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ARTICLE

Construction of banana bunchy top nanovirus-DNA-3 encoding the coat protein gene and its introducing into banana plants cv. Williams

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Abstract Banana (*Musa* sp.) is considered as one of the most important fruit crops worldwide as well as in Egypt. The main goal of this study was to construct the open reading frame (ORF) of banana bunchy top nanovirus (BBTV)-DNA-3 that encodes the viral coat protein (*cp*) gene for banana transformation. The previously sequenced BBTV-G-DNA-3-ORF that cloned into plasmid pH1 was used as a template for PCR amplification using two specific primers containing *Bam* H1 site. A new plasmid called pRHA1 containing the amplified ORF under the control of maize polyubiquitin (*ubi*) promoter was created. The *bar* gene (herbicide-resistance gene as a

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selectable marker) cassette (*bar* gene, Cauliflower mosaic caulimovirus (CaMV) 35S promoter and *nos* terminator) was released from plasmid pAB6 using *Hind* III-digestion and subcloned into the *Hind* III-digested plasmid pRHA1 to create the plasmid pRHA2 via the microprojectile bombardment transformation system. The plasmid pRHA2 was successfully introduced in the applied banana cultivar. Leaf painting test was conducted to confirm the expression of the *bar* gene in the putative transformed banana lines. The presence and expression of BBTV-G-*cp* gene were also detected using some molecular (polymerase chain reaction and dot blot using a cold DNA probe) and serological (ELISA and western blot) techniques, respectively, in the obtained transgenic banana lines.

1. Introduction

Banana (*Musa* sp.) is one of the most important crops in Egypt which is reported to be the fourth country on the global level as produces annuals (728,999) tons [1]. Banana productivity is generally reduced by virus diseases; the most deleterious disease which lemmatizes banana production is banana bunchy top nanovirus (BBTV), the causal agent of banana bunchy top disease (BBTD) [9].

The control of such viruses using insecticides and/or inspection and rouging is ineffective. Production of virus-free banana plants *via* tissue culture technique (TCT) or transgenic resistant cultivars to such viruses would be the most effective means for its control beside the classical means. In many situations this strategy can delay the requirement to use chemical control measures. Unfortunately, TCT will not eliminate all banana viruses, therefore, transgenic varieties that are virus resistance, based on virus-derived transgenes have been widely demonstrated to be an effective strategy for control of such viruses and could increase productivity and reduce inputs. Classical breeding is difficult and not accurate enough for transferring gene(s) to banana and this could be due to the extremely complicated genetic system of *Musa* spp., i.e., different genomic constitutions, heterozygosity, polyploidy, long generation times, and sterility of edible clones [13]. Genetic transformation, in conjugation with pathogen-derived resistance [11] is one potential strategy for developing virus resistance in bananas. One which has proven to be difficult to obtain by conventional breeding [19].

This study was designed to carry out the following items, (a) Preparation of a construct containing the BBTV DNA-3 encoding the open reading frame (ORF) of the viral coat protein (CP). (b) Introducing the BBTV-*cp*-ORF gene into cv. Williams of banana plants. (c) Detection of either presence or expression of the introduced gene(s). (d) Regeneration of the transformed banana plant materials. (e) Acclimatization of the transformed banana plants.

2. Materials and methods

This study was conducted at the Gene Transfer Laboratory (GTL), Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.

2.1. Plant materials

Banana plants cv. Williams were obtained from GTL, AGERI, ARC, Giza, Egypt and subjected to indirect-enzyme linked immunosorbent assay (I-ELISA) detection of BBTV according to [18].

2.2. Antisera

Antibodies specific to BBTV-Australian isolate that used for ELISA detection was kindly provided by Dr. John E. Thomas, Plant Protection Unit, Department of Primary Industries, Queensland, South East Region, Indooroopily, Brisbane, Australia.

2.3. Plasmid vectors

Two plasmids were used, i.e., pAB6, that contains *gus* and *bar* genes [28] and pAHC17 that contains the *ubi* promoter [7]. In addition, plasmid pH1 that contains the ORF of BBTV-G-*cp*, constructed by Molecular Plant Pathogenesis Laboratory (MPPL), AGERI, ARC, Giza, Egypt was also applied.

2.4. Primers used

All primers in Table 1 were synthesized at AGERI, ARC, Giza, Egypt on an AB1392 DNA/RNA Synthesizer (Applied Bio systems, Lincoln Center Drive, Foster City, CA, USA).

2.5. Construction of plasmid pRAH2

The BBTV-G-DNA-3-*cp* gene was cloned into a suitable plant expression vector creating plasmid pRAH2 as follows: The BBTV-G-DNA-3-*cp* encoding ORF was amplified from plasmid pH1 by Add/On-PCR using two primer pairs containing the *Bam* HI site (BBTV-F and BBTV-R, Table 1) according to Wanitchakorn et al. [30]. The plasmid pRAH1 was created by ligation of the *Bam* H1-digested ORF-PCR product and plasmid pAHC17 [16]. *Bar* gene cassette was released from plasmid pAB6 by *Hind* III-digestion and cloned into the *Hind* III digested-plasmid pRHA1 creating plasmid pRHA2. The integrity of the plasmid pRHA2 was confirmed using *Hind* III digestion and PCR analysis.

In order to determine the sense orientation for BBTV-G-*cp* gene in the prepared construct, PCR analysis using two primers, i.e., the BBTV-F and the *nos* terminator reverse primer (5′ TTA TCC TAG TTT GCG CGC TA 3′) was conducted as mentioned before with a template of plasmid pRHA2 DNA.