for the investigation and detection of clinically important viral and fungal pathogens (Buelow et al., 2012; Foord et al., 2013; Mahony et al., 2007). The Luminex bead suspension array technology exhibits rapid hybridization kinetics, flexibility in assay design and the ability to add and subtract assays easily (Dunbar, 2006). The array technology uses proprietary 6.5 μm carboxylated, superparamagnetic microspheres that are internally labeled with a spectrally distinct fluorescent dye signature, each of which is pre-coupled to a unique anti-MagPlex-TAG oligonucleotide sequence. The experimental approach utilized in this study involves a generic multiplexed PCR step, followed by a multiplexed asymmetric PCR step termed Target Specific Primer Extension (TSPE). In this step, a TSPE primer internal to the multiplexed amplification product will hybridize and extend, only when there is a sequence match. Resultant TSPE products are biotinylated and labeled with complementary MagPlex-TAG sequences at their 5′ end. TSPE products are then hybridized to the MagPlex-TAG microsphere mixture (MagPlex-TAG/anti-MagPlex-TAG hybridization), and a fluorescent reporter molecule is used to detect incorporated biotin. The bead-TSPE product complexes are then detected on the Luminex instrument.

The development of a new Luminex bead suspension array is described, which utilizes a novel hierarchical assay design for the precise identification of begomoviruses and *B. tabaci* at the genus, species and strain levels. This hierarchical assay design facilitates a cross-examination strategy to increase the amount of informative data derived for a single sample, while also increasing the identification accuracy. In addition, the array incorporates two internal control assays for the co-amplification of host sample DNA from both plants and *B. tabaci*. The identity of the begomoviruses and *B. tabaci* present in the samples are established by the concordant results for all of the assays in the array.

2. Materials and methods

2.1. Virus and whitefly samples

All virus samples were prepared from freeze-dried virus-infected plant samples (Table 1). A healthy tomato plant grown in an insect-proof glasshouse was used as a negative control. DNA was extracted from 0.01 g of lyophilized leaf tissue using a bead mill (TissueLyser; QIAGEN, Valencia, CA, USA) and a BioSprint 15 DNA Plant Kit (QIAGEN), both used according to the manufacturer's instructions. Adult *B. tabaci* and *Bemisia afer* individuals from wild populations were preserved in 90–95% ethanol and stored at −20 °C (Table 2). DNA from single whitefly adults was extracted using the method of De Barro and Driver (1997), except that 0.45% (v/v) Triton X-100 was used instead of 0.45% NP40 in the lysis buffer. All DNA extracts were stored at −20 °C.

2.2. Virus and whitefly sequence characterization

Sequence data information for all virus and several of the whitefly isolates used in this study have previously been made available (van Brunschot et al., 2013). The mtCOI gene of *B. tabaci* samples BemQ7, BemASIA1 and BemASIA2 (amplified from plant samples Q1575 and Q4504, respectively) was amplified using the PCR primers C1-J-2195/TL2-N-3014 (Simon et al., 1994). PCR products were purified using the QIAquick PCR purification kit (QIAGEN), directly sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and then processed by Greenomics (Wageningen UR, Netherlands). Sequence data of these samples were deposited into the National Center for Biotechnology Information (NCBI) GenBank database, with accession numbers listed in Table 2. The presence or absence of *Tomato yellow leaf curl virus*-Israel in all whitefly samples was confirmed using conventional PCR (van Brunschot et al., 2010).

2.3. Luminex begomovirus–whitefly array design

The begomovirus-specific, whitefly species-specific and internal control assays (specifically TYLCV-IL, BemB, BemQ, TOFL and Bem18S assays) were adapted from previously published TaqMan real-time PCR assays (van Brunschot et al., 2013). To design the universal virus assays (Beta and Beg assays), 211 full-length DNA-A component sequences (both Old World and New World begomovirus species) and 44 full-length betasatellite sequences were downloaded from GenBank and each set of sequences aligned using ClustalW (Thompson et al., 1994), implemented in Geneious version 5.4.3 (Biomatters, Auckland, New Zealand). For the species-specific assays, variable regions were identified manually to differentiate TYLCV-IL from all other related begomovirus species and strains (target C4 ORF), and *B. tabaci* MEAM1 and *B.*

Download English Version:

https://daneshyari.com/en/article/6133823

Download Persian Version:

https://daneshyari.com/article/6133823

Daneshyari.com