A *Cassava vein mosaic virus* promoter cassette induces high and stable gene expression in clonally propagated transgenic cassava (*Manihot esculenta* Crantz)

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ABSTRACT

The study described a T-DNA vector with a *Cassava vein mosaic virus* promoter cassette (pCsVMV) and a kanamycin selectable marker gene driven by the 35S *Cauliflower mosaic virus* promoter with a view to stably express transgenes over repeated cycles of clonal propagation. A *β-glucuronidase* reporter gene under control of pCsVMV (pCsVMV-GUS) was introduced into the cassava landrace 'Tokunbo' via *Agrobacterium*-mediated genetic transformation. Transgenic tobacco plants (*Nicotiana tabacum* SR1) with the same gene construct were also produced. In tobacco, the pCsVMV-GUS was highly expressed in all tissues tested such as leaf, stem, petiole, and roots. In transgenic cassava, the pCsVMV-GUS gene was highly expressed in all tissues and most cell types of in vitro plants including leaf, stem, petiole, and fibrous roots. The pCsVMV-GUS gene was also highly expressed in these tissues as well as in tubers of greenhouse grown cassava. High and stable pCsVMV-GUS gene expression was maintained over 3 cycles of ratooning under greenhouse conditions, thus showing the absence of undesired gene silencing effects after repeated in vitro subculturing and vegetative propagation. From the high constitutive levels of GUS activity observed, the study concluded that the CsVMV promoter cassette was useful for high-level expression in cassava over repeated cycles of clonal propagation.

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is a root crop and serves as an important source of energy in the diet of 600 million people in tropical countries where food deficiency and malnutrition are common. In addition, cassava is used as raw material for industrial products such as starch, flour, and pharmaceuticals (Rickard et al., 1991). The increased cultivation of cassava is attributed to unique characteristics of cassava crop (Nweke et al., 2002). When growth conditions are adequate, it gives high tuber yield. Cassava is tolerant of drought and soil acidity and grows well in less fertile and marginal soils (El-Sharkawy, 2004). Mature cassava tubers can be left in situ in the field until favorable harvesting, processing and marketing conditions are available (Ceballos et al., 2004). There are several major problems that limit the production and use of cassava tubers by farmers and industry. For example, diseases and pests can significantly reduce yield if left uncontrolled. The storage roots of cassava are rich in starch (70–90% of their dry weight) but deficient in protein and other micronutrients (Cock, 1985). Moreover, once harvested, the roots are subjected to rapid postharvest physiological deterioration, which constrains their storage and marketing (Plumbley and Rickard, 1991).

Unlike many of the world's major crop plants, conventional breeding to solve major production problems aforementioned is difficult, long and cumbersome. Genetic improvement of cassava through sexual crosses is limited because many varieties rarely flower and seed production is often low. Also, cassava is highly heterozygous and suffers from high inbreeding depression (Ceballos et al., 2004). In the field, cassava is typically propagated clonally by stem cuttings. This propagation strategy is ideal for a transgenic approach to crop improvement as gene segregation through outcrossing is limited (Ihemere et al., 2006). The successful use of transgenic approach for improvement of cassava depends on the availability and effectiveness of promoters to drive transgene expression to sufficient levels in tissues of interest and on stable transgene expression after multiple cycles of vegetative propagation.

A number of promoters have been identified that confer high level of constitutive expression of heterologous genes in transgenic plants including cassava. The 35S promoter from the Cauliflower mosaic virus (CaMV) in various configurations has been the most widely used constitutive promoter in cassava (Li et al., 1996; Zhang et al., 2000). However,

the repeated (duplication) inclusion of CaMV 35S promoter sequence in plant transformation vectors has been implicated in unwanted gene silencing effects. For instance, a 35S-driven transgene expressing the *Arabidopsis* constitutive photomorphogenesis1 (COP1) gene protein as a fusion to β-glucuronidase (GUS) or green fluorescent protein (GFP) results in the homology-dependent transcriptional silencing of the endogenous *COP1* gene, as evident by a characteristic *COP1* mutant phenotype at the adult vegetative stage (Qin et al., 2003). Similarly, in gentian plant, transcriptional gene silencing by methylation in the promoter sequences of transgenic plants carrying a T-DNA construct consisting of a tandem repeat of two 35S promoter is reported to be homology-dependent gene silencing involving the promoter region (Mishiba et al., 2005). Thus, there is a need to construct transformation vectors with different strong promoters to reduce incidences of gene silencing triggered by repeated promoter sequences. One of such suitable promoters that confer high level of gene expression in transgenic plants is the cassava vein mosaic virus (CsVMV) promoter (Verdaguer et al., 1996). Previous studies showed that the levels of activity and differential expression pattern of a "constitutive" promoter can vary substantially depending on the plant species and tissue type. For example, earlier transformation study on CsVMV promoter in tobacco and rice plants reported that the promoter was active in all plant organs tested and in a variety of cell types, suggesting a near constitutive pattern of expression (Verdaguer et al., 1996). However, in grape, the CsVMV promoter was highly active in somatic embryo tissues but produced low level of expression in roots through all developmental stages (Li et al., 2001). By comparison, the CsVMV promoter drives higher transgene expression than CaMV 35S promoter in the leaves, roots, and nodules of transgenic alfalfa (Samac et al., 2004) and matured soybean root nodules (Govindarajulu et al., 2008). Although several studies examined promoter expression in cassava (Gonzalez et al., 1998; Zhang et al., 2000), the CsVMV promoter has not been examined in stably transformed cassava plants. Additional knowledge on the functionality of alternative promoter like CsVMV in cassava is essential to improve the efficacy of transgene expression in this economically important crop.

This study sets out to: (i) construct a T-DNA vector with a CsVMV promoter cassette to drive expression of a gene of interest in combination with a 35S CaMV promoter driving a kanamycin selectable marker gene; (ii) examine the expression level and pattern of the CsVMV promoter in stably transformed cassava tissues and organs; and (iii) evaluate the expression level of this CsVMV promoter cassette in transgenic cassava plants after repeated cycles of vegetative propagation.

2. Materials and methods

2.1. Plant materials and growth conditions

Cassava cultivar TME 12 plantlets were obtained from the in vitro germplasm collection of Tissue Culture Laboratory, IITA, Ibadan. The plantlets were maintained on MS medium (Murashige and Skoog, 1962; Sigma) supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and adjusted to pH 5.8 before autoclaving for 20 min at 121 °C at 25 ± 2 °C under a 16-h photoperiod (90 μmol m^{-2} s^{-1}) and subcultured at 4-week interval. Tobacco (*Nicotiana tabaccum* cv SRI) were grown from seeds. Growth media and conditions used for tobacco plants were as described by Oyelakin (2009). All somatic embryo cultures were kept in the dark at 25 ± 2 °C and subcultures were made at 4-week interval. Prior to transformation, immature cyclic somatic embryos were incubated under light to generate green somatic embryos. In vitro transformed cassava and tobacco plantlets with well-developed roots were rinsed with water to wash off the agar medium and transplanted to peat pellet (AS Jiffy Products Ltd, Norway) in plastic pots that were covered to maintain high humidity. The plants were grown at 22–26 °C in a containment facility for 3 weeks and transferred to the greenhouse where they were grown to maturity.

2.2. Construction of plant transformation vector

An expression cassette was assembled in silico by merging a 515-bp promoter fragment from CsVMV corresponding to position 7171–7675 of the CsVMV genome (Calvert et al., 1995) to a 265-bp fragment encompassing the 3′end of the *nopaline synthase* gene (Yanisch-Perron et al., 1985) with in between a 45-bp multiple cloning site. This sequence was flanked by *Eco*RI and *Hin*dIII sites at the 5′ and 3′ end respectively. This 837 bp module was synthetized (Genscript, NY, USA) and subcloned into the *Eco*RI and *Hin*dIII sites of pCAMBIA2300 (Jefferson et al., 1987) yielding pING71 (Fig. 1a).

Next, the *Uid*A coding sequence was isolated from pATAG3 (Goossens et al., 1999) via PCR using OLIV3: 5′-TCAAGGTACCAA CC**ATG**GTCCGTCCTGTAGAAAC (start codon in bold) and OLIV16: 5′-TACATATACTGCAG**TCA**TTGTTTGCCTCCCTGCTG (stop codon in bold) and the resulting 2.0-kb fragment was subcloned into *Kpn*I/*Pst*I digested pING71, resulting in pOYE153 (Fig. 1b). The transformation vector was introduced into *Agrobacterium tumefaciens* strain LBA4404 by the freeze–thaw method as described by Burrow et al. (1990).

2.3. Transformation of cassava and tobacco

Primary and secondary somatic embryos were produced as described by Jorgensen et al. (2005). Primary somatic embryos were obtained by isolating enlarged shoot meristem, taken from 4-week old in-vitro plantlets of cassava cultivar TME 12, from incubated nodal stem segment on MS medium (Murashige and Skoog, 1962; Sigma) supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 6-benzylaminopurine (10 mg/l) for seven days. The isolated meristems were incubated on MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 10 mg/l picloram. After 2 weeks, primary somatic embryos were induced. The globular-and-torpedo- shaped embryo matured on MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 6-benzylaminopurine (0.1 mg/l). When embryos reached the cotyledonary stage, the cotyledons were harvested and placed on MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 2,4-dichlorophenoxyacetic acid (6 mg/l) to induce formation of secondary embryos. Mature (green) somatic embryos were produced by transferring cotyledons of secondary somatic embryos on MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 2, 4-dichlorophenoxyacetic acid (6 mg/l) to induce embryos
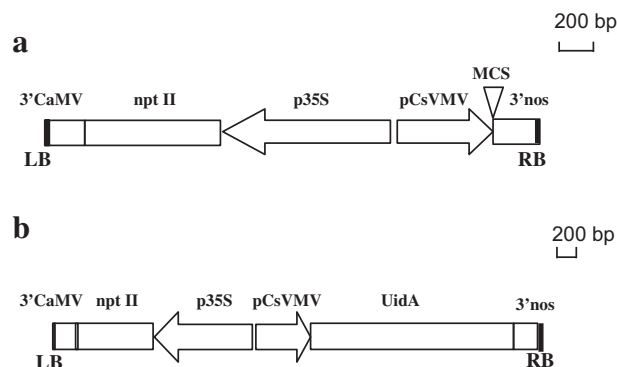

Fig. 1. Physical maps of T-DNA vectors used for transformation. (a) pING71. (b) pOYE135. 3′ UTRCaMV — 3′ untranslated region of cauliflower mosaic virus promoter; NPT II — coding region of neomycin phosphotransferase II gene from *Escherichia coli*; CaMV 35S — coding sequence of 35S promoter from cauliflower mosaic virus. CsVMV — coding region from cassava vein mosaic virus promoter; UidA-protein — coding region for β-glucuronidase from *E. coli*; 3′NOS — polyadenylation signal of the gene for nophaline synthase in the Ti plasmid; LB and RB — left and right borders of T-DNA transformation vector.

which were matured by transferred globular shaped embryos on MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ and 6-benzylaminopurine (0.1 mg/l) with light incubation.

The green cotyledons obtained from cassava mature somatic embryo were used for transformation by *A. tumefaciens*-mediated method according to Li et al. (1996) with NPT II as the selection marker gene. Green somatic cotyledons were infected with *Agrobacterium* suspension (OD$_{600}$ = 0.8–1.00) for 10 min and co-cultivated on co-cultivation medium [MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$, 1 mg/ml BA, 1 mg/ml IBA, 100 mM acetosyringone] for four days. Shoots were selected with G418. The selected shoots were transferred to a medium composed of MS medium supplemented with 2% sucrose, 0.8% agar, 2 μM CuSO$_4$ for rooting. Transformed plantlets were identified by GUS histochemical assays. GUS-positive plantlets were hardened and grown in screen house. Tobacco transformation procedure was as described previously Ingelbrecht et al. (1994).

2.4. Histochemical GUS assay

Histochemical localization of GUS activity in transgenic plants was carried out essentially as described by Jefferson et al. (1987). Plant material was incubated in a GUS assay buffer [10 Mm Na$_2$EDTA·H$_2$0, 0.1% Triton X-100, 0.3% 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc), 0.1 M NaH$_2$P0$_4$, 0.5 M K$_3$Fe(CN)$_6$]. After 2–6 h of incubation at 37 °C, the tissues were cleared with repeated 70% ethanol washes and stored in 70% ethanol. Some tissues were sectioned by hand and visualized in a Stemi 2000 Zeiss stereomicroscope.

3. Results

3.1. Construction of a pCsVMV promoter cassette

The CsVMV promoter was previously shown to direct strong constitutive gene expression in transgenic tobacco and rice but is under developmental regulation in transgenic grape. We have constructed a T-DNA transformation vector, pING71, that contains a CsVMV promoter cassette (pCsVMV) and a kanamycin resistance gene under control of the 35S CaMV promoter (pCaMV) (Fig. 1a). The pCsVMV promoter cassette contains a multiple cloning site to facilitate subcloning a gene of interest as a transcriptional fusion with the CsVMV promoter. The CsVMV promoter cassette and the CaMV35S-nptII gene of the T-DNA transformation vector are transcribed in opposite direction to avoid transcriptional interference. To evaluate this vector and study the expression level and pattern of pCsVMV in transgenic cassava and tobacco, the *Uid*A coding sequence (GUS) was inserted into the MCS of pING71, creating a pCsVMV-GUS chimeric gene in pOYE153 (Fig. 1b).

3.2. Comparison of the CaMV35S and CsVMV nucleotide sequences

Nucleotide sequence comparison of CaMV35S and CsVMV promoter revealed limited homology at +1 to −345 region including TATA, CAAT and CCACT boxes (Fig. 2). They shared homology at only 34.4% of positions shown in Fig. 2. However, beyond these relatively conserved domains the promoter sequences showed much less homology (data not shown). In CaMV35S promoter, the consensus TATA box was 27 bp upstream the translational start (+1) whereas the distance was 148 bp long in CsVMV promoter. The two promoters differed in other respect, for instance, CsVMV contained GT1 element (GAAAT) at position +327 to +333 but not in CaMV35S. Similarly, Dof1 protein binding motif (AAAAGG) is located at position +253 and +258 of the CsVMV promoter sequence but is absent in CaMV35S. There was a higher occurrence of light responsive elements like GATA core element in CaMV35S than in CsVMV.

3.3. Expression of pCsVMV-GUS in farmer preferred transgenic cassava and tobacco

To analyze the activities and expression pattern of CsVMV-GUS *in planta*, pOYE153 was introduced into tobacco by *Agrobacterium*-mediated transformation. Fifteen transgenic tobacco plants were regenerated from transformed leaf discs. Leaves and roots of 4-week-old transgenic tobacco plants were collected for GUS assays. High and constitutive expression of pCsVMV-GUS was observed in all tissues of tobacco leaves, stem and root (Fig. 3a, b, c). The expression pattern of CsVMV-GUS was indicated by intensity of blue staining of different tissues of leaves and roots. Deep GUS staining was observed in the midrib and major veins of leaves (Fig. 3a) and inner tissues of stem and primary root, particularly the vascular stele (Fig. 3b). Intense GUS activity was confined to the cortex in the stem (Fig. 3c). The GUS activity and expression pattern were similar in all transgenic tobacco plants generated.

To further investigate the activities and pattern of expression of CsVMV-GUS, LBA4404 (pOYE 153) was used to transform the cassava landrace 'Tokunbo' by *Agrobacterium*-mediated transformation. Twelve transgenic cassava plants were generated, some of which showed stable expression while others showed chimeric expression patterns. The generation and characterization of these primary cassava transformants will be described in detail elsewhere. One transgenic cassava plant showing uniform expression in all tissues tested, was selected for this study. Leaves, petioles, young stem, primary root and storage root from six-month-old transgenic cassava plants were collected for GUS assay. The same tissues were collected from six-month-old untransformed cassava plants to serve as negative control. A detailed histochemical analysis of GUS accumulation was carried out using hand-cut fresh tissue sections of various organs from primary transgenic plants. The pCsVMV-GUS was highly expressed in leaves and primary root (Fig. 4a, c) as indicated by blue-stained tissues on various parts of different plant organs. The expression pattern of the GUS activity was revealed by blue-stained tissues on leaves, petioles, young stem and leaf midrib (Fig. 4b, d, e, and f). In the leaves, strong GUS activity was observed in the mesophyll tissues but light in the vein network, including major and minor veins (Fig. 4b). As shown in the cross-section of petiole (Fig. 4d), preferential highest GUS expression was noticed in vascular bundle and endodermis, high in epidermis but weak in the pith. The cross-section of young stem (Fig. 4e) showed a strongest GUS activity in cortex and vascular bundle and high GUS stain in the pith area. In the leaf midrib (Fig. 4f), GUS expression was strongest in the vascular bundle and light outside the vascular region.

3.4. High pCsVMV-GUS gene expression is maintained in transgenic cassava after repeated cycles of vegetative propagation

In order to further confirm the pCsVMV-GUS activities and expression in transgenic cassava after vegetative propagation, petioles, stem, primary root and tuberous root from primary transformant, first and second ratooned transgenic cassava plants were collected for GUS assays. Petioles, stem, primary root and tuberous root from untransformed cassava plants were also collected for GUS assays to serve as negative control. Also a detailed histochemical analysis of GUS accumulation was carried out using hand-cut fresh tissue sections of various organs from ratooned transgenic plants. Cross-sections from petioles stem and tubers of negative control plants showed no GUS activity (Fig. 5a, b, c, d, e). In the petioles, strongest pCsVMV-GUS expression was confined to the vascular tissues as well, including vascular cambium, phloem, xylem and related parenchyma cells (Fig. 5a). High GUS expression was observed in pith. The expression of pCsVMV-GUS in petioles of first ratooned plant was unchanged from that observed in petioles of second ratooned plant (Fig. 5a). In the cross-section of young stem (Fig. 5b), GUS activity was strongest in the epidermis and high in the cortex and pith areas. Similar observations were made on GUS expression in young stem of first and second rationed transgenic plants. The GUS
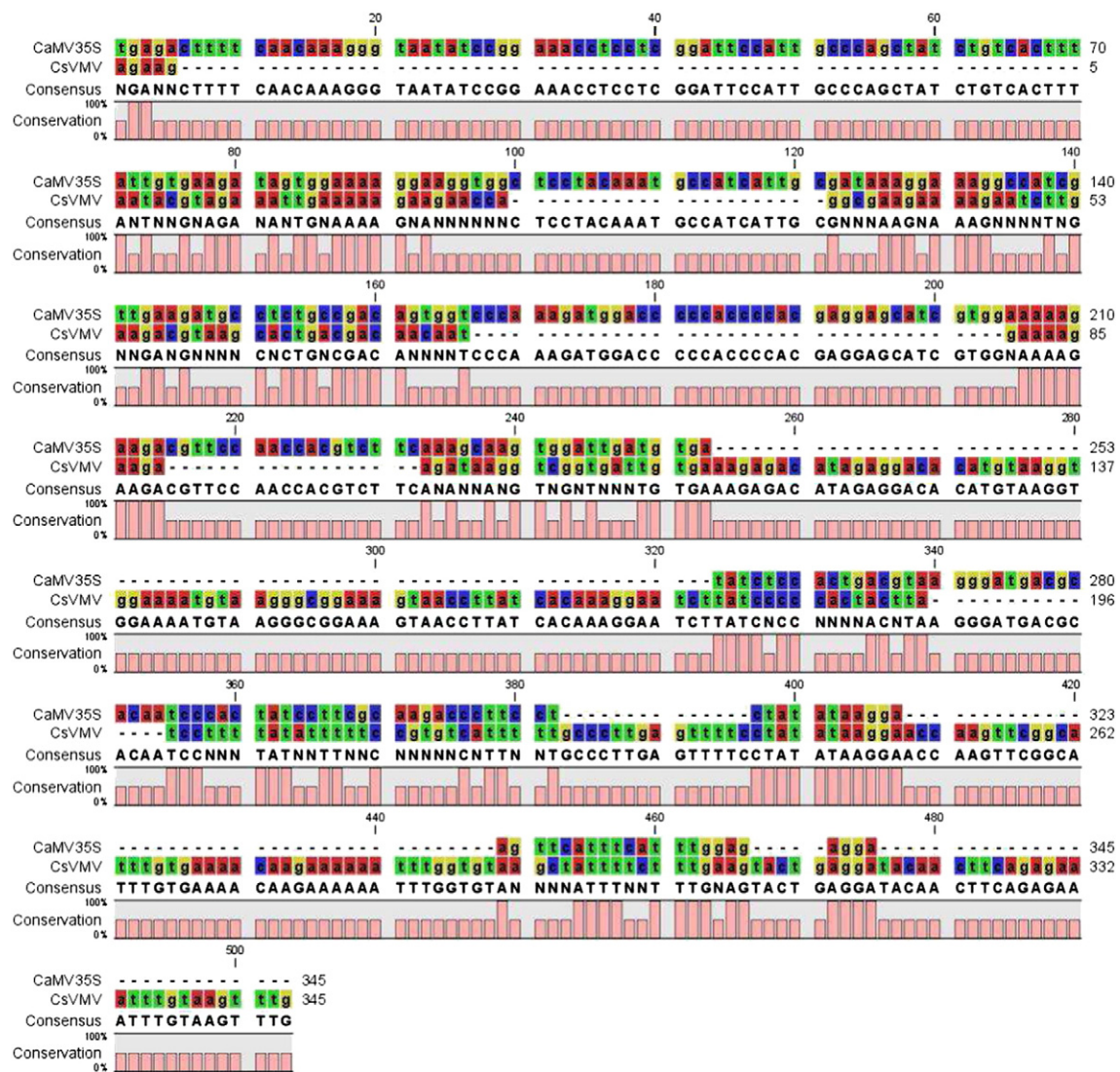
Fig. 2. Alignment of nucleotide sequences (+1 to −345) of CsVMV and CaVMV 35S promoters through conserved TATA, CAAT and CCACT boxes showing limited homology between the sequences.

activity was high in the cortex and vascular tissues in the old stems but slightly detectable in the pith area (Fig. 5c). Similar observation was recorded in primary root of transgenic plants (Fig. 5d). The GUS expressions in old stem and primary root were similar in both first and second ratooned transgenic plants. In mature tubers, cross-section (Fig. 5e) revealed that GUS activity was strong and confined to the cortex but uniform in the vascular bundle. The GUS expression in mature tubers was similar in both first and second ratooned transgenic plants. Cross-sections from different regions of a mature tuber (Fig. 6a) were examined for pCsVMV-GUS expression: GUS activities were similar in all the tuber regions with uniform expression in the vascular tissues including vascular parenchyma, which is the major site for starch accumulation and storage, and in the external phloem. pCsVMV-GUS was strongly expressed in the cortical cells (Fig. 6a).

4. Discussion

We constructed and tested a T-DNA vector with pCsVMV-GUS and CaMV 35S-NPTII cassettes transcribing in opposite direction in tobacco and cassava transgenic plants. We further evaluated the activity, level
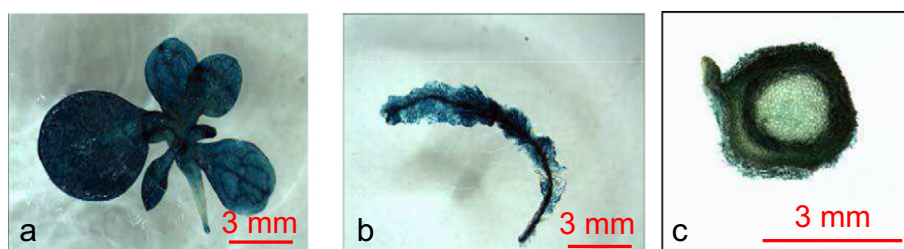

Fig. 3. Histochemical localization of GUS activity in various organs of transgenic tobacco plant. GUS activity is indicated in transgenic tissues by an indigo dye precipitate after staining with X-Gluc. Expression pattern of pCsVMV-GUS in leaves (a), roots (b), and cross-section of stems (c).
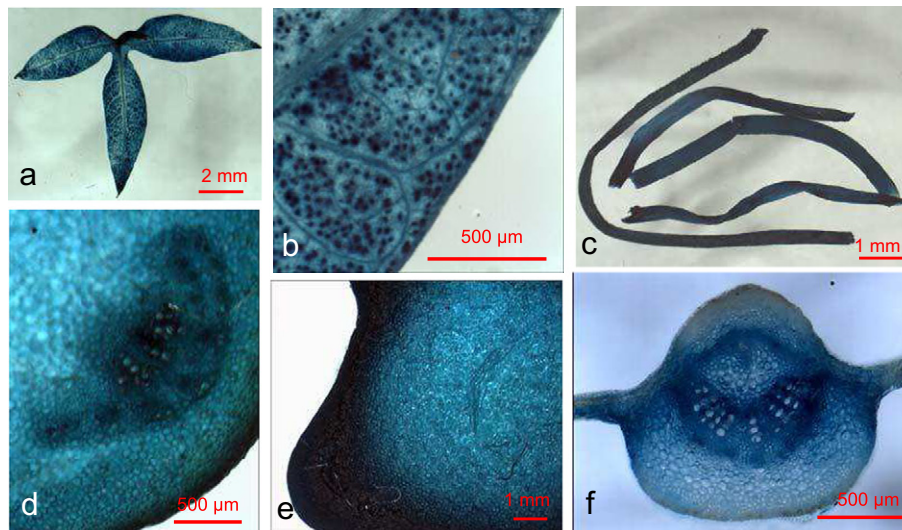
Fig. 4. Histochemical localization of GUS activity in various organs of transgenic cassava plant. GUS activity is indicated in transgenic tissues by an indigo dye precipitate after staining with X-Gluc. (a) Constitutive GUS expression in a leaf (b) close-up of a stained leaf with strong GUS blue staining in vein networks and midrib (c) constitutive GUS expression in a primary root (d) cross-section of petioles with the strongest GUS expression in the vascular bundle (e) cross-section of young stem with strong GUS expression in the cortex (f) cross-section of midrib with preferential GUS expression in the midrib.

and pattern of expression of pCsVMV-GUS in various organs and tissues of clonally propagated transgenic cassava plants. The pCsVMV promoter was active and driven strong GUS expression in various transgenic tobacco parts: leaf, midrib and root. Analysis of transgenic cassava plants indicates that pCsVMV-GUS is active in all organs and various cell types. The pCsVMV-GUS drives strong and constitutive expression in vascular tissues of petiole, stem and tuberous root and in leaf mesophyll tissues and vascular stele of roots of transgenic tobacco and cassava plants. Similarly constitutive expressions of GUS gene driven by CsVMV in rice and tobacco have been reported by Verdaguer et al.
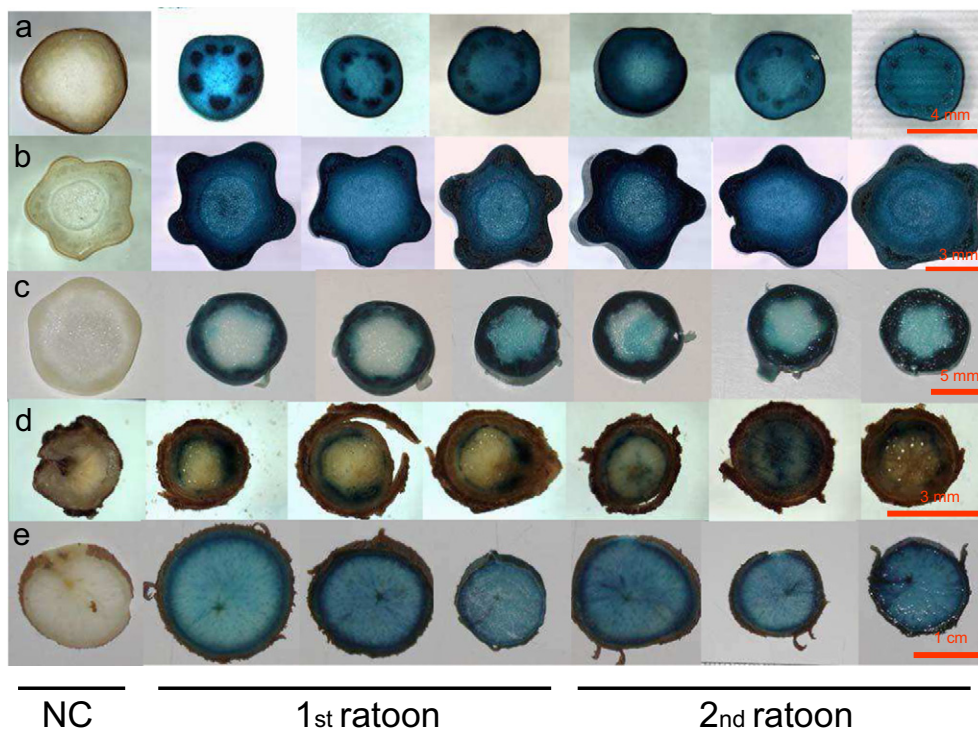

Fig. 5. Histochemical localization of GUS activity in various organs of transgenic cassava plant. GUS activity is indicated in transgenic tissues by an indigo dye precipitate after staining with X-Gluc. (a) cross-sections of petioles of untransformed cassava plants (control) and transgenic cassava types with strong GUS activity in vascular cambium, phloem and xylem; the order of a from left to right is untransformed cassava plants (control) to transgenic types 1–7. (b). Cross-sections of young stems of untransformed cassava plants (control) and transgenic cassava types with deep blue-stained cortical tissues; the order in b is the same as in a. (c) Cross-sections of old stems of untransformed cassava plants (control) and transgenic cassava types with the strongest GUS activity in the cortex; the order in c is the same as in a and b. (d) Cross-sections of primary root of untransformed cassava plants (control) and transgenic cassava types with intensive GUS activity in the cortex; the order in d is the same as in a, b and c. (e) Cross-sections of mature tubers of untransformed cassava plants (control) and transgenic cassava types with uniformed GUS expression in the vascular bundle; the order in e is the same as in a, b, c and d.

Fig. 6. Histochemical localization of GUS activity in various region of mature tuber from transgenic cassava plants. (a) Cross-sections of different regions of mature tuber showing uniform GUS expression in vascular bundle. (b) Close-up cross-section of mature tuber showing strongest expression in the cortex with uniformed expression in the vascular tissues.

(1996). However expression of pCsVMV-GUS in the primary root and tubers of transgenic cassava disagree with lack of expression of CsVMV promoter in transgenic grape plant reported by Li et al. (2001) which they attributed to transcriptional regulation and not gene silencing phenomenon. It is noteworthy that pCsVMV-GUS activity was detected in the midrib and pith of transgenic tobacco and cassava plants corresponding to non-chlorophyllaceous cells in plants in this study. Verdaguer et al. (1996) could not detect GUS activity in non-chlorophyllaceous cells of tobacco pith and cortical parenchyma. This leads to the suggestion by Verdaguer et al. (1996) that CsVMV promoter has two major domains of activity, i.e., vascular elements and the green, chloroplast-containing cells. Our results disagree with the notion that CsVMV-GUS activity is limited to vascular element and green chloroplast-containing cells in plant even the stem pith with the least GUS activity in this study has a weak level of GUS expression. It is possible, according to Verdaguer et al. (1996) that observations are due to the limitations of the staining assay. Large cells with little cytoplasm may appear to contain little or no stain compared with smaller cells with dense cytoplasm. Likewise, cells with different metabolic activities may stain with different intensities (Verdaguer et al., 1996). High pCsVMV-GUS gene activity and expression maintained over three cycles of ratooning under greenhouse conditions indicate the absence of undesirable gene silencing effects after repeated in vitro subculturing and vegetative propagation. The absence of gene silencing effect could be attributed to limited nucleotide sequence homology between CsVMV and CaMV35S promoter sequences (Fig. 2) which implies different mechanism of regulation of transgene expression by promoters (Verdaguer et al., 1996). The opposite orientation of CsVMV-GUS and CaMV 35S-NPTII cassette might have prevented transcriptional interference and enhanced consistent high expression of the CsVMV-GUS clonally propagated transgenic cassava plants. Furthermore, the stable high expression observed in the study could not be due to trans activity of 35S promoter that control NPTII gene as reported by Yoo et al.(2005) in Arabidopsis as selectable markers were placed under 35S promoter without trans activity in cassava (Hankoua et al., 2006; Ihemere et al., 2006). In addition, our transgenic cassava plants were phenotypically identical with themselves and with untransformed cassava plants as opposed to Yoo et al. (2005) observation on transgenic Arabidopsis.

Our work provide alternative promoter for transgene expression in cassava plant with no problem of homology-dependent gene silencing usually caused by duplication of promoter sequences in T-DNA transformation vectors. The unique promoter activity and transgene expression patterns observed in this study provide useful information relevant to the use of this promoter to direct transgenes expression in this economically important crop plant. For effective application of genetic engineering for improvement of cassava against numerous production constraints, availability of promoters that are highly expressed in vital organs and cells of the crop becomes prime importance. As pCsVMV is active in virtually major organs of cassava, it will be useful to drive expression of transgenes aim at removal of these production constraints. Genes involve in starch biosynthesis are expressed in storage roots and leaves (Baguma et al., 2003; Ihemere et al., 2006). High expression of antisense or RNA interference genes driven by pCsVMV-GUS could be achieved in storage roots and leaves of cassava to obtain modified starches with enhanced functionality. Similarly, pCsVMV-GUS could be suitable for improvement of nutritional value of cassava storage roots to drive genes such as gene encoding storage protein rich in essential amino acids (Zhang et al., 2003). There are several insect pests affecting cassavas, foliage and/ or stems, particularly Lepidoptera, Diptera and Hemiptera. There is little or no genetic resistance to these pests and their management is commonly achieved through biological control (Ceballos et al., 2004). The pCsVMV-GUS could find use as promoter in transgenic approach to drive strong expression of cry genes encoding insect-specific endotoxin (Bt toxins) from Bacillus thuringiensis for protection against these insect pests. Another potential use of pCsVMV-GUS could be in the control of post-harvest physiological deterioration of cassava tubers which starts from root xylem and spread to adjacent storage parenchyma. Several tuber deterioration genes have been cloned in cassava, including catalase (Reilly et al., 2001). The inhibition of the activity of the tuber deterioration genes could be achieved by strong expression of their antisense genes under pCsVMV-GUS promoter. African mosaic virus and brown streak mosaic virus diseases are major constraints to cassava production. The viruses are transported through the phloem of vascular tissues during long-distance transport (Hull, 2002; Alicai et al., 2007). Therefore, strong expression of anti-viral genes under the control of pCsVMV-GUS in phloem cells might inhibit the systemic spread the viruses.

Acknowledgment

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