Virology 452-453 (2014) 287–296

Contents lists available at ScienceDirect

Virology

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

Somatic homologous recombination in plants is promoted by a geminivirus in a tissue-selective manner

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ARTICLE INFO

Article history:
Received 19 November 2013
Returned to author for revisions
15 December 2013
Accepted 25 January 2014
Available online 19 February 2014

Keywords:
Geminiviridae
Begomovirus
Euphorbia mosaic virus
Cleome leaf crumple virus
Rolling circle amplification (RCA)
Somatic homologous recombination
Arabidopsis thaliana

ABSTRACT

Four transgenic *Arabidopsis thaliana* lines carrying different reporter gene constructs based on split glucuronidase genes were used to monitor the frequency of somatic homologous recombination after geminivirus infections. *Euphorbia mosaic virus* and *Cleome leaf crumple virus* were chosen as examples, because they induce only mild symptoms and are expected to induce less general stress responses than other geminiviruses. After comparing the different plant lines and viruses as well as optimizing the infection procedure, *Euphorbia mosaic virus* enhanced recombination rates significantly in the transgenic reporter line 1445. The effect was tissue-specific in cells of the leaf veins as expected for this phloem-limited virus. The advantage for geminiviruses to activate a general recombination pathway is discussed with reference to an increased fitness by generating virus recombinants which have been observed frequently as an epidemiologic driving force.

Introduction

DNA damage in plants can be repaired by various eukaryotic protein systems (Bray and West, 2005; Kimura and Sakaguchi, 2006), including somatic homologous recombination (SHR) with high fidelity. Several studies have demonstrated that abiotic and biotic stresses increase the SHR frequency in plants (Boyko et al., 2005; Kovalchuk et al., 2003; Lucht et al., 2002; Molinier et al., 2005; Ries et al., 2000). These stressors trigger either a boost of reactive oxygen species or cause DNA damage directly. For some stress types (*e.g.*, UV-C, elicitor flagellin) the SHR frequency was found to be elevated even within the subsequent, non-stressed plant generation (Molinier et al., 2006). This phenomenon was called "transgenerational stress memory" and is likely an epigenetic effect, because it depends on the functional Dicer-like proteins DCL2 and DCL3 (Boyko et al., 2010; Boyko and Kovalchuk, 2010). However, the transgenerational effect does not occur generally for all stressors (Pecinka et al., 2009).

In the cited studies, transgenic SHR reporter constructs were used to monitor changes of homologous recombination frequencies (HRF). They consist of two non-functional split parts of a reporter gene with partially overlapping sequences of several hundred base pairs of the β-glucuronidase gene (GUS; Fig. 1A). Homologous recombination of the overlapping sequences restores GUS activity which can be detected by histochemical staining. The recombination events monitored as blue spots or sectors in plant tissues allow the quantitative evaluation of SHR. The reporter constructs may be arranged in direct or indirect orientation enabling further insights into the type of the recombination events (Gherbi et al., 2001; Puchta et al., 1995a). They had been integrated into the *Arabidopsis thaliana* genome of two ecotypes at different loci with the help of *Agrobacterium tumefaciens* (Tinland et al., 1994) (Fig. 1A). Consequently, distinct plant lines exhibited different baselines of HRF as well as different responsiveness to stress types which was attributed either to the kind of SHR reporter construction (length or orientation of homologous overlaps), the genomic position or chromatin status of the transgene, the ecotype background, or to a combination of these properties (Pecinka et al., 2009).

Geminiviruses (Jeske, 2009) replicate their circular single-stranded (ss) DNA by three modes of action: complementary strand replication (CSR), rolling-circle replication (RCR) and recombination-dependent replication (RDR) (Alberter et al., 2005; Erdmann et al., 2010; Jeske et al., 2001; Jovel et al., 2007; Preiss and Jeske, 2003). They rely completely on host proteins for replication because they do not encode a DNA polymerase. This is true in particular for the plant homolog of the retinoblastoma protein (pRBR), a cell cycle regulator that blocks replication in differentiated cells (reviewed by Gutierrez et al., 2004; Hanley-Bowdoin et al., 2004). As a consequence host DNA may be

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http://dx.doi.org/10.1016/j.virol.2014.01.024
0042-6822

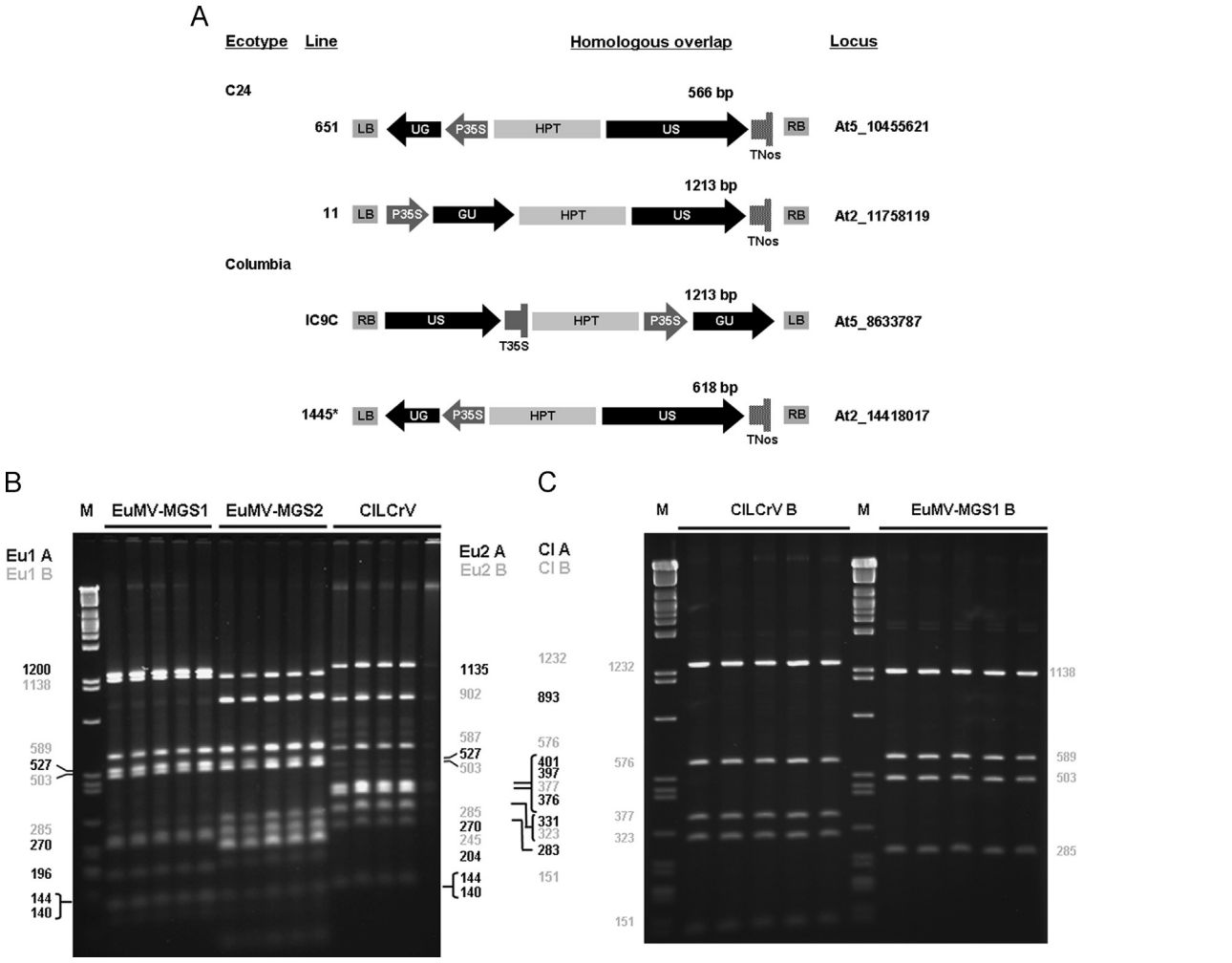

Fig. 1. Prerequisites of the analysis. (A) Schematic representation of SHR reporter constructs present in *A. thaliana* transgenic lines 651 (Puchta et al., 1995b), 11 (Swoboda et al., 1994), IC9C (Molinier et al., 2004) and 1445 (Fritsch et al., 2004; Gherbi et al., 2001; Pecinka et al., 2009). Ecotype background, length (bp) and orientation (direct or inverted) of the GUS overlap construct are indicated for each line. The positions of the genomic integration locus are given according to "The Arabidopsis Information Resource" (TAIR, Apr 02, 2013). LB and RB: left and right border of T-DNA; P35S: cauliflower mosaic virus promoter; T35S: cauliflower mosaic virus terminator; GUS: β-glucuronidase reporter gene; TNos: nopaline synthase terminator; HPT: hygromycin phosphotransferase gene. (B) RFLP analyses to confirm the exclusiveness of full-length DNA A and DNA B from Euphorbia mosaic virus-MGS1 (EuMV-MGS1), Euphorbia mosaic virus-MGS2 (EuMV-MGS2) or Cleome leaf crumple virus (ClLCrV) in the applied inocula. RCA products of viral DNAs from systemically infected wild-type *A. thaliana* plants are shown, which were treated with *Hpa*II (five technical replicates) for each virus. Restriction fragments were separated in 2% agarose gels, with 600 ng of *Pst*I-digested λ DNA as molecular weight marker (M) and staining with ethidium bromide afterwards. Black and gray numbers indicate the expected fragment sizes for DNA As and DNA Bs, respectively. The corresponding undigested RCA products were used for biolistic inoculation. (C) Characterization of the mock-inoculum by RCA/RFLP as in (B). In order to generate RCA products containing only DNA B, restriction enzymes were chosen to linearize only DNA B, and the resulting fragment was gel-purified, recircularized and amplified by RCA. This product was digested with the diagnostic restriction enzyme showing the absence of DNA A or satellite DNA.

re-replicated, as has been shown for plants (Nagar et al., 2002) and yeasts (Kittelmann et al., 2009).

Recombination is an important factor for the evolution and epidemics of geminiviruses (van der Walt et al., 2009, and references therein). At the same time the RDR mode provides an efficient mechanism for early recombination during infection. This motivates our current study on whether host recombination can be influenced by geminiviruses. A transcriptome analysis of *A. thaliana* after geminivirus (cabbage leaf curl virus) infection revealed several changes in the expression of SHR pathway factors (Ascencio-Ibanez et al., 2008). It is therefore plausible that geminivirus infection may influence this host pathway.

Most of the geminiviruses are confined to the phloem tissue (Horns and Jeske, 1991; Wege et al., 2001) allowing us to differentiate between direct effects in the infected tissue and general, stress-induced effects in the whole plant for the first time. The results show that geminiviruses are indeed promoting SHR in phloem tissue under defined experimental conditions.

Results

Monitoring SHR by the help of the reporter constructs as presented in Fig. 1A has been shown to be dependent on the physiological condition of the plants. Most reports have used young plants in axenic cultures for optimal differentiation of baseline and stress-induced SHR. Moreover, the kind of the stressor is important for the outcome of the assay. On the other hand, infection of non-transgenic *Arabidopsis* with the geminiviruses used in this study was found to be optimal at later stages of development in potted plants with vigorous vegetative growth (Paprotka et al., 2010). It was therefore necessary to find a compromise between the optimal experimental conditions for monitoring SHR efficiency and for viral infection. A second difference between this study and previous ones is the phloem-limitation of many geminiviruses. If this tissue tropism is true for the investigated geminiviruses, it would allow discrimination of changes in SHR originated by general stresses from those specifically induced by virus infection in phloem cells.