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Comparing the Effect of Two Promoters on Cassava Somatic Embryo at Transient GUS Assay Level

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Author's contribution

This work is a portion of the M.Sc. Dissertation of the author OOO. The other authors read and approved the final manuscript.

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ABSTRACT

35S promoter from the Cauliflower Mosaic Virus (pCaMV) is a constitutive promoter commonly used in plant genetic transformation while *Cassava Mosaic Virus* (pCsVMV) is another promoter which is underutilized. The combination of the two promoters was used to form (pOYE153). The method adopted includes the insertion of a β–glucuronidase reporter gene (UidA) into a promoter cassette comprising the CsVMV promoter. The second construct (pCAMBIA2310) had (pCaMV) used for the selectable marker and gene of interest. This construct was mobilized into *Agrobacterium tumefaciens* strain LBA4404 and then tested for expression of the UidA gene in transient assays in cassava somatic embryos. After co-cultivation of these *Agrobacterium* with the plant tissues, histochemical β–glucuronidase (GUS) assays were performed to determine the level of UidA gene expression in transient assays. The results showed that the pCsVMV was able to drive high gene expression of β–glucuronidase reporter gene (UidA) in the transient assays in cassava somatic embryo. Expression of the gene also increases with the increase in the day of co-cultivation and likewise expression of the gene was higher for the sample in the light than the dark.

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1. INTRODUCTION

Promoters are regions of the DNA that are located upstream of a coding region and which have specific sequences, recognized by proteins involved in the initiation of transcription of DNA to mRNA [1]. Promoters are important in the control of overall expression profile of a gene; it drives transcription at appropriate times and places. The basal promoter is located between 20-40 base pairs upstream of the start of the transcription and the promoter usually extends to about 200 base pairs or more upstream. The different types of promoters include constitutive promoters, tissue and cell-specific promoters, inducible and synthetic promoters. Promoters are used in the development of plant products and in research setting, so the type of promoter should be selected based on the application and desired expression pattern of the transgene in the plant [2]. Promoters comprise a set of transcription control modules clustered around the initiation site of RNA polymerase II [3].

The type of constructs and area of gene expression will determine the type of promoter to be used; some promoters express well in green tissue, root or reproductive tissues of the plant i.e. anther and ovary promoters that can confer tissue specific or temporal expression. Plant and viral promoters that drive high, constitutive expression have become valuable tools in plant genetic engineering. Among the noticeable promoters frequently used in plant genetic transformation is the *Cauliflower Mosaic Virus* 35S promoter CaMV35S [4].

Plant genetic transformation is a powerful means of studying gene expression in plants. It also allows for manipulation of biochemical processes that cannot be easily manipulated through conventional breeding. Genetic transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. *Agrobacterium tumefaciens* is a plant pathogenic bacterium, which has become the most frequently used agent for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. *Agrobacterium tumefaciens* naturally infects the wound sites in dicotyledonous plant causing the formation of the crown gall tumors.

The GUS reporter system (*GUS*: beta-glucuronidase; *uid*A) is a reporter gene system, particularly useful in plant molecular biology. Gene silencing refers to the inactivation of a transgene in a plant or animal cell. Gene silencing can occur at the transcriptional or post-transcriptional level. Gene silencing has also been called “homology-dependent” [5] or “repeat Induced” gene silencing [6]. It can involve the interaction between two unlinked loci in which one transgene locus is capable of trans-inactivating the second [7].

Gene silencing is found in plants and animals and is responsible for many important functions like defense against foreign organisms such as viruses [8]. Gene silencing can be caused by the presence of homologous sequences in a genome, for example, duplication of a gene in the genome can result in gene silencing. The duplication of promoter sequences has been shown to result in silencing of the genes controlled by these promoters [5]. The 35S promoter of the *Cauliflower Mosaic Virus* (CaMV) is considered a constitutive promoter and is widely used as the promoter for the marker gene and the gene of interest.

2. MATERIALS AND METHODS

2.1 Plasmids Description

The first plasmid was (pOYE153) comprises of pCaMV 35S used to drive the selectable marker while pCsVMV was used drive the gene of interest, the two promoters operated in opposite direction. The second plasmid was (pCAMBIA2301) where the selectable marker and the gene of interest were driven by pCaMV 35S and it was in the same direction.

2.2 Preparation of Cassava Somatic Embryo

Nodes were removed from in-vitro cassava plants and cultured on meristem enlargement medium for a week. The meristem was removed and it was placed on primary induction medium which contains picloram (10 mg/L) for 10 days in the dark so as to induce somatic embryo, after which the induced embryo was moved to

secondary induction medium also in the dark for another 2.5 weeks [9,10]. The embryo matured after 17 days and it thereafter moved to maturation medium in the light for 2 weeks (Fig. 1).

2.3 Preparation of the Two Infection Media

The two bacteria constructs were inoculated into 3 mls $YEB_{Rif50\ Sm300Km25}$ overnight and from the 3 mls culture, 1 ml solution was taken to inoculate 20mls $YEB_{Rif50\ Sm300Km25}$. The next day, the bacteria grew and it was ready for infection. The bacteria constructs were allowed to grow to Optical Density OD_{600} of about 0.8. They were later spun at 5,000 rpm for 15 mins and redissolved in $YEB_{Rif50\ Sm300Km25}$ with 5 mM Acetosynringone and grown for another 2 hrs so that the OD increased to about 1.0 - 1.2. The constructs were spun and redissolved in plant medium with OD diluted to 0.5 for transformation [11].

2.4 Transformation of Cassava Somatic Embryo

The matured somatic embryos were harvested and it was chopped into smaller pieces for transformation. The somatic embryos were added into the diluted bacteria in the plant medium. The mixture was then placed on orbit shaker for 45 mins – 60mins so as to allow infection to take place very well. The somatic embryos were taken out and blotted on sterile paper for 5-10mins, and it was transferred to co-cultivation medium. The infected plant tissue on co-cultivation was placed at 22ºC for 4 days. Transfer of T-DNA is optimal at 22ºC – 24ºC, some were placed in the dark and the rest in the light. After 2, 4 and 6 days histochemical GUS assay was done on the infected materials in the dark and in the light [11].

2.5 Histochemical Gus Assay

The histochemical GUS assay was done using [12] method. The assay was done somatic embryo. Few tissues of the infected somatic cotyledons of cassava were placed in 200 ul GUS solution, vacuum infiltrated for 1 minute, and incubated at 37ºC for 4-8 hrs. Then the GUS solution was removed and chlorophyll in the tissues was removed with repeated 70% Ethanol washes.

3. RESULTS AND DISCUSSION

3.1 Expression of GUS Gene Using pCAMBIA2301

Genetic transformation of cassava somatic embryo with pCAMV was done with LBA4404(pCAMBIA2301). The constructs gave positive expression of GUS gene. The transient expression of GUS assay exhibited by cassava somatic embryo was blue with the constructs. There was increase in the number of somatic embryo that were stained blue with increase in the number of day of co-cultivation likewise co-cultivation of the explants in the light increases the number of explants that were stained blue. In the dark, 10% of the explants showed GUS gene expression after 2 days; after 4 days, the number of the explants increased to 40% while after 6 days 50% of the explants showed GUS gene expression. In the light, 30% of the explants showed GUS expression after 2 days; 50% showed GUS expression after 4 days and 65% expressed GUS gene after 6 days for the first cassava variety TME12 (Table 1). For the second cassava variety Albert, in the dark, 15% of the explants showed GUS gene expression after 2 days; after 4 days, the number of the explants expressed GUS gene increased to 40% while after 6 days 55% of the explants showed GUS gene expression. In the light, 25% of the explants showed GUS expression after 2 days; 50% of the explants expressed GUS gene after 4 days and 65% after 6 days (Table 2).

3.2 Expression of GUS Gene Using pOYE153

Genetic transformation using pOYE153 also showed expression of the GUS gene, the intensity using pCsVMV was 5 times deeper than the intensity expressed with pCAMV and as the number of the days of co-cultivation increases the colour intensity increase so therefore, the higher the number of day of co-cultivation the better the infection with *Agrobacterium.* The GUS gene expression in cassava somatic embryo increases as the number of days of co-cultivation increases, 6 days is better than 4 days and 4 days is better than 2 (Figs. 2 and 3). Samples that were co-cultivated in the light had higher GUS gene expression than the samples co-cultivated in the dark. The GUS staining expression was in parches for most of the explants. In the dark, 20% of the explants showed GUS gene expression after 2 days; after

4 days, this increased to 60% while after 6 days about 70% of the explants showed GUS gene expression. In the light, 40% of the explants showed GUS expression after 2 days; 75% after 4 days and 85% after 6 days for the first cassava variety TME12 (Table 1). For the second cassava variety Albert, In the dark, 20% of the explants showed GUS gene expression after 2 days; after 4 days, this increased to 55% while after 6 days about 70% of the explants showed GUS gene expression. In the light, 35% of the explants showed GUS expression after 2 days; 70% after 4 days and 90% after 6 days (Table 2). Overall, these experiments showed that both light and prolonged co-cultivation had a positive effect on gene expression in transient gene assays. 4% of the number of the explants used were 100% blue for the explants that were co-cultivated in the light.

The 35S promoter from CaMV is one of the most commonly used promoters in plant genetic transformation studies because it has the ability to drive high, constitutive gene expression in different tissues of the plant. In some *Agrobacterium* transformation vectors, the CaMV35S promoter is used to drive both the selectable marker and the gene of interest resulting in duplication of promoter sequences. This repetition of promoter sequences can cause gene silencing. Previous constructions were made either with the two CaMV 35S promoters following each other serially or with CaMV 35S facing each other [13]. This configuration causes overlapping transcription which can lead to transcriptional interference or gene silencing.

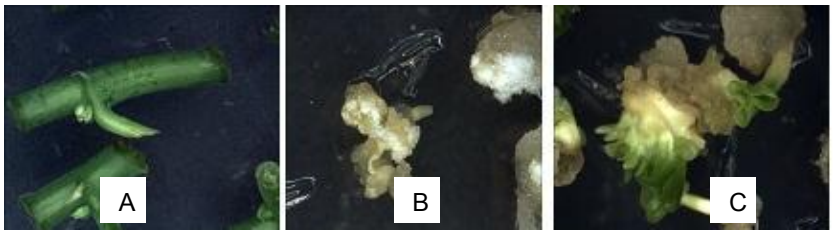

Fig.1. Preparation of cassava somatic embryo for genetic transformation
A- cassava nodal cuttings, B- induced somatic embryo, C- matured somatic embryo

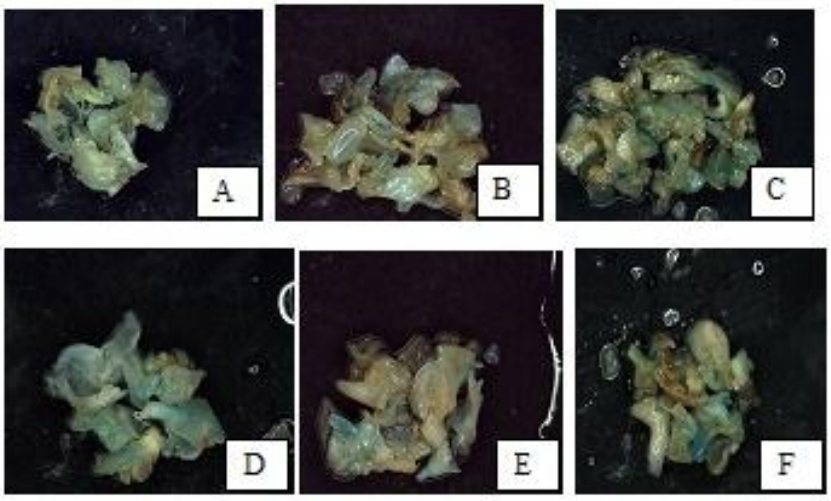

Fig. 2. Transient GUS gene expression of cassava somatic embryo in dark
A- GUS assay after 2 days on co-cultivation medium with Albert, B- GUS assay after 4 days on co-cultivation medium with Albert, C- GUS assay after 6 days on co-cultivation medium with Albert, D- GUS assay after 2 days on co-cultivation medium with TME 12, E- GUS assay after 4 days on co-cultivation medium with TME 12, F- GUS assay after 6 days on co-cultivation medium with TME 12

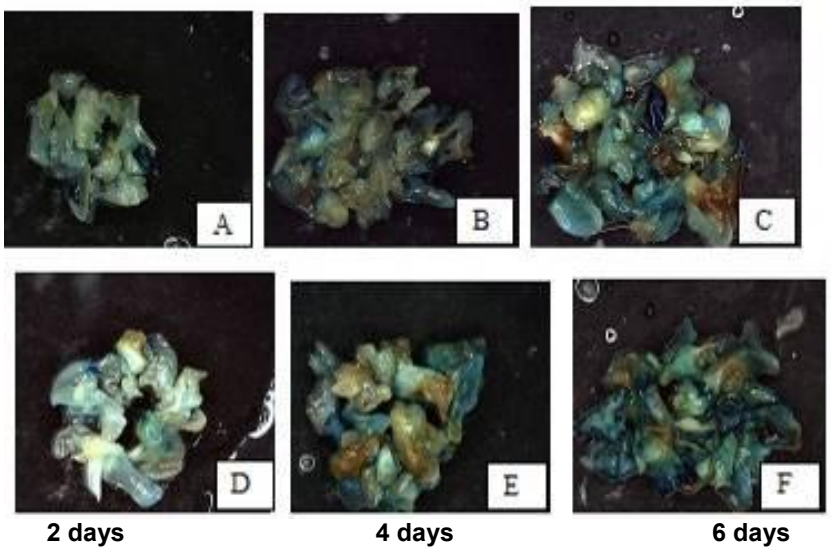

Fig. 3. Transient GUSgene expression of cassava somatic embryo in light.
A- GUS assay after 2 days on co-cultivation medium with Albert, B- GUS assay after 4 days on co-cultivation medium with Albert, C- GUS assay after 6 days on co-cultivation medium with Albert, D- GUS assay after 2 days on co-cultivation medium with TME 12, E- GUS assay after 4 days on co-cultivation medium with TME 12, F- GUS assay after 6 days on co-cultivation medium with TME 12

Table 1. Percentage of the transformed explants for TME12

Number of days on co-cultivation	pCAMBIA2301 construct		pOYE153 construct	
	Dark (%)	Light (%)	Dark (%)	Light (%)
2	10	30	20	40
4	40	50	60	75
6	50	65	70	85

Table 2. Percentage of the transformed explants for Albert

Number of days on co-cultivation	pCAMBIA2301 construct		pOYE153 construct	
	Dark (%)	Light (%)	Dark (%)	Light (%)
2	15	25	20	35
4	40	50	55	70
6	55	65	70	90

This study revealed that pOYE153 with two different promoters: pCsVMV isolated from *Cassava Mosaic Virus* and pCaMV isolated from *Cauliflower Mosaic Virus.* The promoters pCsVMV designed in opposite directions; thereby transcription is in opposite direction further reducing the chances of gene silencing. The *Uid*A gene is used as the reporter gene in this project. The pCsVMV promoter has previously been tested by [14] and was found to be constitutive in tobacco and rice. Expression of GUS gene at transient level in cassava shows that pOYE153 was expressed at a higher level compared to the second construct pCAMBIA2301 with the GUS gene under control of pCaMV. The experiment has shown that pCsVMV can be used to drive any other gene of interest. The project also showed that co-cultivation under light conditions aid genetic transformation and that longer periods of co-cultivation result in enhanced gene expression in transient assays.

4. CONCLUSION

This study has revealed that pCsVMV is a promoter that can drive the gene of interest just like the commonly used pCAMV. This also showed that the expression of GUS gene in explants can be improved by increasing the

number of days in co-cultivation medium to six days as well doing the co-cultivation in the light.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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