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Expression of a begomoviral DNAβ gene in transgenic *Nicotiana* plants induced abnormal cell division*

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Abstract: An increasing number of monopartite begomoviruses are being identified that a satellite molecule (DNAβ) is required to induce typical symptoms in host plants. DNAβ encodes a single gene (termed βC1) encoded in the complementary-sense. We have produced transgenic *Nicotiana benthamiana* and *N. tabacum* plants expressing the *βC1* gene of a DNAβ associated with *Tomato yellow leaf curl China virus* (TYLCCNV), under the control of the *Cauliflower mosaic virus* 35S promoter. Transgenic plants expressing βC1 showed severe developmental abnormalities in both species. Microscopic analysis of sections of both transgenic and non-transgenic *N. tabacum* leaves showed abnormal outgrowths of transgenic *N. tabacum* to be due to disorganized cell division (hyperplasia) of spongy and palisade parenchyma. Immuno-gold labeling of sections with a polyclonal antibody against the βC1 protein showed that the βC1 protein accumulated in the nuclei of cells. The possible biological function of the βC1 protein was discussed.

Key words: *Tomato yellow leaf curl China virus* (TYLCCNV), DNAβ, *βC1* gene, Transgenic plant, Cell division
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INTRODUCTION

Geminiviruses within the genus *Begomovirus* cause many destructive diseases in dicotyledonous crops throughout the world, wherever their whitefly vector, *Bemisia tabaci*, is prevalent. Many begomoviruses have genomes consisting of two species of circular single-stranded DNA (DNA-A and DNA-B) encapsidated in characteristic geminate particles. However, an increasing number of begomoviruses were found to have only one genomic molecule (monopartite) which resembles DNA-A (Hanley-Bowdoin *et al.*, 1999; Fauquet *et al.*, 2003). In recent years, satellite DNA molecules, referred to as DNAβ, were found in association with some monopartite begomoviruses and found to be required for inducing yellow vein in *Ageratum*, leaf curl in cotton, yellow vein mosaic in bhendi and yellow leaf curl in tomato (Saunders *et al.*, 2000; Briddon *et al.*, 2001; Jose and Usha, 2003; Zhou *et al.*, 2003). DNAβ is a circular single-stranded DNA of approximately 1350 nucleotides. Several putative genes have been noted on the virion-sense or complementary-sense strand of DNAβ, but only the *βC1* gene, located on the complementary-sense strand, is conserved in position and size in all DNAβ species (Saunders *et al.*, 2000; Zhou *et al.*, 2003). We report here that the expression of the *βC1* gene of DNAβ associated with *Tomato yellow leaf curl China virus* (TYLCCNV) isolate Y10 (TYLCCNV-Y10) in *Nicotiana* plants induced abnormal cell division.

MATERIALS AND METHODS

The *βC1* gene (381 nucleotides) and its frame-shift mutant version were PCR-amplified from

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plasmid pGEM-Y10β containing TYLCCNV-Y10 DNAβ and pGEM-Y10βC1T containing a stop codon at 45 nucleotides downstream of the first start codon in *βC1* gene (Zhou *et al.*, 2003). The amplified DNA fragments were inserted between a duplicated *Caulifolwer mosaic virus* (CaMV) 35S promoter and the nopaline synthase terminator (*nos*) in the expression vector pBin438, to produce pBin-Y10βC1 and pBin-Y10βmC1, respectively. These two recombinant plasmids were introduced into *Agrobacterium tumefaciens* EHA105 by triparental mating and were used for transformation. *N. benthamiana* and *N. tabacum* plants transformation were performed with the *Agrobacterium*-mediated leaf disc procedure. The expressions of the *βC1* gene and its mutant version were confirmed by PCR and Northern blot analyses.

Tissue fragments with outgrowth or normal portion from transgenic *N. tabacum* plants were fixed in 2.5% (v/v) glutaraldehyde and 2% (v/v) polyformaldehyde overnight in 50 mmol/L phosphate buffer (pH 6.8) at 4 °C. Thereafter the samples were thoroughly rinsed with 50 mmol/L phosphate buffer (pH 6.8) and post-fixed with 1% (w/v) osmium tetroxide in the same buffer for 2 h at room temperature. All samples were then dehydrated in a graded ethanol series, embedded in Spurr resin (Polysciences Inc.). Semi-thin (1.5 μm) sections were mounted in phosphate-buffered glycerol and examined with a light microscope after staining with toluidine blue. The similar tissues were also embedded in LowicrylK4M resin, and ultra-thin sections were probed by an immuno-gold probe labeled with a polyclonal antibody against the βC1 protein, which were produced by this lab. The grids were then examined under electron microscopy (JEM-1200EX, JEOL, Japan).

RESULTS AND DISCUSSION

We previously demonstrated that the frame-shift mutagenesis of ATG in the *βC1* gene of TYLCCNV-Y10 DNAβ abolished disease symptoms in *N. benthamiana* (Zhou *et al.*, 2003). The *βC1* gene, therefore, may play a major role in symptom induction. To test this possibility, *N. benthamiana* and *N. tabacum* plants were transformed with *A. tumefaciens* containing construct pBin-Y10βC1. About 40% lines of T_0 transgenic *N. benthamiana* and *N. tabacum* plants display abnormal phenotypes, including leaf distortion, upward leaf-curling and blistering of leaves (Figs.1a and 1b). In addition, abnormal phenotypes such as interveinal protuberances or small interveinal tissue outgrowths could be observed on the undersides of some leaves of transgenic *N. tabacum* plants (Fig.1c). In contrast, both *Nicotiana* plant species transformed with *A. tumefaciens* containing the construct pBin-Y10βmC1 developed normally and remain symptomless.

Semi-thin leaf sections of transgenic *N. tabacum* having outgrowths on the undersides of a leaf revealed that the outgrowths resulted from important internal tissular modifications (Fig.1d). Typically, leaf tissues reorganization is shown by the emergence of an additional palisade parenchyma over the abaxial epidermis (Fig.1d). The observed congregation of the cells in spongy and palisade parenchyma implied rapid unregulated cell division in these transgenic tissues. Another striking modification is the thickened cell wall not only in the cell of spongy and palisade parenchyma but also in the vascular system, which probably resulted from lignification of cell walls (Fig.1d). In contrast, no abnormal cell division and cellular modification was found in the leaf tissues from healthy, non-transgenic *N. tabacum* plants (Fig.1e).

Expression of the *βC1* gene in *Nicotiana* plants induced abnormal cell division, suggesting that the βC1 protein may interfere with endogenous gene expression regulation. To determine how the βC1 protein played a role in this process, its expression and localization in transgenic abnormal tissues showing outgrowths were analyzed by immuno-gold label. Gold labels were detected primarily in the nucleus from the epidermis, spongy and vascular cells (Fig.1f and Fig.1g), suggesting that the βC1 protein was expressed and accumulated in the nucleus of these tissues. No label was found in the nucleus of healthy, non-transgenic cells (Fig.1h). Similarly, labels were not detected on sections treated with buffer, secondary antibody (no primary antibody), preimmune antiserum or with anti-TMV antiserum (data not shown), indicating that background was minimal. Further work must be done to investigate how the nuclear-localized βC1 protein interfered with plant cell division.

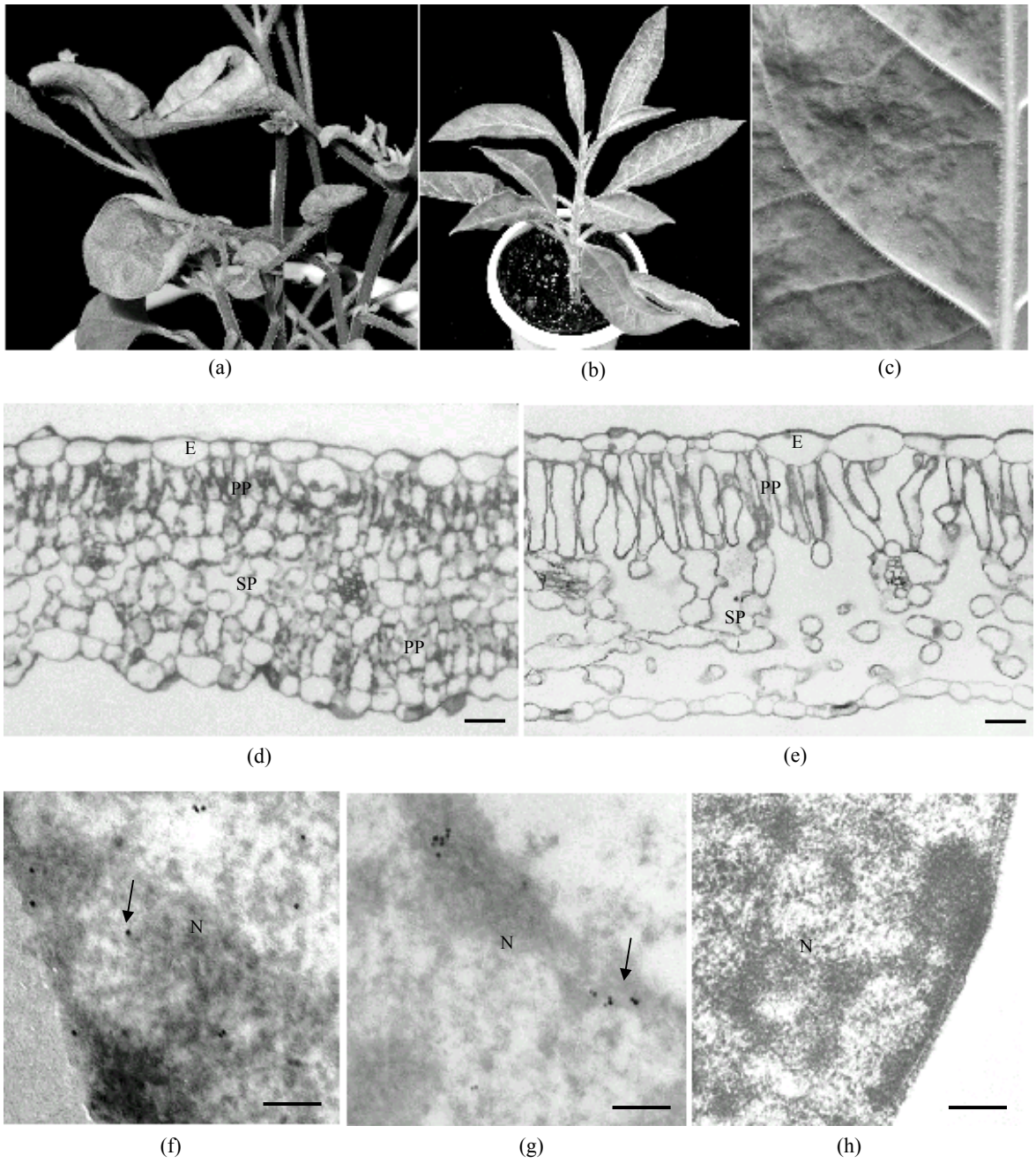

Fig.1 Transgenic plants expressing the *βC1* gene of TYLCCNV-Y10 DNAβ induced abnormal cell division

(a) Transgenic *N. benthamiana* plant showing severe leaf distortion and curling phenotype; (b) Transgenic *N. tabacum* plant showing leaf distortion and curling phenotype; (c) Abnormal tissue outgrowths on the underside of a transgenic *N. tabacum* leaf; (d) Light microscopy of the section through the outgrowths on the underside of a transgnic *N. tabacum* leaf; (e) Light microscopy of the section through the healthy, non-transgenic *N. tabacum* leaf tissue. E, epidermis; PP, palisade parenchyma; SP, spongy parenchyma. Bars in (d) and (e) represent 20 μm; (f) The nucleus of epidermis cell from abnormal transgenic *N. tabacum* tissue probed with gold-labeled antibody against the βC1 protein; (g) The nucleus of vascular cell from abnormal transgenic *N. tabacum* tissue probed with gold-labeled antibody against the βC1 protein; (h) The nucleus of epidermis cell from healthy, non-transgenic *N. tabacum* tissue probed with gold-labeled antibody against the βC1 protein. N, nucleus. The arrow indicates the gold particle. Bars in (f), (g) and (h) represent 500 nm

The *βC1* gene of TYLCCNV-Y10 DNAβ possesses a coding capacity of 126 amino acids (14.6 kDa). We showed here that transgenic expression of this gene in *Nicotina* plants induced abnormal cell division in the absence of virus infection. All other reported βC1 proteins have a similar size (Zhou *et al.*, 2003; Briddon *et al*., 2003), suggesting that the conserved biological function may be attributed to this protein. Actually, severe developmental abnormalities were also reported in *N. benthamiana* plants containing a dimeric *Ageratum yellow vein virus* DNAβ transgene (Saunders *et al.*, 2004). The effect of the βC1 protein on tissue development may provide insight on plant cell cycle regulation and plant developmental process. It is interesting to investigate the interaction between the βC1 protein and host factors.

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