Characterization, detection and identification of transgenic chili pepper harboring coat protein gene that enhances resistance to cucumber mosaic virus

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Abstract Previously, two events (H15 and B20) of transgenic pepper (*Capsicum annuum* L.) that enhanced resistance to *Cucumber mosaic virus* (CMV) by the introduction of CMV coat protein (CP) gene were constructed. Presently, a single copy number of the CP gene was revealed in H15 and B20 by Southern blot. To predict possible unintended effects due to transgene insertion in an endogenous gene, we carried out sequencing of the 5'-flanking region of the CP gene and a Blast-based search. The results revealed that insertion of the transgene into genes encoding putative proteins may occur in the H15 and B20 transgenic event. Multiplex polymerase chain reaction (PCR) for simultaneous detection and identification of transgenic pepper was conducted with a set of nine primers. Both transgenic event were differentiated from non-transgenic

event by the presence of 267 bp and 430 bp PCR products indicative of CP gene specific primer pairs and primer pairs targeting the CP gene and 35S promoter. H15 and B20 uniquely possessed a 390 bp and 596 bp PCR product, respectively. The presence of a 1115 bp product corresponding to intrinsic pepper actin gene confirmed the use of pepper DNA as the PCR template. The primer set and PCR conditions used presently may allow the accurate and simple identification of CMV resistant transgenic pepper.

Keywords *Capsicum annuum* L., *Cucumber mosaic virus*, GMO, Multiplex PCR

Introduction

Since transgenic plants were first commercialized in 1997, their global use has progressively increased, exceeding 50-fold as of 2005. Transgenic soybean, maize, cotton and canola constitute almost 100% of the cultivated area of transgenic crops, with the remainder being comprised of *Bacillus thuringiensis* rice and virus resistant squash and papaya (James et al. 2005). Since the introduction of transgenic crops, concern has been expressed about the consequences of the unintentional environmental introduction of transgenic genes. Transgenic introduction of a gene can potentially influence the function of the surrounding sequence (insertion effect) or visa versa (position effect). The unintended effect of DNA insertion can be partly predicted on the basis of knowledge concerning the location and function of the inserted DNA (Kuiper et al. 2001).

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Multiplex polymerase chain reaction (PCR), which is performed by use of a combination of several primer pairs in the same reaction tube, produces more information in a shorter time than single end point PCR with less consumption of reagents (Germini et al. 2004). Since its first description in 1988, this method has been successfully applied in many areas of DNA testing (Henegariu et al. 1997). Recently, several studies have exploited multiplex PCR for the efficient and reliable detection and/or identification of transgenic sugar beet shoots (Mannerliöf and Tenning 1997), and genetically modified varieties of maize (Matsuoka et al. 2001, James et al 2003), canola and soybean (James et al. 2003).

Previously, transgenic pepper eventtolerant to the new pathotype *Cucumber mosaic virus* CMVP1 was developed by genetic transformation of a coat protein (CP) gene from CMVP0 (Lee et al. 2009). The present study was undertaken to provide more information about newly developed transgenic pepper event. Specifically, we carried out DNA blot analysis to determine the transgene copy number, performed sequencing of the flanking regions of the inserted DNA to predict unintended effects in transgenic event and used multiplex PCR to detect two transgenic event and also to identify each two event.

Materials and Methods

DNA isolation

Genomic DNA was isolated from leaf tissues of the non-transgenic event #2377 (parental event of H15) and #915 (parental event of B20), and from the transgenic event H15 and B20 as previously described (Kim and Hamada 2005). Sample genomic DNA concentration was determined by measuring the ultraviolet (UV) absorption at 260nm and purity was ascertained by measuring the absorbance due to protein at 280 nm and 230 nm, and calculating the A260/A280 and A260/A230 ratios. All measurements utilized a Beckman DU 700 UV/vis spectrophotometer (Beckman Instruments, Fullerton, CA)

Southern blot analysis

Genomic DNA (30 g) was digested with *EcoR* and *HindIII* to cut the inserted CP gene. The resulting DNA fragments were separated by electrophoresis on a 0.8% agarose gel and there-solved fragments were transferred to a positive-charged nylon membrane (Roche Molecular Biochemicals, Mannheim, Germany) by capillary transfer using 0.4N NaOH. DNA probes for the NPTII or CMV CP gene cDNA were prepared from digoxigenin-PCR probe synthesis kit (Roche Molecular Biochemicals), Pre-hybridization, hybridization, and post-hybrid-

ization procedures were done according to the manufacturer's instructions.

PCR-based genome walking

Genomewalking was performed using the protocol supplied by the vendor of the GenomeWalker Universal kit (Clontech Laboratories, Palo Alto, CA). Genomic DNA was digested with *AluI*, *DraI*, *EcoRV*, *HpaI*, *PvuII* and *SnaI*, and was ligated with the GenomeWalker adaptor (Clontech Laboratories). PCR were carried out using each enzyme-digested DNA library, 10 pM AP1 primer, 10 pM CP gene specific reverse primer, 0.2 mM dNTP mixture, 2.5 U of EX Taq polymerase (TaKaRa Shiga, Japan) and 10× EX Taq buffer (TaKaRa) in a 50 µl reaction volume using a xp thermal cycler (Bioer Technology, Hangzhou, China). Cycling condition consisted of 5 cycles of 94°C for 25 s and 72°C for 3 min followed by 20 cycles of 94°C for 25 s and 67°C for 3 min, with a final cycle at 67°C for 7 min. Semi-nested PCR was performed under the same conditions using 1/10 of the primary PCR product as DNA template and using the AP2 primer.

Multiplex PCR and sequencing of PCR product

Multiplex PCR reactions were carried out in the presence of 40 ng of genomic DNA, 10 pM of the five primer pairs, 0.2 mM dNTP mixture, 1.5U of EX Taq polymerase and 10× EX Taq buffer in a 50 µl reaction volume. Thermal cycling was using the aforementioned xp thermal cycler at 94°C for 3 min for predenaturation, 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 1 min, with a final cycle at 72°C for 5 min. The amplified PCR products were separated by electrophoresis on a 0.8% agarose gel. To identify optimal condition of multiplex PCR, the concentration of each primer was adjusted depending on the linear range of PCR amplification prior conducted. Each PCR fragment was ligated into a T&A vector (Real Biotech, Taipei, Taiwan) and sequenced. The obtained sequence was aligned with vector and/or flanking region sequence with the CLC Sequence Viewer 6.0.2 program (CLC bio, Katrinebjerg, Denmark) and the kalign program (<http://www.ebi.ac.uk/Tools/kalign/>)

Results and Discussion

Southern hybridization

DNA blot analyses were performed with probe derived from the CP gene and NPTII kanamycin resistant gene. When genomic DNA digested with *EcoRI* and *HindIII* was hybridized

Fig. 1 Southern blot analysis of transgenic pepper and non-transgenic pepper. (A) Hybridization with coat protein specific probe. (B) Hybridization with NPTII gene for kanamycin resistance specific probe

with NPTII specific probe, a single band was evident in the H15 and B20 transgenic event, but no band was discernable in the parental event (Fig. 1). Hybridization with the CP gene specific probe also produced a single band when digested with *Eco*RI but two bands were apparent when digested with *Hind* III. This is explainable because the CP gene specific probe was made from full CP gene DNA sequence and the CP gene in the genomic DNA of transgenic plants contains one *Hind* III restriction site.

Flanking region and prediction of unintended effect

In *Agrobacterium*-mediated transformation, insertion of transferred DNA with specific plant genomic DNA sequences occurs randomly and can produce unusual, insertion-related outcomes. The unusual changes of transgenic plants can be detected by morphological and chemical analyses, although this approach can be onerous and is hindered by incomplete knowledge of unknown plant traits or chemicals. Localization of insertion location is most direct approach to predicting and identifying the possible occurrence of unintended effects; this can be achieved by direct sequencing of flanking DNA, despite the still-limited knowledge of plant genomes (Kuiper et al. 2001). The 5'-flanking sequence of the transgene in the H15 and B20 transgenic event were determined by PCR-based genome walking. Flanking sequences of about 3000 bp in H15 and 1000 bp in B20 were obtained. A 300 bp DNA flanking region adjacent to the vector region was used to determine the insertion site (Fig. 2). These processes were performed by searching known DNA sequences in an expressed sequence tag database using the BLAST algorithm. The search revealed

that the H15 transgene could be inserted into or adjacent to unidentified *Capsicum annuum* cDNA (Genebank accession no. GD102816 or CO907187). But, no significant similarity was found in a nucleotide database. The B20 transgene also was capable of similar insertions involving an ortholog in pepper of unidentified tomato or potato cDNA (Genebank accession nos. BG627797, CV505761, BG097242, or BI434579), and the insertion site displayed significant similarity with mRNA from root of *Solanum lycopersicum*. Thus, unintended effects are conceivable using the H15 and B20 event, and may be at least partly predictable based on knowledge of the insertion location, defined as a loss of function of the gene in the insertion region.

Multiplex PCR for detection and identification of transgenic plants

Detection of transgenic plants can be conducted by protein-based method such as Western blot and enzyme-linked immunosorbent assay (ELISA), or DNA-based methods including Southern blot and PCR (Ahmed 2002). Western and Southern blots and ELISA are highly specific but suffer from being comparatively time-consuming, costly and technical. In contrast, the PCR-based approach, especially multiplex PCR, is a simple and sensitive means of distinguishing transgenic plants (Yamaguchi et al. 2003). Multiplex PCR has been used to detect or identify three major transgenic crops: soybean (Roundup ready), maize (MON810, Bt11, event 176, T14/25 and GA21) canola (GT73, HCN92/28, MS8/RF3, Oxy235) (James et al. 2003, Margarit et al. 2005, Germini et al. 2004, Matsuoka et al. 2001, Yamaguchi et al. 2003).

vectors were used to differentiate transgenic event from non-transgenic event. Especially, two specific sense primers, pepper 15 F for H15 event and pepper 20 F for B20 event, which target the flanking region of each event, were used to identify the H15 and B20 event. Also, two specific primers for intrinsic pepper actin gene were used to confirm that the pepper DNA was used as the template for multiplex PCR. In both the H15 and B20 event, CP gene R2 (antisense primer) targeted the CP gene and P35S F (sense primer) targeted the 35s promoter primer pairs, CP gene F and CP gene R primer pairs targeted the CP gene, and Pepper actin F and Pepper actin R primer pairs targeted the gene encoding actin, with the amplified PCR products being 267 bp, 430 bp, and 1115 bp respec-

Table 1 Sequence and concentration of primers and template DNA concentration for the multiplex PCR.

Template DNA (ng)	Primer name	Sequence (5'-3')	Primer concentration (pmol)	Product length (bp)
40	Actin F	ATGGCAGATGAAGATATTCA	15	1115
	Actin R	GCACTGTATGACTGACACCA	15	
	CP gene F	GTAGGGAGTGAACGCTGTAGACC	6	430
	CP gene R	GAAGTACTAGCTCGTCCGTCTCG	6	
	P35s F	ACTATCCTTCGCAAGACCCTTC	14	267
	CP gene R2	CTACAGCGTTCCTCCCTACAAAG	14	
	Pepper 15 F	CACAGGATCGTGCTAAAGACCT	14	390
	P35s R	GTAAGCCTCTCTAACCATCTGTGG	5	
	Pepper 20 F	GCCATGACATGAATCGTTGC	10	596
	P35s R	GTAAGCCTCTCTAACCATCTGTGG	5

Fig. 3 Multiplex PCR analysis. (A) Primer design for multiplex PCR. (B,C) Multiplex PCR detection and identification of two transgenic peppers. Lanes: M, 1kb marker 1 and 7, actin (1115 bp) 2 and 8, P35S-B20 flanking region (596 bp) 3 and 9, CP gene (430 bp) 4 and 10, P35S-H15 flanking region (390 bp) 5 and 11, CP gene-P35S (267bp) 6 and 12, multiplex PCR by a set of nine primers

gene F (expected) and 677 bp product with P35S-F (unexpected) in both transgenic event, while the CP gene R2 primer resulted in a 1372 bp product with pepper 15 F (unexpected) product only in the H15 event. These results confirmed the specificity of two reverse primer targeting the CP gene se-

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per_product      cacagagatcgtgctaaagacctgggaaatgaggaaaggtgcccatagtggggaaacca
pepper_15       cacagagatcgtgctaaagacctgggaaatgaggaaaggtgcccatagtggggaaacca
pGWB506         -----
P3SS           -----

per_product      tggggcgccgacggggcagaattgtaaacatgatagtttaaacatgaaggcgggaacacg
pepper_15       tggggcgccgacggggcagaattgta-----aacatgatagtttaaacatgaaggcgggaacacg
pGWB506         -----
P3SS           -----

per_product      aatcgtatccaagctcaagctgctctagcatcggcatcaagctgcgcaactgtgggg
pepper_15       aatcgtatccaagctcaagctgctctagcatcggcatcaagctgcgcaactgtgggg
pGWB506         -----
P3SS           -----

per_product      agggcgatcgtgcggggcctctcgtattacgcacagctggcgaagggggagtgtcgc
pepper_15       agggcgatcgtgcggggcctctcgtattacgcacagctggcgaagggggagtgtcgc
pGWB506         -----
P3SS           -----

per_product      aaggcgataagtgggtaacgccagggtttcccgactcacgacgtgtgtaaacacgacgc
pepper_15       aaggcgataagtgggtaacgccagggtttcccgactcacgacgtgtgtaaacacgacgc
pGWB506         -----
P3SS           -----

per_product      cagtcgcaagcttgcatgectgcaggtccccagattagccttttcaatttcagaagaat
pepper_15       cagtcgcaagcttgcatgectgcaggtcccc-----agattagccttttcaatttcagaagaat
pGWB506         -----
P3SS           -----

per_product      gctaacccacagatggttagagagacttac
pepper_15       gctaacccacagatggttagagagacttac
pGWB506         -----
P3SS           gctaacccacagatggttagagagacttac

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E	<p>pcr_product pepper_20 pWB506 P35S</p> <p>gcacatgcatgaatcgttgcacacatcaatttttttttttgcgttttgcgttaataatcgc gcacatgcatgaatcgttgcacacatcaatttttttttttgcgttttgcgttaataatcgc -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>aatttttttttgcgttttttttcagaagagaaagagagagagagagagagagagagag aatttttttttgcgttttttttcagaagagaaagagagagagagagagagagagagag -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>tgaacaaacttgcgtgaatttactatcaagatcaaaagctctctcttttgcgtttctct tgaacaaacttgcgtgaatttactatcaagatcaaaagctctctcttttgcgtttctct -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>tctatcaagtttctatgctgaatttgcgttttgcgtttgtaaaactgtgaagagctc tctatcaagtttctatgctgaatttgcgttttgcgtttgtaaaactgtgaagagctc -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>ttttcagaatcaatagatatttgcgtcaaatctctctctctctctctctcaaatga ttttcagaatcaatagatatttgcgtcaaatctctctctctctctctctcaaatga -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>tagtctaaactgaagcgggaagagacatctgacccaagctcaagctgctctagacttc tagtctaaactgaagcgggaagagacatctgacccaagctcaagctgctctagacttc -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>gcacatcaggtgcgaactgttgggaagggcgtcgttgcggcctctctctctctctct gcacatcaggtgcgaactgttgggaagggcgtcgttgcggcctctctctctctctct -----</p>	<p>pcr_product pepper_20 pWB506 P35S</p> <p>ccagctggcgaaggggagctgctgcgaagcgtgaagtgggtgaagcagaggttttc -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>ccagtcacagcgttgaacacagcggcagtcgaacgtgaactgactgcaggtccccag ccagtcacagcgttgaacacagcggcagtcgaacgtgaactgactgcaggtccccag -----</p> <p>pcr_product pepper_20 pWB506 P35S</p> <p>attagccttttcaatttcagaagagatgaacccaagatggttagagagctctac attagccttttcaatttcagaagagatgaacccaagatggttagagagctctac -----</p>
F	<p>pcr_product P35S cp_gene</p> <p>actatccttcggaagacacttcctctatataaggaagctctctctcttcggaagacac actatccttcggaagacacttcctctatataaggaagctctctctcttcggaagacac -----</p> <p>pcr_product P35S cp_gene</p> <p>ggggagctctgaatgacaaactcgaatcaacagctgctggtcgaacgtgcagctgc g-----atgacaaactcgaatcaacagctgctggtcgaacgtgcagctgc</p> <p>pcr_product P35S cp_gene</p> <p>tcgcgtcgtggttcgcgttcgcgttcgcgttcgcgttcgcgttcgcgttcgcgttcgc tcgcgtcgtggttcgcgttcgcgttcgcgttcgcgttcgcgttcgcgttcgcgttcgc</p> <p>pcr_product P35S cp_gene</p> <p>gcagcagctttgcgacttaataagagcttgcagcgtggtcgtccaaactataacaccc gcagcagctttgcgacttaataagagcttgcagcgtggtcgtccaaactataacaccc -----</p> <p>pcr_product P35S cp_gene</p> <p>aaacttgcaggagtggaagctgtgagactgggtacagcttcaactctataacccaaa aaacttgcaggagtggaagctgtgagactgggtacagcttcaactctataacccaaa -----</p> <p>pcr_product P35S cp_gene</p> <p>gcacacaaactagacgtgggtctctatcaggttaaaaggttgtactaactgactcagt gcacacaaactagacgtgggtctctatcaggttaaaaggttgtactaactgactcagt -----</p> <p>pcr_product P35S cp_gene</p> <p>cacggaatataaagaagcttgcgttcgcgttcgaacttcaggttaactccttcgcgaa cacggaatataaagaagcttgcgttcgcgttcgaacttcaggttaactccttcgcgaa -----</p> <p>pcr_product P35S cp_gene</p> <p>attgattctcagctgtgggtgacagctcgttaaggttcctgcctcctcagacttactcgt attgattctcagctgtgggtgacagctcgttaaggttcctgcctcctcagacttactcgt -----</p>	<p>pcr_product P35S cp_gene</p> <p>tgcgcacact tgcgcacact</p> <p>pcr_product P35S cp_gene</p> <p>cgcactcgggtccagcgaacacaaactgtgtgactccttcggcgtgcagctgcga cgcactcgggtccagcgaacacaaactgtgtgactccttcggcgtgcagctgcga</p> <p>pcr_product P35S cp_gene</p> <p>tatagtgacatgagaagtagcgtcctcgtgactcgaagagcagctgcgtcagagac tatagtgacatgagaagtagcgtcctcgtgactcgaagagcagctgcgtcagagac</p> <p>pcr_product P35S cp_gene</p> <p>ggagcagctagtacttc ggagcagctagtacttc</p>
G	<p>pcr_product pWB506 P35S cp_gene</p> <p>ccagagctcgtgtaagagctgggaatgaggaagctgcccactgttgggggaacac -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>tgggctgcaggcgaagctgtaaacactgactggtttaaactgaagcgggaagcagc -----aaactgactggtttaaactgaagcgggaagcagc</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>aaactgacacagctcgaactgctcgaacttgcgttcgaactggaagctggaagctgga aaactgacacagctcgaactgctcgaacttgcgttcgaactggaagctggaagctgga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>aggcgatcgttggggcgttgcgttctcgaacttgcgttcgaactggaagctggaagctgga aggcgatcgttggggcgttgcgttctcgaacttgcgttcgaactggaagctggaagctgga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>aagcgatcagcttgggtgaagcagaggttttccagtcacagctgtgaacacagcggc aagcgatcagcttgggtgaagcagaggttttccagtcacagctgtgaacacagcggc -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>cagtcgaagcttgcgttcgaactcgcaggtccccagctgagcttctcaattcagaagagat cagtcgaagcttgcgttcgaactcgcaggtccccagctgagcttctcaattcagaagagat -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gctaacccacagctgttagagaggttagcagcagctctctcgaagcagctacccg -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>agcaaatctccaggaatcaaatcctctccaggaaggttagagctgagctcagaaga agcaaatctccaggaatcaaatcctctccaggaaggttagagctgagctcagaaga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>ttcagacttaactgcatcagaacacagaagagatatttctcagactcagaagact ttcagacttaactgcatcagaacacagaagagatatttctcagactcagaagact -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>attccagctatgagactcagagctgttctcgaacacagcaggttaagagacttga attccagctatgagactcagagctgttctcgaacacagcaggttaagagacttga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gtctctaaaaggtagtctccactgaacacagcaggttagctcagaactcgaagag gtctctaaaaggtagtctccactgaacacagcaggttagctcagaactcgaagag -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gactaacagactcgcgttaagactgggaagcttcaacagagctctctcagactc gactaacagactcgcgttaagactgggaagcttcaacagagctctctcagactc -----</p>	<p>pcr_product pWB506 P35S cp_gene</p> <p>aatgacagagaaactctcgtcgaactggtggaagcagcacttgcctactcaca aatgacagagaaactctcgtcgaactggtggaagcagcacttgcctactcaca -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>aatatcagaactcagctctcagaagacaaaggtctgagactttcacaacaggtga aatatcagaactcagctctcagaagacaaaggtctgagactttcacaacaggtga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>atatcgggaactcctcaggttccacttgcgcagctacttgcctctctctgagagta atatcgggaactcctcaggttccacttgcgcagctacttgcctctctctgagagta -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gtggaagagaggtggttctcgaactcgaactcgaactcgaactcgaactcgaactcga gtggaagagaggtggttctcgaactcgaactcgaactcgaactcgaactcgaactcga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gaagatgctcctggaagaggtggtcccaagctggaccccccacagcagagatctg gaagatgctcctggaagaggtggtcccaagctggaccccccacagcagagatctg -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gaaagagagagcttccacacagctctcgaagcagctggtgactgactcctcact gaaagagagagcttccacacagctctcgaagcagctggtgactgactcctcact -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gaactgaaggtggaacacacacactcctctcgaagcacttctctatataagga gaactgaaggtggaacacacacactcctctcgaagcacttctctatataagga -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>agttacttctcttgggaagacaggggagctcagactggaacactgactcaca agttacttctcttgggaagacaggggagctcagactggaacactgactcaca -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>gtcgtggtggaagcagagctcgtcgcgtggtggttcgcgttcgcgttcgcgttcgc gtcgtggtggaagcagagctcgtcgcgtggtggttcgcgttcgcgttcgcgttcgc -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>cggatgcttaactttagagctcttgcgcagagcttctgcgacttaacagagcttagag cggatgcttaactttagagctcttgcgcagagcttctgcgacttaacagagcttagag -----</p> <p>pcr_product pWB506 P35S cp_gene</p> <p>ctgggtcgaacttgaacacacacacttcttggaggtgaagctggtg ctgggtcgaacttgaacacacacacttcttggaggtgaagctggtg -----</p>

Fig. 4 Sequence comparison between PCR product and transgenic event harboring gene. (A) Actin (1115 bp). (B) CP gene (430 bp). (C) CP gene – P35S (267 bp). (D) P35S-pepper 15 flanking region (390 bp). (E) P35S-pepper 20 flanking region (596 bp). (F) cp gene-P35S (677 bp). (G) Cp gene-pepper 15 flanking region (1372 bp)

quence and so the reliability of our results, and also reveal another difference between the H15 and B20 event.

Cloning and sequencing of each band fractionated by electrophoresis was next performed. The sequence data confirmed that each band was due to primers used in this study, with each PCR product containing the proper sequence of vector and/or flanking sequence of the transgenic event (Fig. 4). Ideally, a multiplex PCR system capable of detecting transgenic plant targets like promoter and/or selection markers that is also capable of identifying specific event would be user-friendly and capable of simplifying the process of transgenic crop detection and identification (James et al. 2003). Also, the use of specific primer pairs targeting both the transgene and the flanking region of each transgenic event provides reliability because the possibility of missed analysis due to spontaneously infected virus is excluded.

Conclusion

We used primer pairs targeting promoter and transgene, and primers specificity targeted to both transgenic event, the efficient, reliable and simple detection and identification of virus resistance pepper transgenic event was achieved. A set of nine primers and PCR conditions used in this study may offer appropriate and simple detection and identification against virus resistance pepper transgenic event.

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