Journal of Zhejiang University SCIENCE
 ISSN 1009-3095
<http://www.zju.edu.cn/jzus>
 E-mail: jzus@zju.edu.cn

Science Letters:

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

CUI Xiao-feng (崔晓峰), LI Yun-qin (李云琴), HU Dong-wei (胡东维), ZHOU Xue-ping (周雪平)[†]

(Institute of Biotechnology, Zhejiang University, Hangzhou 310029, China)

[†]E-mail: zzhou@zju.edu.cn

Received Oct. 15, 2004; revision accepted Nov. 20, 2004

Abstract: An increasing number of monopartite begomoviruses are being identified that a satellite molecule (DNA β) is required to induce typical symptoms in host plants. DNA β encodes a single gene (termed β C1) encoded in the complementary-sense. We have produced transgenic *Nicotiana benthamiana* and *N. tabacum* plants expressing the β C1 gene of a DNA β associated with *Tomato yellow leaf curl China virus* (TYLCCNV), under the control of the *Cauliflower mosaic virus* 35S promoter. Transgenic plants expressing β C1 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 β C1 protein showed that the β C1 protein accumulated in the nuclei of cells. The possible biological function of the β C1 protein was discussed.

Key words: *Tomato yellow leaf curl China virus* (TYLCCNV), DNA β , β C1 gene, Transgenic plant, Cell division
 doi:10.1631/jzus.2005.B0083 Document code: A CLC number: Q78

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 DNA β , 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; Bridson et al., 2001; Jose and Usha, 2003; Zhou et al., 2003). DNA β 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 DNA β , but only the β C1 gene, located on the complementary-sense strand, is conserved in position and size in all DNA β species (Saunders et al., 2000; Zhou et al., 2003). We report here that the expression of the β C1 gene of DNA β associated with *Tomato yellow leaf curl China virus* (TYLCCNV) isolate Y10 (TYLCCNV-Y10) in *Nicotiana* plants induced abnormal cell division.

MATERIALS AND METHODS

The β C1 gene (381 nucleotides) and its frame-shift mutant version were PCR-amplified from

* Projects (Nos. 30270062 and 30125032) supported by the National Natural Science Foundation of China

plasmid pGEM-Y10 β containing TYLCCNV-Y10 DNA β and pGEM-Y10 β C1T containing a stop codon at 45 nucleotides downstream of the first start codon in β C1 gene (Zhou et al., 2003). The amplified DNA fragments were inserted between a duplicated *Cauliflower mosaic virus* (CaMV) 35S promoter and the nopaline synthase terminator (*nos*) in the expression vector pBin438, to produce pBin-Y10 β C1 and pBin-Y10 β mC1, 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 β C1 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 μ m) 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 β C1 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 β C1 gene of TYLCCNV-Y10 DNA β abolished disease symptoms in *N. benthamiana* (Zhou et al., 2003). The β C1 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-Y10 β C1. About 40% lines

of T₀ 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-Y10 β mC1 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 β C1 gene in *Nicotiana* plants induced abnormal cell division, suggesting that the β C1 protein may interfere with endogenous gene expression regulation. To determine how the β C1 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 β C1 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 β C1 protein interfered with plant cell division.

Fig.1 Transgenic plants expressing the $\beta C1$ gene of TYLCCNV-Y10 DNA β 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 transgenic *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 $\beta C1$ protein; (g) The nucleus of vascular cell from abnormal transgenic *N. tabacum* tissue probed with gold-labeled antibody against the $\beta C1$ protein; (h) The nucleus of epidermis cell from healthy, non-transgenic *N. tabacum* tissue probed with gold-labeled antibody against the $\beta C1$ protein. N, nucleus. The arrow indicates the gold particle. Bars in (f), (g) and (h) represent 500 nm

The $\beta C1$ gene of TYLCCNV-Y10 DNA β 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 $\beta C1$ 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* DNA β transgene (Saunders *et al.*, 2004). The effect of the $\beta C1$ 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 $\beta C1$ protein and host factors.

References

- Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S., Saunders, K., Stanley, J., Zafar, Y., Malik, K.A., Markham, P.G., 2001. Identification of DNA components required for induction of cotton leaf curl disease. *Virology*, **285**:234-243.
- Briddon, R.W., Bull, S.E., Amin, I., Idris, A.M., Mansoor, S., Bedford, I.D., Dhawan, P., Rishi, N., Siwatch, S.S., Abdel-Salam, A.M., Brown, J.K., Zafar, Y., Markham, P.G., 2003. Diversity of DNA β , a satellite molecule associated with some monopartite begomoviruses. *Virology*, **312**:106-121.
- Fauquet, C.M., Bisaro, D.M., Briddon, R.W., Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C., Stanley, J., 2003. Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. *Arch. Virol.*, **148**:405-421.
- Hanley-Bowdoin, L., Settledge, S.B., Orozco, B.M., Nagar, S., Robertson, D., 1999. Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Cri. Rev. Plant Sci.*, **18**:71-106.
- Jose, J., Usha, R., 2003. Bhendi yellow vein mosaic disease in India is caused by association of a DNA β satellite with a Begomovirus. *Virology*, **305**:310-317.
- Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M., Stanley, J., 2000. A unique virus complex causes *ageratum* yellow vein disease. *Proc. Natl. Acad. Sci. USA*, **97**:6890-6895.
- Saunders, K., Norman, A., Gucciardo, S., Stanley, J., 2004. The DNA β satellite component associated with *ageratum* yellow vein disease encodes an essential pathogenicity protein ($\beta C1$). *Virology*, **324**:37-47.
- Zhou, X.P., Xie, Y., Tao, X.R., Zhang, Z.K., Li, Z.H., Fauquet, C.M., 2003. Characterization of DNA β associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. *J. Gen. Virol.*, **84**:237-247.

Welcome contributions from all over the world

<http://www.zju.edu.cn/jzus>

- ◆ The Journal aims to present the latest development and achievement in scientific research in China and overseas to the world's scientific community;
- ◆ JZUS is edited by an international board of distinguished foreign and Chinese scientists. And an internationalized standard peer review system is an essential tool for this Journal's development;
- ◆ JZUS has been accepted by CA, Ei Compendex, SA, AJ, ZM, CABI, BIOSIS (ZR), IM/MEDLINE, CSA (ASF/CE/CIS/Corr/EC/EM/ESPM/MD/MTE/O/SSS*/WR) for abstracting and indexing respectively, since started in 2000;
- ◆ JZUS will feature **Sciences in Engineering** subjects in Vol. A, 12 issues/year, and **Life Sciences & Biotechnology** subjects in Vol. B, 12 issues/year;
- ◆ JZUS has launched this new column "Science Letters" and warmly welcome scientists all over the world to publish their latest research notes in less than 3-4 pages. And assure them these Letters to be published in about 30 days;
- ◆ JZUS has linked its website (<http://www.zju.edu.cn/jzus>) to **CrossRef**: <http://www.crossref.org> (doi:10.1631/jzus.2005.xxxx); **MEDLINE**: <http://www.ncbi.nlm.nih.gov/PubMed>; **HighWire**: <http://highwire.stanford.edu/top/journals.dtl>; **Princeton University Library**: <http://libweb5.princeton.edu/ejournals/>.