Li H-Q., Sautter C., Potrykus I. & Pounti-Kaelas J. 1996. Genetic transformation of cassava (*Manihot esculenta* Crantz.) Nature Biotechnology 14: 736–740.

Murashige T. & Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant. 15:* 473–497.

Raemakers C.J.J.M., Rozenboom-Schippers M.G.M., Jacobsen E. & Visser R.G.F. 1996. Maturation of Friable Embryos and Germination into Plantlets (in this proceedings)

Schenk R. & Hildebrandt A. 1972. Medium and techniques for induction and growth of monocotyledonousand dicotyledonous plant cell cultures. Can. J. Bot. 50: 199–204.

Schöpke C., Taylor N.J., Carcamo R., Konan N.K., Marmey P., Henshaw G.G., Beachy R.N. & Fauquet C. 1996. Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz.) from microbombarded embryogenic suspesnion cultures. Nature Biotechnology 14: 731–735.

Taylor N.J., Clarke M.& Henshaw G.G. 1993. The induction of somatic embryogenesis infifteen African and one South American cassava cultivars. In: Roca W.R. & Thro A.M. (eds.) Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, 25 – 28th August 1992. Cali, Colombia: Centro International de Agricultura Tropical 1993.

Taylor N.J., Edwards M., Kieran R.J., Davey C.D.M., Blakesley D. & Henshaw G.G. 1996. Development of friable embryogenic callus and suspension culture systems in cassava (*Manihot esculenta* Crantz). Biotechnology 14: 726–730.

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The cassava vein mosaic virus promoter: A new promoter for cassava genetic engineering

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Abstract/Résumé

The cassava vein mosaic virus (CVMV) is a double stranded DNA virus which infects cassava plants in northern Brazil and has been characterized as a plant pararetrovirus belonging to the caulimovirus subgroup. Two DNA fragments, CVPI of 388 nucleotides from position -368 to +20 and CVP2 of 511 nucleotides from position -443 to +72, were isolated from the viral genome and fused to the *uidA* reporter gene to test promoter expression. Both promoter fragments were able to cause high levels of gene expression in protoplasts isolated from cassava and tobacco cell suspensions. The expression pattern of the CVMV promoters was analyzed in transgenic tobacco and rice plants, and revealed that the GUS staining pattern was similar for each construct and in both plants. The two promoter fragments were active in all plant organs tested and in a variety of cell types, suggesting a near constitutive pattern of expression. Particle bombardments of cassava tissue provide the first evidence that the CVMV promoter is strongly expressed in this important crop. An *nptII*:CVMV fusion gene construct used for selection of cassava transgenic tissue is the first biotechnology application of this new promoter.

Key words/Mots clés: cassava, cassava vein mosaic virus promoter, genetic engineering

Le promoteur du virus de la mosaique des nervures du manioc: un nouveau promoteur pour le genie genetique du manioc

Le virus de la mosaïque des nervures du manioc (CVMV) est un virus à ADN bicaténaire qui infecte les plants de manioc dans le Nord du Bresil. Ce virus est un pararetrovirus et un membre possible du genre *Caulimovirus*. Plusieurs promoteurs transcriptionnels puissants ont été isolés des génomes de pararetrovirus de plantes. Le promoteur 35S du virus de la mosaïque du choufleur est le meilleur exemple d'un tel promoteur. On pouvait donc s'attendre a ce que CVMV contienne un promoteur fort pour l'expression de gènes dans le manioc. Les auteurs ont isolé deux fragments d'ADN de la region presumee promotrice du génome du CVMV, de tailles différentes et contenant un motif du type TATA. Leur aptitude à exprimer des gènes dans les plantes a été testée grace a un gène de fusion *uidA* (codant pour la β-D-glucuronidase, GUS). Les deux fragments d'ADN ont induit l'expression de genes dans des protoplastes isolés de suspensions cellulaires de manioc et de tabac, à de très hauts niveaux. Le fragment le plus large a conféré un niveau d'expression du même ordre de grandeur que le promoteur 35S amélioré. Le mode d'expression du promoteur du CVMV fusionné au gène *uidA* a ete aussi analyse dans les plantes transgeniques de tabac et de riz. Chez les deux espèces, les fragments d'ADN viral ont induit l'expression de gènes, aussi bien dans tous les organes de la plante testée que dans plusieurs types cellulaires, suggérant un mode d'expression constitutif. L'activité GUS est la plus élevée dans les éléments vasculaires, les cellules du mésophylle et les apex racinaires. La preuve de l'activité du promoteur dans les plantes de manioc a été aussi fournie par microbombardement de plantules *in vitro*. Un construit CVMV: gène de fusion *nptII*, pour la résistance a la kanamycine, a aussi été testé dans des expériences de transformation de manioc. Ce construit a été utilisé avec succès pour sélectionner des tissus transgeniques de manioc. Ces données suggerent que le promoteur du CVMV est très actif chez le manioc. Il représente donc une alternative au promoteur CaMV 35S pour conduire l'expression de gènes étrangers dans cette importante culture alimentaire.

Introduction

The cassava vein mosaic virus (CVMV) is a pararetrovirus which infects cassava plants in northeastern regions of Brazil. The CVMV is considered to be a member of caulimovirus group. Indeed, the CVMV has spherical particles of 50–60 nm diameter occurring in cytoplasmic inclusion bodies (Kitajima et al. 1966) and contains a circular double stranded DNA genome of 8158 nucleotides. However, the molecular characterization of the CVMV genome (Calvert et al. 1995), organized in five open reading frames is different from other characterized plant pararetroviruses. Several transcriptional promoters which are capable of causing high levels of gene expression in transgenic plants have been isolated from pararetrovirus genomes. The 35S promoter directing the genome length RNA transcription of the cauliflower mosaic virus (CaMV) (Benfey & Chua 1990) has been widely used in plant genetic engineering and is among the strongest plant transcriptional promoters. As a caulimovirus infecting cassava, the CVMV is a strong candidate to isolate a high expressing promoter for foreign genes in this important crop.

The present study was undertaken to identify the promoter region of the cassava vein mosaic virus and to determine its ability to promote gene expression in transgenic plants. In this report we describe the isolation of two CVMV promoter fragments and provide evidence of promoter activity using uidA gene fusion constructs. The strength of these promoters was evaluated in electrophorated protoplasts of cassava and tobacco cell suspension cultures. The pattern of expression was characterized in stably transformed rice and tobacco plants. Evidence of promoter activity in cassava plants was provided by micro-bombardments of embryogenic suspension culture and in vitro plantlets.

Materials and Methods

Isolation of promoter fragments and plasmid constructions. Nucleotide numbers refer to the cassava vein mosaic virus genome nucleotide sequence reported by Calvert et al. (1995). The original CVMV full length genomic clone was provided by Dr. R.J. Shepherd (University of Kentucky). Restriction fragments derived directly from the genomic clone were cloned into pUC119 plasmid. Using these subclones, two viral DNA fragments containing a consensus TATA box motif were isolated. A fragment designated CVP1 encompassed CVMV nucleotides 7235 to 7623 and was obtained by *Alu*I enzymatic digestion. A larger fragment containing CVMV nucleotides 7160 to 7675 and designated CVP2, was isolated by PCR amplification using specific primers containing restriction sites at their 5′ ends. Plasmid pILTAB:CVP1 and pILTAB:CVP2 (Figure 1A) were constructed by ligating the promoter fragments into pGN100, a pUC 119 derived plasmid containing the uidA coding sequence linked to the 3′ polyadenylation signal of the nopaline synthase gene (nos 3′). The CVP2 promoter was fused with the coding sequence of the *nptII* gene and cloned in a pMOM 999 vector (Monsanto Corp.) deleted of its 35S CaMV promoter. This modified plasmid was designed pILTAB-CVN (Figure 1B). The plasmid pe35GN contains the enhanced 35S promoter (Kay et al. 1987) and the uidA coding sequence linked to the nos 3′ end. The plasmid

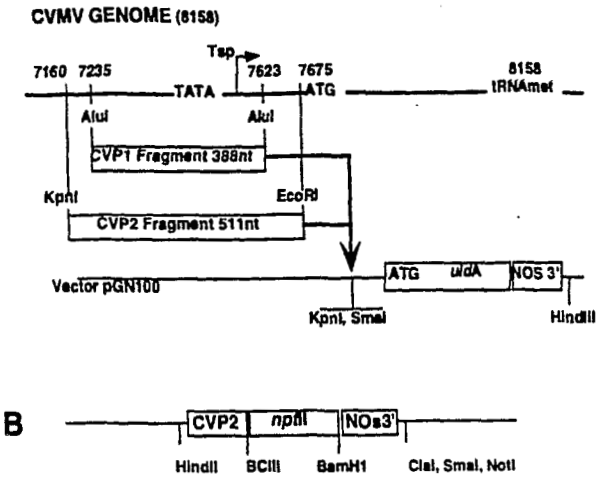

Figure 1. A) Construction of CVMV fusion genes. Positions of the promoter fragments are numbered in the CVMV genomic DNA. The CVP1 fragment was isolated using *Alu*I restriction sites while the CVP2 fragment, which included an additional 75 nucleotides at the 5′ end and 52 nucleotides at the 3′ end, was obtained by PCR amplification using appropriate primers. The 3′ end of CVP2 is just upstream of the first ATG codon in the viral genome. The transcription start site is indicated. This chart is not drawn to scale. (Tsp: transcription start site). B) Schematic representation of pILTAB-CVN.

pDO432 contains the luciferase coding sequence from *Photinus pyralis* under the control of the 35S promoter (Ow et al. 1986).

Plant transformation. Transformed tobacco plants (*Nicotiana tabacum* cv. Xanthi NN) were produced via *Agrobacterium tumefaciens* according to the procedure described by Horsch et al. (1988). Seven independent transgenic lines containing the CVP1 construct and eight containing the CVP2 construct were produced. Seven transgenic rice lines (*Oryza sativa* L. Taipei 309) were obtained via particle bombardment as described by Li et al. (1993) using pILTAB:CVP2 plasmid.

Histochemical localization of ß-glucuronidase activity. Histochemical analysis of GUS activity was performed essentially as described by Jefferson et al. (1987). Hand-cut tissue sections were taken and cleared in 70% ethanol. Stained sections were visualized in a Zeiss microscope.

Protoplast isolation, treatment and culture. Tobacco protoplasts were isolated from BY-2 (*Nicotiana tabacum* L. cv. Bright Yellow) cell suspension cultures and transfected with DNA essentially as described by Kikkawa et al. (1982). Cassava protoplasts were prepared from *Manihot esculenta* L. cv. TMS 60444 embryogenic cell suspension cultures (Taylor et al. 1996). Cells were collected from 50 ml of a 10 day old culture and digested in 50 ml of medium containing 0.55 M mannitol, 3.2 g/l Schenk and Hildebrandt salts (Sigma), 1 x Murashige and Skoog vitamins (Sigma), 20 mM $CaCl_2$, 2% cellulase Onozuka RS and 0.1% Pectolyase Y 23, pH 5.8 (medium A). After 4 hours at 27°C, the incubation mixture was filtered sequentially through sieves of 100 µM and 70 µm. Protoplasts were washed 3 x by centrifugation at 100 x

g for 10 min in medium A without the enzymes. The purified protoplasts were resuspended to final density of 10^6 cells/ml in electroporation buffer containing 5mM MES, 130 mM NaCl, 10 mM $CaCl_2$, 0.45 M mannitol, pH 5.8. Two hundred µl of electroporation buffer containing 30 µg of each plasmid was added to 800 µl of protoplast suspension in a 0.4 cm path-length cuvette. DNA uptake was carried out using a Gene Pulser instrument (Biorad) delivering a 300 V pulse at a capacitance of 500 µF. After electroporation, the protoplasts were incubated in ice for 30 min, after which they were resuspended at a density of 10^5 cell/ml in culture medium A supplemented with 20% sucrose and $5x10^{-5}$ M Pichloran. After 24 hours of incubation in the dark at 27°C, the protoplasts were collected by centrifugation (10 min at 100 x g) and resuspended in GUS extraction buffer (Jefferson et al. 1987) pH 7.7.

LUC and MUG assays. Transfected protoplasts were lysed by vortexing for 2 min in GUS extraction buffer, pH 7.7. Extracts were clarified by centrifugation (5000 x g, 5 min) at 4°C in a microcentrifuge. The supernatant was recovered and used for MUG and LUC assays. GUS activity was determined using 4-methyl-umbelliferyl-ß-D-glucuronide (MUG-Sigma) by the method of Jefferson (Jefferson et al. 1987) and quantified for 50 µl of extract as pmol methylumbelliferone (MU) per hour. LUC activity was determined on 50 µl of the same protein extract with a luminometer (Monolight 2010) using a luciferase assay (Analytical Luminescence Laboratory, San Diego, CA). Cotransfection of cells with *uidA* gene plus a luciferase plasmid allowed us to normalize variations of GUS activity between experiments.

Particle bombardment. Leaves and stems were cut from cassava plantlets (cultivar MCol 1505) grown in vitro and subsequently arranged in the center of 9 cm petri-dishes containing solidified culture medium. Micro-bombardment was performed with an helium-driven particle delivery system (PDS 1000/He-Biorad). Preparation of gold particles (average diameter 1.6 µm) and coating particles with DNA were carried out essentially as described by the instruction manual (Bio-Rad). The target plates were installed in the gun chamber as indicated by Schopke et al. (1966). Each plate was shot twice at a pressure of 1100 PSI with approximately 1 µg of plasmid DNA. Explants were incubated 2 days in the dark at 25°C prior to histochemical GUS assays. Bombardment of embryogenic suspension cultures (cultivars TMS 60444, TMS 60142, Bonoua Rouge, Kataoli, and Okouta) were carried out as described (Schopke et al. 1996).

Results and Discussion

Isolation of the CVMV promoter. Sequence analysis of the CVMV genomic clone allowed us to identify in the viral genome, an AT rich region similar to that of the plant pararetroviruses TATA box regions. The location of the putative TATA box sequence in CVMV is similar to the promoters that control the transcription of the genome length transcripts in other caulimoviruses. This information allowed us to predict the location of a putative promoter in the CVMV genome. Two DNA fragments were subsequently isolated and tested for promoter expression: CVP1 comprising 387 nucleotides from position -368 to +20 (+1 corresponds to the transcription start site), and CVP2, a fragment of 514 nucleotides from position -443 to +72 (Figure 1). Both fragments were cloned into a plasmid that contains the uidA coding sequence for analysis of promoter activity. Sequence analysis showed only limited homology with other promoters suggesting that we had isolated a distinct plant pararetrovirus promoter. The transcription start site of the CVMV promoter was determined by primer extension analysis (Verdaguer et al. 1996) using total RNA recovered from transgenic tobacco plants which harbored the CVP1: *uidA* fusion gene. A single major extension product was detected and mapped to an adenine residue (nt. 7604) located 35 nucleotides downstream of the putative TATA box.

Expression of the CVMV promoter in protoplasts. We used in this experiment the plasmids pILTAB:CVP1 and pILTAB:CVP2 The plasmid pe35GN, containing the *uidA* sequence under the control of the enhanced 35S promoter (e35S), served as a positive control. Each plasmid was cointroduced into protoplasts with a plasmid containing a luciferase gene under the control of the CaMV 35S promoter. The GUS/LUC ratio was determined after each transfection experiment. Four independent transfection experiments, summarized in Figure 2, were carried out and gave similar results. In tobacco protoplasts the GUS/LUC ratio for the CVP1 promoter was 0.58, or about 50% of the level of expression determined by the e35S promoter (1.32). However, when the CVP2 fragment was used, the ratio was 1.3, or two fold more active than CVP1. The difference between the two fragments indicated that CVP1 lacks one or more important element(s) for high level expression. The difference in the level of expression could be due to a transcriptional enhancer in the 5′ region of the larger fragment or to the larger untranslated leader sequence. The CVP2 and e35S promoters yielded similar GUS activity indicating that the CVMV promoter is a strong promoter in tobacco protoplasts. Similar studies in cassava protoplasts gave results comparable to those

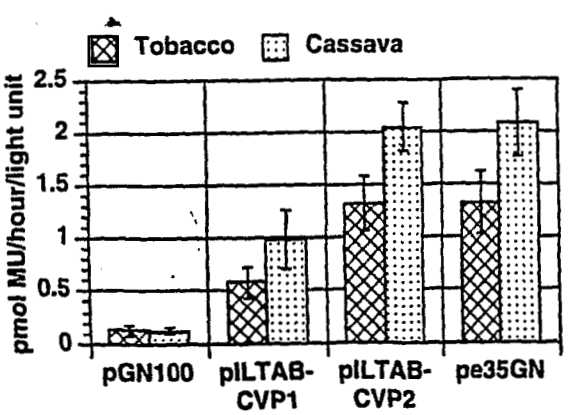

Figure 2. Expression of the CVMV promoters in tobacco and cassava protoplasts. Promoter activity is expressed as a ratio between GUS activity and LUC activity of the same protein extract. As a consequence, GUS activity is measured as pmol 4-methylumbelliferyl-ß-D-glucuronide (MUG) per hour per unit of light emitted. Bars represent the average of four independent experiments +/- standard errors. pe35GN contains the enhanced 35S promoter (see Materials and Methods) while pGN100 contains a promoter-less *uidA* gene.

in tobacco showing that the CVMV promoter is also very effective in these cells.

Expression of the CVMV promoter in transgenic tobacco and rice plants. When this study was initiated the cassava genetic transformation was still to be achieved. Therefore we chose to express the CVMV promoter in "heterologous" plants as tobacco and rice which are easily transformed. The analysis of the CVMV expression pattern in these two different systems gave us good indications about promoter activity in transformed plants. A detailed histochemical analysis of GUS accumulation was carried out using hand-cut fresh tissue sections of various organs from primary transformed plants and their R1 progeny. All transformed tobacco plants containing either the CVP1 or CVP2 fragment had essentially the same gene expression pattern while intensity of staining varied among transformants. Also, the general expression pattern of the CVP2 promoter-uidA gene was quite similar in rice and tobacco, despite the differences in anatomy of these plants. In tobacco leaves, intense staining was observed in phloem tissues, in the cells of the palisade layer and the spongy mesophyll. The parenchyma cells of the midrib did not contain detectable GUS activity (Figures 3A, 3B) except for the chlorenchyma cells just below the epidermis (Figure 3C). In the epidermis, guard cells and trichomes developed an intense staining. Cross-sections of tobacco stems showed strong staining of the internal and external phloem cells (Figure 3C). GUS staining was not detectable in pith cells or in cortical parenchyma cells of the stem. Root tissues incubated with X-Gluc revealed a blue stained vascular cylinder (Figure 3D). The root tips stained the most intensely of any region of the root. As observed in tobacco plants, transverse sections of rice leaves incubated with X-Gluc substrate resulted in strong staining in the vascular bundles and in the mesophyll cells (Figure 3E). Bundle sheath cells, bulliform cells and sclerenchyma fibers showed no staining.

Analysis of transgenic plants indicated that the CVMV promoter, as is the case with other caulimovirus promoters, is active in all organs, in various cell types and is not dependent on viral trans-acting factors. The CVMV promoter is strongly

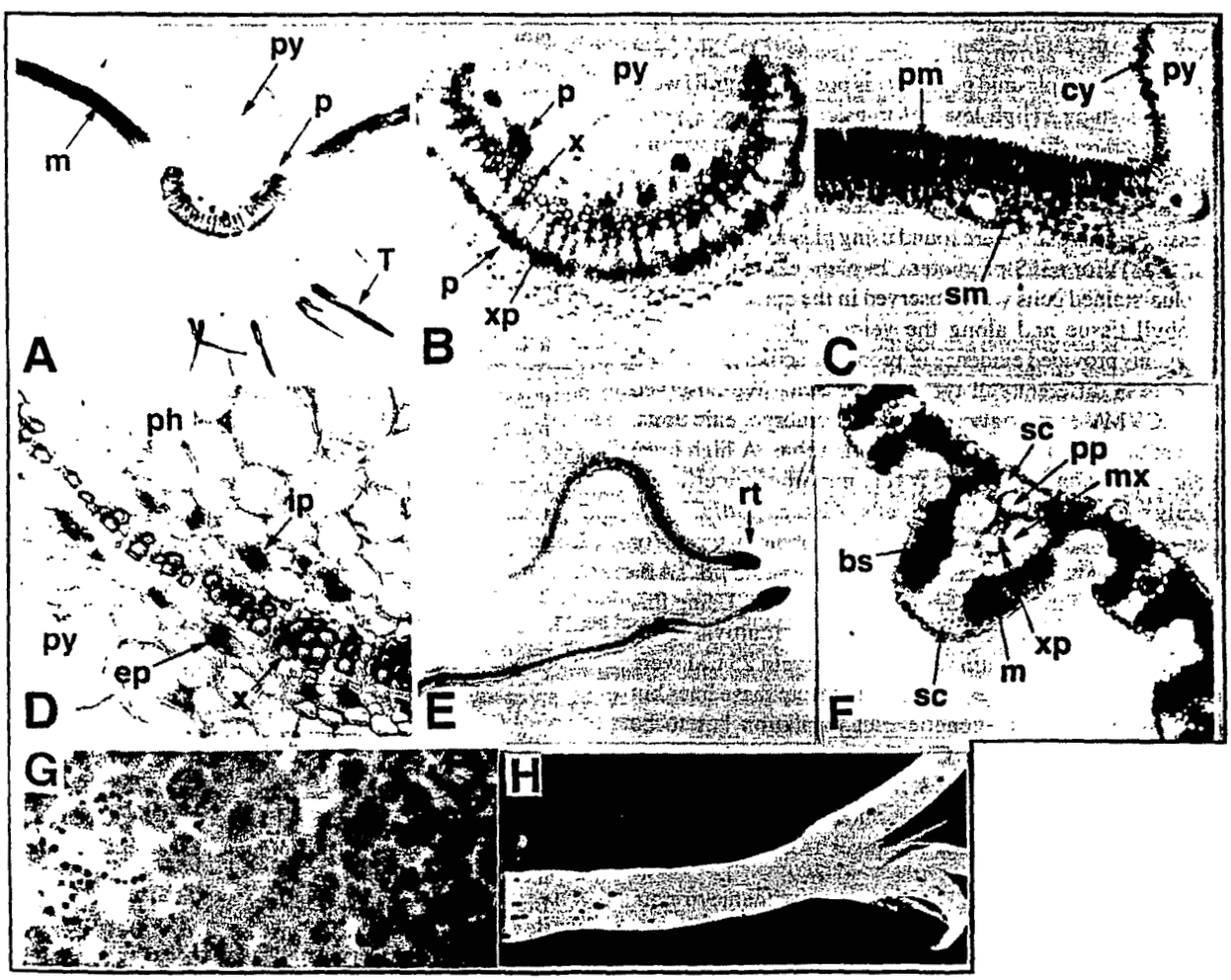

Figure 3. GUS expression in plant tissues containing CVMV promoter-*uidA* gene fusion. GUS activity is indicated by an indigo dye precipitate after staining with X-Gluc. A: Transgenic tobacco leaf section. B: Detail of transgenic tobacco leaf section showing vascular tissues of the midrib (x10). C: Transverse section through transgenic tobacco leaf lamina (x30). D: Tobacco roots (x10). E: Cross-section of a transgenic rice leaf sheath (x50). F: GUS transient expression in embryogenic suspension culture. G: GUS transient expression on cassava stem from in vitro plantlet (x5). bs: bundle sheath; cy: chlorenchyma; ep: external phloem; ip: internal phloem; m: mesophyll; mx: metaxylem; p: phloem; ph: pith; pm: palisade mesophyll; pp: phloem parenchyma; py: parenchyma; rt: root tip; sc: sclerenchyma; sm: spongy mesophyll; x: xylem; xp: xylem parenchyma.

expressed in vascular tissues, in leaf mesophyll cells, and in the root tips or rice and tobacco plants. However, GUS activity was nondetectable in non-chlorophyllaceous cells of tobacco pith and cortical parenchyma. This could indicate that the CVMV promoter has two major domains of activity, i.e., the vascular elements and the green, chloroplast-containing cells. However, we cannot exclude the possibility that these observations are due to the limitations of the staining assay.

GUS activity in extracts prepared from different organs was determined among tobacco and rice transformed plants using the 4-methylumbelliferyl-ß-D-glucuronide (MUG) fluorescence assay (not shown). The result of this experiment indicated that the CVMV promoter (CVP1 and CVP2 constructs) is expressed in all organs of transformed rice and tobacco confirming the histochemical analysis previously described.

Activity of the CVMV promoter in cassava plant explants. Particle bombardment experiments were undertaken on embryogenic suspension cultures as well as on various explants from *in vitro* grown cassava plantlets. These experiments were initiated to obtain preliminary data on CVMV promoter activity in cassava tissues. The plasmid pILTAB:CV2 and plasmid pe35GN (as positive control) were used in this study. High level of transient gene expression was observed on different suspension cultures from various cultivars (see Materials and Methods). Approximately the same number of intensely blue-stained foci showing GUS expression (Figure 3F) were found using plasmids containing either CVMV or e35S promoter. In plant explants (Figure 3G), blue-stained cells were observed in the epidermis layer, mesophyll tissue and along the veins of leaflets. These experiments provided evidence of promoter activity for CVP2 fragments in different cell types of cassava. We observed that the CVMV is strongly expressed in embryogenic units, preferential target for cassava transformation. A high level of gene expression at this stage is very important for efficient and early selection of transformed cells. Also, an *nptII*:CVMV fusion gene (coding for the neomycin phosphotransferase for resistance to kanamycin) was constructed (plasmid pILTAB-CVN) to be used in transformation experiments. Using this plasmid, several putative transformed lines (cultivar TMS 60444) growing on medium with paromomycin 25 µM were produced. Some plants were regenerated from these lines but molecular evidence for genetic transformation has to be provided. Efficiency of the CVMV promoter construct for selection of transgenic tissues has to be confirmed by a comparison analysis with other promoters (namely the 35S CaMV promoter and the nos promoter from the nopaline synthases gene).

Conclusion

We have isolated a new promoter from the genome of the cassava vein mosaic virus. This promoter is able to drive high level gene expression. In protoplasts, the larger CVMV promoter fragment conferred a gene expression level in the same range than the enhanced 35S CaMV promoter. In plants the CVMV promoter was also strongly expressed with an apparent constitutive nature. Preliminary experiments suggest that the CVMV promoter is also very active in cassava. This promoter is a new alternative to the largely used 35S promoter and will be used by ILTAB to express genes of interest in transgenic cassava plants.

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References

Banfey P.N. & Chua N.H. 1990. The cauliflower mosaic virus 35S promoter: combinatorial regulation of transcription in plants. Science 250: 959–96.

Calvert L., Ospina M. & Shepherd R.J. 1995. Characterization of cassava vein mosaic virus: a distinct plant pararetrovirus. Journal of General Virology 76: 1271–1276.

Horsch R.B., Fry J., Hoffmann N., Neidermeyer J., Rogers S.G. & Fraley R.T. 1988. Leaf disc transformation. In: Gelvin S.B. & Schilperoort R.A. (eds.). Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht. p. A5/1-A5/9.

Jefferson R.A., Kavanagh T.A. & Brevan M.W. 1987. Gus fusion: ß-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. Embo Journal 6: 3901–3907.

Kay R., Chan A., Daly M. & McPherson J. 1987. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 236: 1299–1302.

Kikkawa H., Nagata T., Matsui C. & Takebe I. 1982. Infection of protoplasts from tobacco suspension culture by tobacco mosaic virus. Journal of General Virology 63: 457–467.

Kitajima E.W. & Costa A.S. 1966. Particular esferoidais associadas ao virus do mosaico das nervuras da mandioca. Bragantia 25: 211–221.

Li L., Qu R., de Kochko A., Fauquet C. & Beachy R.N. 1993. An improved rice transformation system using a biolistic method. Plant Cell Reports 12: 250–255.

Ow D.W., Wood H.V., Deluca M., de Wet J.R., Helinski D.R. & Howell S.H. 1986. Transient and stable expression of the firefly luciferase gene in plants. Science 234: 856–859.

Schopke C., Taylor N.J., Carcamo R., Konan K., Marmey P., Henshaw G., Beachy R.N. & Fauquet C. 1996. Regeneration of transgenic cassava plants (*Manihot esculenta* Crantz) from microbombarded embryogenic suspension cultures. Nature Biotechnology 14: 731–735.

Taylor N.J., Edwards M., Kiernan R.J., Davey C.D., Blakesley D. & Henshaw G.G. 1996. Development of friable embryogenic callus embrogenic callus and embryogenic suspension culture systems in cassava (*Manihot esculenta* Crantz). Nature Biotechnology 14: 726–730.

Verdaguer B., de Kochko A., Beachy R.N. & Fauquet C. 1996. Isolation and expression in transgenic tobacco and rice plants, of the cassava vein mosaic virus (CVMV) promoter. Plant Molecular Biology 31: 1121–1139.