African Journal of Biotechnology Vol. 12(13), pp. 1467-1474, 27 March, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1122
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Arabidopsis and *Musa cyclin D2* expressed in banana (cv. "Sukali Ndiizi"- AAB) enhances regeneration efficiency

Samukoya, Clara[1,2*], Mutumba, Gerard[2], Nanteza, Ann[2] and Tushemereirwe, Wilberforce[1]

[1]National Crops Resources Research Institute (NaCRRI), P.O. Box 7065 Kampala, Uganda.
[2]Makerere University, Kampala, Uganda.

Accepted 6 August, 2012

Genetic transformation of banana is important because of its polyploidy, sterility and long generation time of most cultivars which limit conventional breeding. However, transformability and regeneration of transgenic lines remains low in bananas. This research reports on the potential of *CycD2* genes to improve transformation and regeneration efficiency of banana (cv. "Sukali Ndiizi"). Two genes *Arath;CycD2;1* and *Musa;CycD2;1* were evaluated for cell cycle modification of the embryogenic cell suspension that is conventionally used in banana genetic engineering at the National Biotechnology Centre, Kawanda. The *UidA* (GUS) gene was used as reporter gene to establish transient transformation frequency by fusing it with each of the *CycD2; 1* genes and Cauliflower mosaic virus 35S promoter in the binary vector, pC1305.1. The transformed "Sukali Ndiizi" cells were cultured on selection media and the hygromycin resistant clones developed into shoots. The Gus assay analyses showed a success rate of 80 to 90% for all the constructs including the control, transformed with the empty vector without *CycD2; 1* gene. Also, the Gus assay of the regenerants showed that the gene was expressed in different parts of the plants (roots, corm and leaves). Polymerase chain reaction (PCR) analysis of the regenerated shoots gave the regeneration frequency of the embryogenic clones of *Arath; CycD2; 1* and *Musa; CycD2*; 1 gene was 47 and 62%, respectively. This was much higher than that of the control clones without *CycD2; 1* (18%). The results show that *CycD2; 1* genes have the potential to significantly improve regeneration efficiency of "Sukali Ndiizi" cells".

Key words: Cell cycle genes, reporter gene, genetic transformation, regeneration efficiency.

INTRODUCTION

Plant transformation is the introduction of at least two genes, a selectable marker gene and a gene of interest (Sági et al., 1997). On the other hand, regeneration is the organogenesis or embryogenesis from the transformed cells (Arias et al., 2006). In Uganda, banana suffers a range of production constraints including pests, especially weevils (*Cosmopolites sordidus* Germar) and root nematodes, diseases, particularly black sigatoka (*Mycosphaerella fijensis*), Fusarium wilt, bacterial wilt, frequent droughts and reduced soil fertility (Gold et al., 1993; Tushemereirwe et al., 2004; Talwana et al., 2003). These cause serious yield losses and shorten the plantation lifespan.

Breeding for disease-resistant banana cultivars using classical methods remains a tedious endeavour because of high sterility, polyploidy and long generation times of most of edible cultivars (Arinaitwe, 2008). Genetic transformation provides an opportunity for single genes to be extracted from the genome of the source organism and transferred directly into the genome of the desired variety. This allows the candidate variety to retain all its original characteristics, with only simple addition of the desired trait (Sági et al., 1997).

*Corresponding author. E-mail: csamukoya@kari.go.ug. Tel: (+256) 782 748 744. Fax: 256 414566381.

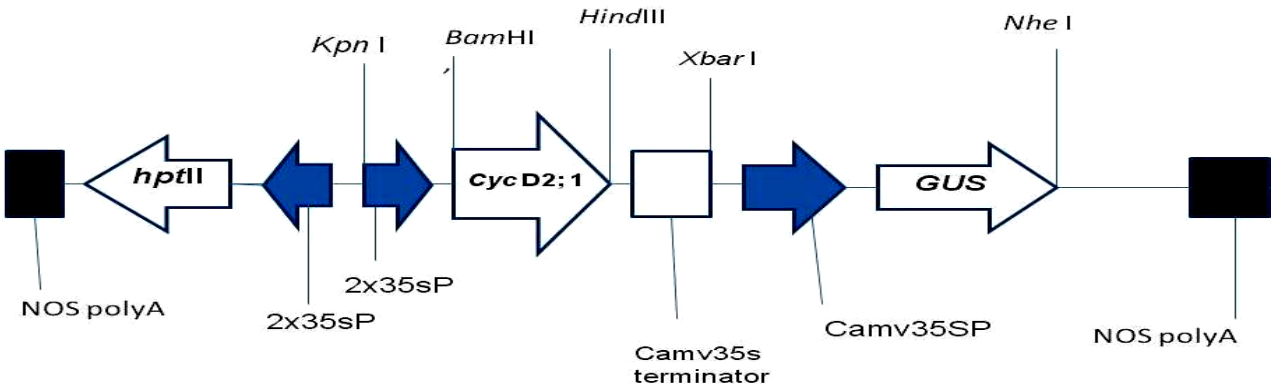

Figure 1. Vector map for gene construct with *Musa*; C*ycD2; 1 or Arath; CycD2; 1* and *GUS* genes fused together. Both the *GUS* and *CyclinD2; 1* genes are driven by the 35S CaMV promoter.

Among the available deoxyribonucleic acid (DNA) delivery techniques, *Agrobacterium*-mediated transformation is widely used due to: (i) the simplicity and low cost of the technique, (ii) low copy numbers of the transgene and (iii) ability to transfer large DNA segments with minimal rearrangement (Cheng et al., 2004; Jones, 2005). Refinement of banana transformation protocols has been done through improvement of bacterial contact with the banana cells (Khanna et al., 2004), incubation temperature and plant cell volume (Arinaitwe, 2008).

Culturing cells on auxins to stimulate cell division prior to transformation is routinely used but the improvement of the transformation frequency has not been significant (Villemont et al., 1997). Based on this fact, upregulating genes that directly modulate the cell cycle, as proposed by Arias et al. (2006), needs to be explored.

MATERIALS AND METHODS

Suspension cultures

Embryogenic cell suspensions of banana cultivar 'Sukali Ndiizi' (*Musa spp*. AAB group) was initiated and maintained in M2 medium (khanna et al., 2004). Five days after sub culturing the cells were collected in a 50 ml falcon tube and allowed to settle. The settled cells were pooled to avoid variations and 5 ml media containing 0.5 ml small-cell variants (SCV) of cells was aliquoted out into sterile 50 ml falcons and used as starting material for further experiments.

Agrobacterium and plasmids

Agrobacterium tumefaciens strain AGL-1 was used in this study. Schematic representation of the binary vector, pCambia 1305.1, is shown in Figure 1. The vectors containing *CyclinD2* inserts were transformed into *Agrobacterium* via freeze thaw method.

Cloning of *Arath; CycD2; 1* and *Musa; CycD2; 1* gene constructs

Primers were designed with restriction sites *Bam*HI and *Hind*III to pick out the *Arath; CycD2; 1* and *Musa; CycD2; 1* using polymerase chain reaction (PCR). The amplicon was cloned using a Topo TA cloning kit (Invitrogen). The Topo vector was restricted using *Bam*HI and *Hind*III enzymes and restriction reaction run on agarose gel to isolate the band of interest. The band was purified using Qiagen gel purification kit and ligated in PLBR19 vector that has cauliflower mosaic virus (CaMV) 35S promoter and terminator.

The ligate was transformed into *Escherichia coli* (strain JM109) and selected on Luria Bertani (LB) medium (Bacto Trypton, 10 g/L, Bacto yeast extract 5 g/L, sodium chloride, 10 g/L and bacterial agar, 15 g/L (for LB agar), pH 7.0) containing 100 µg/ml of ampicilin. Using enzymes *Kpn*I and *Xba*I, the genes with the CaMV35S promoter and terminator were restricted and ligated into pC1305.1 binary vector containing *UidA* (GUS) reporter gene. The resultant vectors were transformed into competent JM109 by heat shock method and selected on LB with kanamycin 100 µg/ml. Three colonies were picked, grown overnight in liquid medium at 200 rpm and plasmid extracted using Qiagen kit and protocol. At all stages, PCR, restriction digestion and gel electrophoresis were done to confirm the presence of inserts in vectors.

The restriction digestion reaction used to detect recombinant *E. coli* was composed of 5.8 µl of water, 2.0 µl of 10 X Tango buffer, 1.0 µl *Hind*III (20 U/µl), 1.0 µl *Bam*HI (20 U/µl) and 0.2 µl of 1 x bovine serum albumin (BSA). The same volume of enzymes and buffers were used for the case of *Kpn1* and *XbaI* (New England Biolabs® Inc.). Agarose (1%) was used to run all gels in 1 X Tris-acetate-EDTA (TAE) pH 8.0. The gels were run at 100 v for 1 h, stained in ethidium bromide solution (0.5 µg/ml) for 15 min and viewed using the gel documentation system.

Plasmid purification of *E. coli*

Single bacterial colonies were picked and cultured in 5 ml of selective LB medium (containing kanamycin 100 µg/ml). Cultures were incubated at 37ºC with shaking at 200 rpm overnight. Plasmid isolation was done using the QIAprep spin Miniprep Kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. To elute plasmid DNA, 50 µl of sterile water pre-warmed at 70ºC was added. The column was placed into a 1.5 ml microfuge tube, left to stand for 5 min, and centrifuged at 13000 rpm for 1 min. The isolated plasmid DNA was stored at -20ºC for future use.

Transformation of "Sukali Ndiizi" cells

The embryogenic cell suspensions (ECS) of 'Sukali Ndiizi' (AAB) were transformed using the centrifugation assisted *Agrobacterium*-

Table 1. PCR primers used in the study.

Primer name	Sequence (5'-3')
Arath CycD2Fw	CCCAACTTATGAGTCCAAGTT
Arath CycD2Rv	CGCGGATCCTCATCTGGTTGT
MusaCycD2Fw	CGCGGATCCTCATCTGGTTGT
MusaCycD2Rv	CGCGGATCCTTATGGATGG
HYGFw	CTATCGGCGAGTACTTCTACACAG
HYGRev	CCCATGTGTATCACTGGCAAAC

mediated banana transformation protocol of Khanna et al. (2004).

Selection and regeneration of "Sukali Ndiizi" transgenic plants

After five days of co-cultivation, infected ECS were washed with liquid M2 medium supplemented with timentin at 200 ug/ml. Infected ECS were sub-divided and aspirated on nylon mesh. The mesh with embedded cells was transferred to semi-solid M3 media supplemented with timentin (200 µg/ml) to kill off bacteria and hygromycin (50 µg/ml) to select for transformed cells. The cells were transferred onto selective M3 medium (Khanna et al., 2004) on 50 µM nylon mesh every two weeks until observation of embryogenic cell clones.

The cell clones were individually transferred onto selective semi-solid embryo initiating medium (RD1) (MS salts 2.15 g/L, MS vitamins 103 g/L, ascorbic acid 40 mg/L, myo-inositol 100 mgL, sucrose 30 g/L, phytagel 2.3 g/L, pH 5.8) containing timentin (200 mg/L) and hygromycin (50 mg/L) for embryo initiation. This was followed by transferring the cells onto shoot germination medium (M4) (Khan et al., 2004). The regenerated shoots were grown on Murashige and Scoog, 1962 (MS) medium supplemented with 5 mg/L benzylaminopurine (BAP), 30 g/L sucrose, pH 5.8 as per Vuylsteke (1989) proliferation medium in the tissue culture laboratory and used for further analysis.

β-glucuronidase histochemical assay

Histochemical gus assays were conducted essentially as described by Jefferson (1987). Briefly, hygromycin resistant tissues or putative transgenic cells were incubated overnight at 37ºC in X-Gluc solution composed of 0.1% (w/v) 5-bromo-4-chloro-3-indoyl-β-glucuronic acid, 100 µm sodium phosphate (pH 7), 0.5 µm potassiumferrocyanide, 0.5 µm potassium ferricyanide, and 10 µm ethylenediaminetetraacetic acid (EDTA). Plant cells and tissues were scored as GUS positive if any deep indigo blue color was present.

DNA isolation and conventional and PCR analysis "Sukali Ndiizi" plants

Total DNA was extracted from 30 mg leaf tissue of transformed plants and untransformed controls using the modified miniprep protocol of Dellaporta et al. (1983). The DNA pellet was re-suspended in 20 µl of Milli-Q water containing 1 mg/ml RNAse, treated for 15 min at 37ºC. DNA was used in PCR analysis or stored at -20ºC for future use.

PCR was used to detect the presence of *Hygromycin B phosphotransferase* (*hptII*) selectable marker and the *CycD; 1*. Using specific primers, a specific segment of *CycD2; 1* and *hpt*II coding sequences were amplified. The presence of the *CycD2* gene was indicated by positive PCR amplification signals after agarose gel analyses. Amplifications were performed using Eppendorf master cycler (EP-AG 5341 012727, H Hamburg, Germany).

The PCR reaction contained 20 ng of plant DNA 1.2 mM $MgCl_2$, 0.4 µM of each of the primer pairs, 1 x PCR buffer, 0.24 mM dNTPs and 0.25 Units of Taq polymerase with 1 x 2.5 mM$MgCl_2$/reaction of 20 µl. The primers and their sequences are shown in Table 1.

The reaction mixture was subjected to an initial denaturation step of 94ºC for 3 min followed by 35 cycles of 94ºC for 30 s; annealing temperatures of 60ºC for *hpt* and 62ºC for *CycD2; 1* for 1 min; 72ºC for 1 min and a final extension step of 72ºC for 10 min. The plasmid vector DNA was included as positive control as well as two negative controls (water and untransformed plant DNA). Amplified DNA was detected by using ultraviolet light after electrophoresis on 1% agarose/ethidium bromide gels using 1 x Tris acetic-EDTA and gel pictures captured with gel documentation system (G-box Syngene).

RESULTS

Cloning of *Arath; CycD2; 1* and *Musa; CycD2; 1* genes

Prior to transformation of "Sukali Ndiizi" cells, restriction digestions and PCR were done, as described in chapter three, to confirm presence of the *Cyclin*D2. Restriction enzymes used included *Bam*HI*, Hind*III, *Kpn*I and *Xba*I. After double digestion of Topo with *Hind*III and *Bam*HI, fragments of expected band sizes (4 kb backbone and 1 kb for *Arath; CycD2; 1* and *Musa; CycD2; 1*) were obtained.

The restriction digestion of PLBR19 and pC1305.1 with *Kpn1* and *Xba1* gave expected band sizes of 1919 and 9446 bp, respectively for the backbones and those of 2400 bp for *Arath; CycD2; 1* or *Musa; CycD2; 1* containing the promoter and terminator.

PCR using gene specific primers at different stages also gave expected bands on electrophoresis gels as shown in Figures 2B and D. The results indicate that the binary pC1305.1 which had *UidA* (GUS) reporter gene carried the genes of interest, *Arath; CycD2; 1* or *Musa; CycD2; 1*.

Transformation and regeneration of "Sukali Ndiizi" embryogenic cells with *Arath; CycD2; 1* and *Musa; CycD2; 1*

Histochemical GUS assay of transient CycD2-GUS gene transformed banana cells

Using *Agrobacterium* mediated transformation system, ECSs of banana cultivar "Sukali Ndiizi" was co-cultivated with AGL-1 strain harbouring a binary vector pC1305.1 carrying *Arath; CycD2; 1*, or *Musa; CycD2; 1* gene. Samples were selected randomly and histochemically stained for the expression of GUS gene as shown in Figure 3.

To effectively compare gene transfer efficiency associated with the two *CycD2; 1* genes, quantitative analysis of blue foci obtained by histochemical GUS assay of transformed ECS cells was performed. The *GUS* assay,

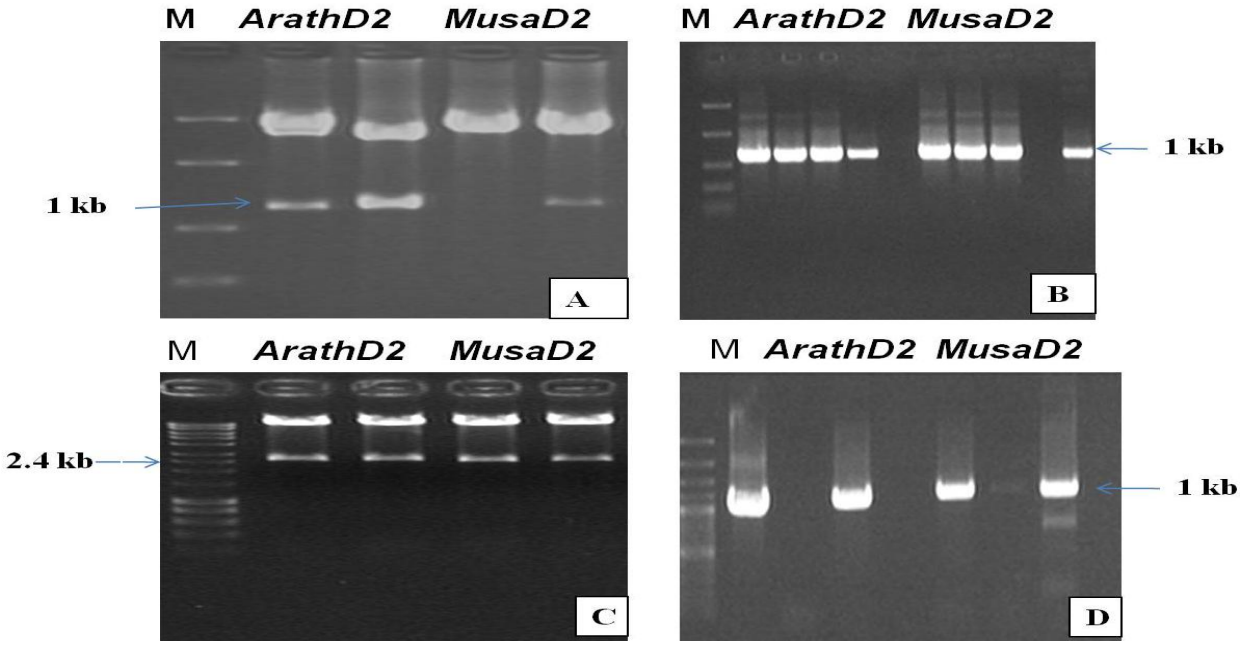

Figure 2. Gel electrophoresis of PCR and restricted plasmid DNA on a 1% agarose gels. **A.** Restriction of Plasmid DNA from putatively transformed *E. coli* using Bam HIand *Hind*III enzymes. **B.** PCR of the inserts in PLBR19 vector containing the promoter and the terminator. **C.** Restriction digestion of pC1305.1 binary vector using *Kpn1* and *Xba1* enzymes to confirm the presence of the inserted constructs containing the promoter and terminator. **D.** PCR of plasmid extracted from AGL-1 after inserting the gene constructs.

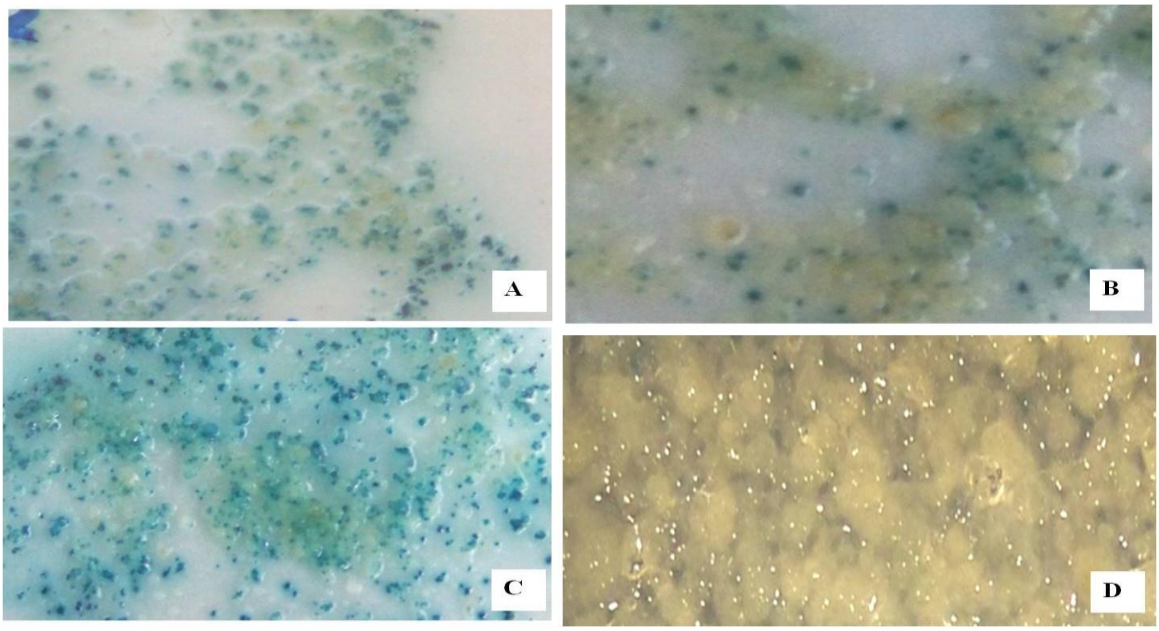

Figure 3. Histochemical assay for transient expression of GUS gene in Ndiizi ECS transformed with different *CycD –GUS* gene after overnight incubation at 37**º**C. **A.** *Arath CycD2; 1*. **B.** *Musa CycD2; 1.* **C.** Control with only pC1305.1 vector without C*ycD* genes. **D.** Non transformed control.

expressed as the average number of counted blue spots per treatment, was observed to be 155, 81 and 171 for *Arath CycD2*, *Musa CycD2* and control without *CycD* gene (binary vector only), respectively. Three randomly selected plates were counted and each plate contained 100 µl of PCV of cells. There was no statistical difference

Table 2. Enhanced transformation in "Sukali Ndiizi" using *CycD2* genes.

Gene type	Number of embryos/0.5 PCV	Shoots/100 embryos	Regeneration frequency (%)	Plant estimate/0.5 PCV
Arath CycD2	1043	62	62	647
*Musa Cyc*D2	801	47	47	376
pC1305.1	96	18	18	26
Untransformed	2100	89	89	8900

($p \leq 0.166$) in transformation efficiency among the different genes.

Selection and regeneration of "Sukali Ndiizi" transformants

Transformed ECSs were transferred to selective M3 media and incubated in the dark at 25±2ºC for three months with sub-culture interval of two weeks. After three weeks in culture, the ECSs turned brown due to necrosis and massive death of untransformed embryogenic cells. One month later, numerous whitish cell clumps (embryogenic cell colonies) appeared on the surface. This response occurred in all gene constructs. The embryogenic colonies were picked, quantified and transferred onto selective RDI medium with selection. Cell clones began to germinate in three weeks. These were transferred onto M4 (regeneration medium) and incubated in the light for further development of the shoots. Significant differences in the number of surviving clones of cells transformed with *CycD2; 1* ($p \leq 0.001$) as well as regenerated plants ($p \leq 0.001$) were observed. The un-transformed control had 89% regeneration. This is expected because these cells were neither subjected to any stress of antibiotics nor *Agrobacterium* infection like the transgenic counterparts. Cells transformed with both *Arath;CycD2; 1* and *Musa; CycD2; 1* had higher numbers of surviving embryos (1043 and 801, respectively) as well as regenerated plants (62 and 47%, respectively) compared to the cells transformed with only pC1305.1 (containing only *GUS* gene but without the cell cycle gene) that had only 18% regeneration.

It was however noted that there was no significant difference between the colonies and shoots regenerated in the *CycD2; 1* genes from *Arabidopsis* and *Musa spp*. More importantly, it was observed that even though the control with the vector only scored the highest average number of blue spots per plate (171), it had the least number of regenerates (18%). On the other hand, *Musa; CycD2; 1* had the least average number of blue spots per plate, but the number of regenerated shoots were far higher (almost the same as its counterpart from *Arabidopsis*) than those of the control with the vector only (Table 2).

Characterisation of "Sukali Ndiizi" transgenic lines

Hygromycin resistant plants were analysed to determine whether they contained the inserted gene constructs. Histochemical GUS assay was used to detect the presence of *UidA* (GUS) reporter gene. Genomic DNA was then extracted and PCR using gene specific primers followed to establish the presence of *CycD2; 1* genes.

Histochemical GUS assay of transformed lines

10 transformed plants (three months after regeneration) per construct were randomly selected and tested for β-glucuronidase (GUS) enzyme. Different tissues (leaves, corms and roots) were tested and intense blue staining was readily observed in all the positive plant tissues and no staining in control tissues as shown in Figure 4.

PCR analysis of "Sukali Ndiizi" regenerates

Genomic DNA from independently transformed and non-transformed (control) tissues was subjected to PCR. Figure 5 shows that the samples from transformed tissues gave the predicted DNA fragment bands of 1 kb for cyclinD2 gene (upper lanes) and 0.5 kb for *hpt*II gene (lower lanes). Of the 50 randomly selected plants, 74 and 78% for *Arath; CycD2; 1* and *Musa; CycD2; 1*, respectively had co-existence of both *hpt*II and *CycD2* genes. The control which had only the binary vector, pC1301.5, minus *CycD2* gene had 88% amplification for *hpt*II gene. The few lines that did not amplify for *CycD2* genes did not also amplify for *hpt*II primers, indicating that they were escapes. No amplified product was observed in case of the non-transformed plants (negative control) and the PCR negative control (Figure 6).

DISCUSSION

A number of genes have been used in genetic transformation of plants including banana. Several of these genes have been cloned, and their expression regulated by Cauliflower 35S mosaic virus promoter (CaMV). Regeneration efficiency of 47 to 62% as observed was far higher than that of the control with an empty vector (18%). The reported regeneration frequency was higher than what was found in cultivar 'mas' which is AAB genotype (Jalil et al., 2003).

The absolute requirement of S-phase (DNA duplication) for *Agrobacterium* mediated transformation was demon-

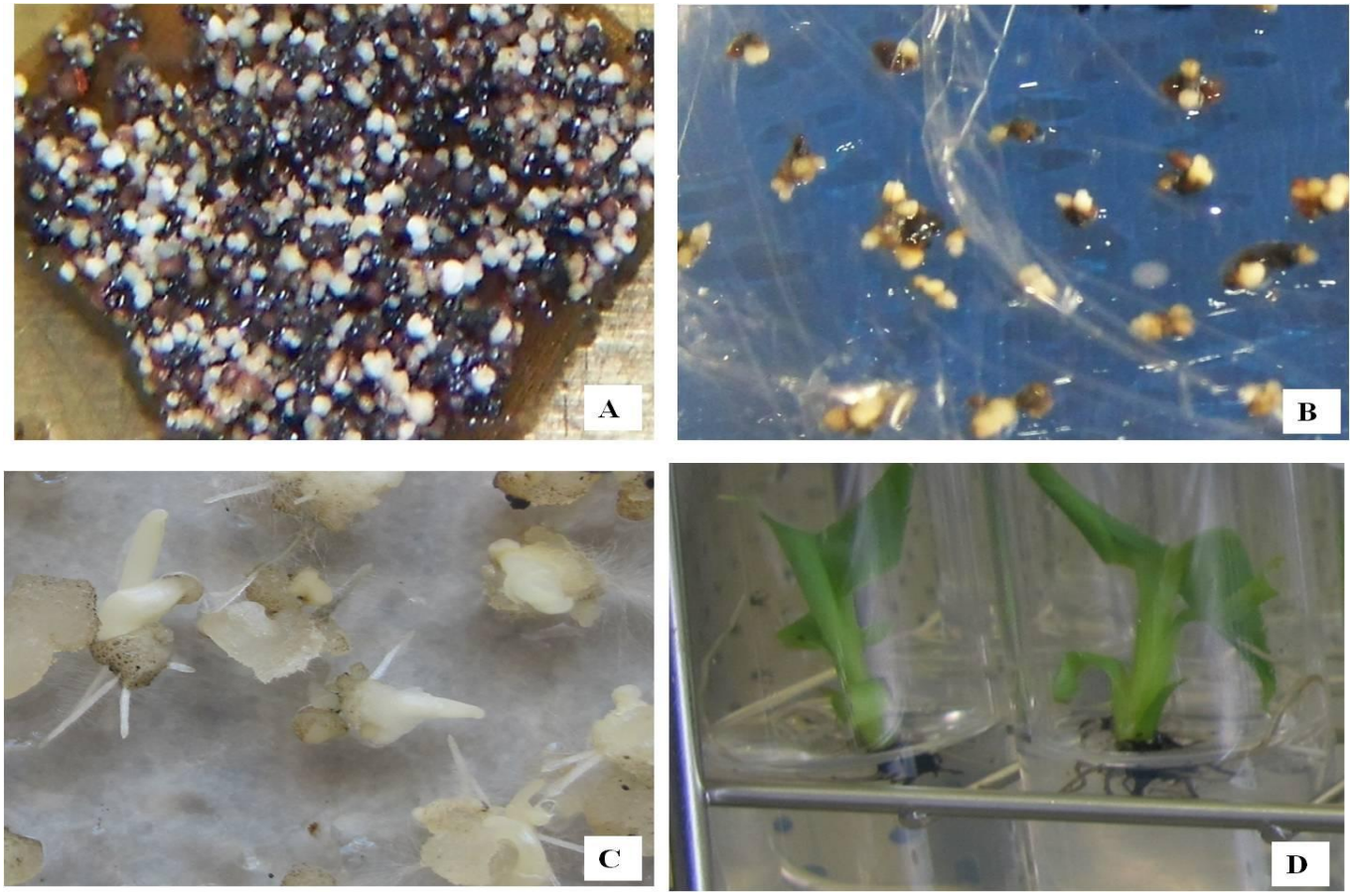

Figure 4. Regeneration stages of transgenic banana cells. **A.** Three months old cultures of 'Sukali Ndiizi' transformed cells on selective M3 medium. White spots are the hygromycin resistant cells with a black background of dead cells. **B.** Embryos on embryo RDI development medium. **C.** Germinating embryos on M4 medium. **D.** Shoots growing on non-selective M4 medium.

strated (Villemont et al., 1997). Although there was no significant difference in GUS staining in cells transformed with *CycD2; 1* gene compared to that of an empty vector, post infection viability was highly improved. This is a significant observation and explains why despite high initial reporter gene expression in cells of many plants, especially monocots like banana, cells do not regenerate. *CycD2;1* genes could have increased stable transformation by promoting cell division and proliferation which are required for stable transformation (Villemont et al., 1997).

Exploitation of highly dividing cells has been reported by several authors. These include reports of increased gene transfer by wounding, pre-culture of explants on auxin rich media, and use of previously sub-cultured plant cells. The observed increase in transformation efficiency has been attributed to be due to stimulation of cell division (Sangwan et al., 1992) and activation of DNA replication machinery. Chateau et al. (2000) observed similar effects in *Arabidopsis*.

Recently, several *Agrobacterium* gene transfer system reviews have highlighted the importance of cell division during gene transfer (Tzfira et al., 2002; Gelvin, 2003; Arias et al., 2006). Arias et al. (2006) particularly emphasized on the importance of cell division, explaining that cell cycle phases S-M was vital for plant cell transformation. The reasoning is based on the fact that plant cell DNA repair machinery is more active during cell division due to on-going DNA replication processes (Tzfira et al., 2002). In the related study, ectopic expression of *Arath;CycD2;1* in transgenic tobacco (*Nicotiana tabacum*) and *Arabidopsis thaliana* plants led to accelerated development and a faster growth rate attributable to a reduction in cell cycle length caused by a reduced G1-phase duration (Cockcroft et al., 2000).

Observed lack of significant difference in the performance of *Arath; CycD2; 1* and *Musa; CycD2; 1* contributes to the growing evidence that genes that control the cell cycle are conserved across the evolutionary distances (Arias, 2006) contributes to the growing evidence that genes that control the cell cycle are conserved across the evolutionary distances (Arias, 2006).

The results presented here shows that "Sukali Ndiizi" cells are highly competent and transformable by *Agrobacterium* mediated transformation system. Regardless of the origin, *CycD2* genes have the potential to significantly improve regeneration frequency of "Sukali Ndiizi" cells.

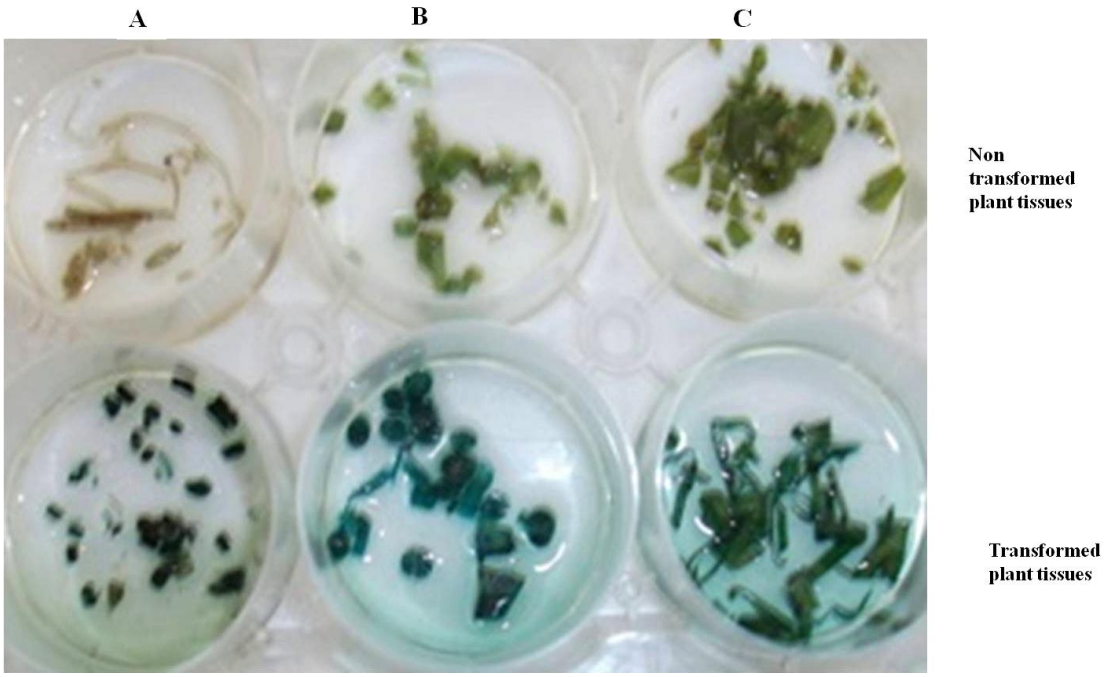

Figure 5. Histochemical GUS assay with X-Gluc-CHAPS of pieces of root (A), corm (B) and leaf (C) of "Sukali Ndiizi" transgenic plants. There was no significant variation in the staining pattern of different tissues.

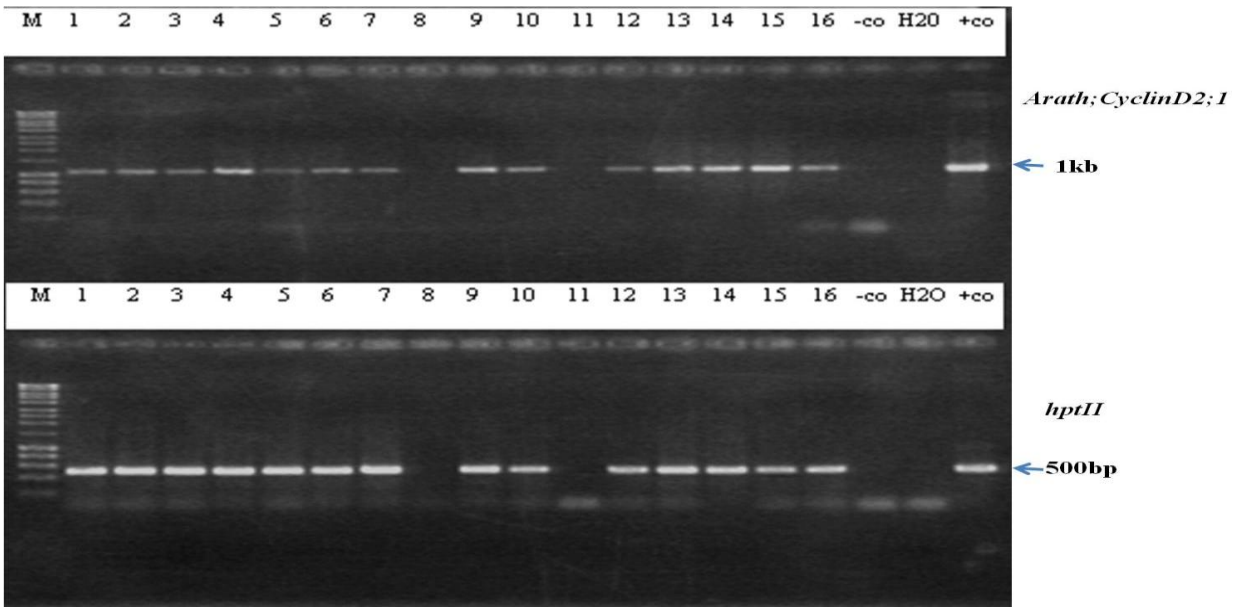

Figure 6. Agarose gel for analysis of representative transgenic plants from 'Sukali Ndiizi' containing *CycD2* together with *hptII* genes by PCR. M, DNA sizing marker (Hyperladder1) of 200 to 10,000 bp from bioline; -Co, negative control, non-transformed banana plant; +Co, positive control (plasmid DNA); Upper panel and lower panel are *Arath CycD2; 1* and *hpt*II genes respectively. Water was also included to check for any contamination. Lanes 1 to 16 are independent regenerates.

Conclusion

In conclusion, Arath *CYCD2;1* gene and Musa *CYCD2;1* were fused together with *GUS* reporter gene and used to transform 'Sukali Ndiizi' embryogenic cell suspensions. A significant difference was observed in the regeneration of cells with *CYCD2;1* constructs as compared to those with empty vector. These genes were expressed in all tissues as shown by GUS assay on roots, shoots and corms. These two genes gave the same result regardless of the

source. This study contributes to the current need of information about improvement of transformation and regeneration efficiency of bananas and highlights the potential of C*ycD2* genes in the improvement of regeneration of plants.

ACKNOWLEDGEMENTS

We appreciate the contribution of the National Banana Research Program to this work especially in the acquisition of the genes used. This work was done with financial support from Biosciences for Eastern and Central Africa (BecA Net).

REFERENCES

Arias Renée S, Sergei AF, Steven HS (2006). Divide and conquer: development and cell cycle genes in plant transformation. Trends in Biotechnology. 24:267-273.

Arinaitwe G (2008). An improved *Agrobacterium* mediated transformation method for banana and plantain (*Musa* spp). A PhD thesis submitted to the Catholic University of Leuven. 158p.

Chateau S, Sanguan, RS, Sangwan NBS (2000). Competence of *Arabidopsis thaliana* genotypes and mutants for *Agrobacterium tumefaciens* mediated gene transfer: Role of phytohormones. J. Exp. Bot. 51:1961-1968.

Cheng M, Lowe BA, SpenserTM, Ye X, Armstrong CL (2004). Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. *In vitro* Cellular and Developmental Biology-Plant 40:31-45.

Cockcroft CE, den Boer BGW, Healy JMS, Murray JAH (2000). Cyclin D control of growth rate in plants. Nature, 405: 575-579.

Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA mini preparation: version II. Plant Mol. Biol. Rep. 1(4): 19-21.

Gelvin SB (2003). Improving plant genetic engineering by manipulating the host. Trends Biotechnol. 21:95-98.

Gold CS, Ogenga-Latigo MW, Tushemereirwe WK, Kashaija IN Nankinga C (1993). Farmers' perception of banana pest constraints in Uganda. In: Gold CS, Gemmil B (Eds). Biological and integrated control of highland banana pests and diseases. Proceedings of a research co-rdination meeting, Cotonou, Benin, 12-14 November 1991. International Institute of tropical Agriculture, Ibadan, Nigeria, 3-24.

Jalil A, Khalid N, Othman RY (2003). Plant regeneration from embyogenic suspention cultures of Musa acuminate cv. Mas (AA). Plant cell tissue and organ culture 75:209.214.

Jefferson RA (1987). Assaying chimeric genes in plants: The GUS gene fusion system.PlantMol. Biol. Rep., 5: 387-405.

Jones HD (2005). Wheat transformation: current technology and applications to grain development and composition. J Cereal Sci. 41:137–147.

Khanna H, Jean-Yves Paul, Robert M. Harding, Martin B, Dickman, James L Dale (2007). Inhibition of *Agrobacterium*-Induced Cell Death by Antiapoptotic Gene Expression Leads to Very High Transformation Efficiency of Banana. MPMI 20: 1048–1054.

Khanna H, Becker D, Kleidon J, Dale J (2004). Centrifugation assisted *Agrobacterium tumefaciens*-mediated Transformation of embryogenic cell suspensions of Banana. Mol. Breed. 14:239-252.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol.Plant.15:473-97.

Ságí L, Swennen R (1997). Genetic transformation for the improvement of bananas - A critical assessment Focus Paper II. Networking Banana and Plantain: INIBAP Annual Report 1997, INIBAP, Montpellier, France (1998) 33-35.

Sangwan RS, Bourgeois Y, Brown S, Vasseur G, Sangwan-Norreel B (1992). Characterization of competent cells and early events of *Agrobacterium*-mediated genetic transformation in *Arabidopsis thaliana*. *Planta* 188: 439–456.

Talwana HAL, Speijer ER, Gold CS, Swennen RL, De Waele D (2003). A comparison of the effects of the nematodes *Radopholus similis* and *Pratylenchus goodeyi* on growth, root health and yield of an East African Highland banana (*Musa* AAA-group). International Journal of Pest Management, 49(3):199-204. Tobacco tissue cultures. Physiol. Plant. 15:473-97, 1962.

Tushemereirwe W, Kangire A, Ssekiwoko F, Offord LC, Crozier J, Boa E, Rutherford M and Smith J (2004). First report of *Xanthomonas campestris pv. musacearum* on banana in Uganda. *Plant Pathology*, 53:802.

Tzfira T, Vaidya M, Citovisky V (2002). Increasing plant susceptibility to *Agrobacterium* infection by over expression of *Arabidopsis* VIPI gene. Proc Acad Sci. 99:10435-10440.

Villemont E, Dubois F, Sangwan RS, Vasseur G, Bourgeois Y, Sangwan-Norreel BS (1997). Role of the host cell cycle in *Agrobacterium*-mediated genetic transformation *of* petunia: Evidence of an S-phase control mechanism for T-DNA transfer. Oxford J. 1:1333-1961.

Vuylsteke D (1989). Shoot-tip culture for the propagation, conservation, and exchange of *Musa* germplasm. Practical manuals for handling crop germplasm *in vitro*. IITA, Ibadan.