ASIAN J TROP BIOTECHNOL
Volume 18, Number 2, December 2021
Pages: 55-68

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c180202

Genetic transformation of banana with Extracellular Secreted *Plant ferredoxin-like protein* (ES-*Pflp*) gene

MACHARIA SARAH WANJIKU, STEVEN RUNO, LEENA TRIPATHI♥
School of Pure and Applied Sciences, Kenyatta University. P.O. BOX 43844-00100, Nairobi, Kenya. Tel. 8710901-19 Ext. 339,
♥email: l.tripathi@cgiar.org

Manuscript received: 17 August 2021. Revision accepted: 14 October 2021.

Abstract. *Wanjiku SM, Runo S, Tripathi L. 2021. Genetic transformation of banana with Extracellular Secreted* Plant ferredoxin-like protein *(ES-*Pflp*) gene. Asian J Trop Biotechnol 18: 55-68.* Banana *Xanthomonas* Wilt (BXW) is the most damaging disease of banana (*Musa* spp.) in East Africa. It is caused by *Xanthomonas campestris* pv. *musacearum* (Xcm). This disease has no effective control strategies. All banana cultivars are susceptible to BXW disease. Due to their sterility and long generation life cycle, most cultivated cultivars are triploids, hence difficult to enhance through conventional breeding. Genetic engineering provides an alternative and successful method of BXW disease prevention. The purpose of this work was to express the Extracellular Secreted *Plant ferredoxin-like protein* (ES-*Pflp*) gene in bananas and to assess the resistance of transgenic lines to Xcm. A signal peptide guides the *Pflp* to the extracellular area of the cell. In *Arabidopsis*, ES-*Pflp* has been found to provide bacterial resistance. Thus, overexpression of ES-*Pflp* in a banana is expected to boost resistance to Xcm. Co-cultivation of Gross Michel and Sukali Ndizzi Embryogenic Cell Suspensions (ECSs) with *Agrobacterium* strain EHA105 carrying the binary vector pBI-ES-*Pflp*, followed by the selection of kanamycin-resistant calli and regeneration of plantlets. Transgenic banana plants producing an ES-*Pflp* gene were produced using the constitutive promoter of the Cauliflower mosaic virus 35S. Polymerized Chain Reaction (PCR), Southern blot hybridization, and Reverse Transcription Polymerized Chain Reaction (RT-PCR) studies of transgenic lines demonstrated sustained transgene incorporation and expression. Growth research shows that most transgenic lines grew similarly to non-transgenic plants under glasshouse conditions and thus were proceeded to screening for resistance to BXW disease. Agronomic data were collected on all screened lines and compared to control lines. Transgenic lines were screened for increased resistance to BXW disease by artificial inoculation with Xcm. Developing banana cultivars resistant to BXW would increase banana production and help farmers who rely on bananas as a staple food and income crops maintain food security.

Keywords: Banana *Xanthomonas* Wilt, disease, ES-*Pflp*, genetic, *Xanthomonas campestris* pv. *musacearum*

Abbreviations: BRM: Bacterial Resuspension Medium, BBTV: Banana Bunchy-Top Virus, BSV Banana Streak Virus, BXW: Banana *Xanthomonas* Wilt, ECSs: Embryogenic Cell Suspensions, ES-*Pflp*: Extracellular Secreted *Plant ferredoxin-like protein*, HR: Hypersensitive Response, PCR Polymerized Chain Reaction, RT-PCR: Reverse Transcription Polymerized Chain Reaction, SAR: Systemic Acquired Resistance, Xcm: *Xanthomonas campestris* pv. *musacearum*

INTRODUCTION

Bananas and plantains are the world's sixth-largest agricultural crop, trailing maize, rice, wheat, potato, and cassava, with an estimated annual global production of 139 million tons (FAOSTAT 2012). A third of this is produced in Africa, with East Africa producing and consuming the most. India is the world's largest producer of bananas, followed by Uganda, which has 24.7 million tons and 9.77 million tons sequentially (FAOSTAT 2012). Over 130 countries in the tropics and subtropics farm this crop (Pachuau et al. 2014), making it the most domesticated clonal crop (De Langhe et al. 2010). Bananas are a year-round crop (Namuddu et al. 2012). They can grow on steep slopes (Karamura et al. 1998) providing a staple food source (Hasanah et al. 2017) and source of income for millions of people, particularly in Africa, with approximately 87% of production remaining in domestic markets (Roux et al. 2008).

Numerous diseases and pests adversely affect banana production, including black sigatoka (*Mycosphaerella fijiensisi*), Banana *Xanthomonas* Wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm), *Fusarium* Wilt (*Fusarium oxysporum f.* sp. *Cubense*), viruses [Banana Bunchy-Top Virus (BBTV), genus *Nanavirus* and Banana Streak Virus (BSV), genus *Badnavirus*], weevils and nematodes (Jones 2000; Tushemereirwe et al. 2004; Tripathi et al. 2008; Hadiwiyono 2011). Given the banana's global importance, there is considerable opportunity for developing disease-free and high-yielding cultivars for growers. Resistant cultivars would be the most cost-effective and environmentally friendly way to overcome these impediments. Conventional breeding has had limited success in improving banana genetics due to long generation durations, a lack of genetic variety, an undesirable gene pool, and time-consuming screening methods (Namuddu et al. 2012), and the triploidy of many farmed bananas (Vuylsteke 2000). Therefore, while a few

diploid clones produce viable pollen, most of the commercial clones' germplasm is both male and female-sterile (Novak et al. 1989). Genetic engineering is a possible alternative technique for creating superior agronomic features in bananas and plantains.

Plants have many defense systems in place to stave against disease invasion. The Hypersensitive Response (HR) is an induced resistance mechanism characterized by fast, localized cell death in response to infection by a microbial pathogen (Goodman and Novacky 1994; Dangl et al. 1996). HR-induced cell death creates a physical barrier that prevents further pathogen infection. Additionally, a local HR is frequently accompanied with the activation of plant defense responses in adjacent and even distal uninfected plant sections, resulting in the establishment of Systemic Acquired Resistance (SAR). Human resistance is frequently observed in disease-resistant plants. It often occurs before a slower systemic (whole plant) response, resulting in SAR (Freeman 2003). It has been demonstrated that the *Plant ferredoxin-like protein* (*Pflp*) activates HR, which is characterized by planned cell death in response to microbial infection. It has been shown that constitutive expression of the *Pflp* gene in transgenic tobacco, *Arabidopsis*, and banana plants confers increased resistance to various diseases (Huang et al. 2004; Lin et al. 2010; Namukwaya et al. 2012). Additionally, it was discovered that extracellular *Pflp* released by the cell increased resistance to *Ralstonia solanacearum* in *Arabidopsis* (Huang et al. 2004). As a result, the purpose of this study was to convert bananas with Extracellular Secreted *Plant ferredoxin-like protein* (ES-*Pflp*) and determine the amount of resistance to BXW of transgenic lines.

Banana (*Musa* spp.) is a major global food crop grown in over 130 tropical and subtropical nations. However, BXW disease has had a significant impact on banana output in Eastern Africa, jeopardizing the livelihoods of millions of farmers (Tushemereirwe et al. 2003). BXW is a highly damaging disease that affects all banana kinds, including dessert, frying, roasting, and beer variants (Ssekiwoko et al. 2006). The effects of BXW disease are severe and quick, in contrast to the impact of other diseases, which induce progressive increases in mortality over the years. The economic consequence of BXW disease includes yield loss in absolute terms and the mortality of mother plants that would otherwise contribute to ratoon plant production cycles (Tripathi et al. 2009). Over a decade, economic losses are anticipated to total $2 billion due to price rises and considerable production decreases (Abele and Pillay 2007). Due to the lack of known natural sources of resistance in any cultivated banana, genetic modification is widely regarded as the most viable strategy for developing bacterial wilt-resistant bananas, especially given the availability of an efficient and reliable banana transformation protocol (Tripathi et al. 2012). As such, the purpose of this work was to determine the possibility of adopting genetic engineering approaches to reduce BXW sickness by utilizing the ES-*Pflp* gene.

The specific objectives of this study were: i) to transform and create transgenic banana cultivars Gross Michel and Sukali Ndizzi with ES-*Pflp* gene; ii) to determine the effect of overexpression of ES-*Pflp* gene on resistance to BXW disease in transgenic Gross Michel and Sukali Ndizzi plants grown in glasshouse conditions; iii) to compare the degrees of BXW resistance in Gros Michel ES-*Pflp* and Gros Michel *Pflp* transgenic lines.

MATERIALS AND METHODS

Plant materials and explant preparation for transformation experiments

IITA donated Embryogenic Cell Suspensions (ECSs) of the banana cultivars Sukali Ndizzi and Gros Michel for this study. Tripathi et al. (2010; 2012) outlined the process for the subculture of ECSs. Sukali Ndizzi ECSs were subcultured in MA2 medium. In contrast, Gros Michel ECSs were subcultured in ZZ medium [A settled cell volume of 1 mL of ESCs was subcultured in 50 mL of their respective media for 5 days before transformation to maximize cell transformation competence and efficiency (Tripathi et al. 2012).

Agrobacterium and plasmid

The pBI-ES-*Pflp* vector was utilized in this research. It contained the ES-*Pflp* gene driven by the CaMV35S promoter and the nptII gene as a selection marker (Figure 1). The International Institute of Tropical Agriculture purchased this construct from Academia Sinica in Taiwan via the African Agricultural Technology Foundation. The vector-bearing *Agrobacterium* strain EHA105 was grown on LB medium supplemented with kanamycin (50 mg/L) and rifampicin (50 mg/L). To prepare for transformation, the bacterial culture was cultivated for two days at 28°C with shaking in liquid LB medium supplemented with the required antibiotics. A day before transformation, approximately 500 mL of the *Agrobacterium* culture was added to 20 mL of LB medium containing suitable antibiotics and then cultured overnight at 28°C in a shaking incubator (200 rpm). On the 21st day after transformation, the bacteria were harvested by centrifuging at 5,000 rpm for 10 minutes at 4°C, and the pellet was re-suspended in 25 mL of Bacterial Resuspension Medium (BRM) (Tripathi et al. 2012) and shaken at 70 rpm for 2 hours at 25°C for induction. The optical density of the culture at 600 nm was determined and adjusted to 0.6 using BRM media.

Transformation, selection, and regeneration of transgenic plants

In the transformation studies, ECSs of Sukali Ndizzi and Gros Michel's banana cultivars were employed as explants. They transferred the ECSs into falcon tubes and allowed the cells to settle. Excess media was discarded. Ten milliliters of the appropriate culturing media were added, warmed to 45°C, mixed, and incubated for 5 minutes. The medium was decanted and 110 µL of 2% pluronic acid and the pre-induced *Agrobacterium* suspension were added to ECSs. Next, the mixture was centrifuged at 900 rpm for 6 minutes at 25°C and the contents of the tube left tosettle. After that, the cells were

allowed to stand for 30 minutes for infection. The liquid was then decanted, and agro-infect cells were co-cultured for 3 days in the dark on a bacterial co-culture medium (Tripathi et al. 2012).

Following co-cultivation, the Agro-infected ECSs were washed three times with MA2 for Sukali Ndizzi and ZZ liquid for Gros Michel, supplemented with 300 mg/L cefotaxime, i.e., after plating the cells on a sterile nylon mesh, they were transferred to MA3 medium with 300 mg/L cefotaxime and 100 mg/L kanamycin and maintained at 26°C in the dark. Every 14 days, the cells on the sterile mesh were transferred to fresh media. After two to three months of selection, embryos were moved to RD1 medium supplemented with 300 mg/L cefotaxime and 100 mg/L kanamycin and housed at 26ºC in the dark. After germination in the dark at 26ºC, mature embryos were transferred to MA4 medium supplemented with 300 mg/L cefotaxime and 100 mg/L kanamycin. Sukali Ndizzi germinated plantlets were placed to proliferation media, while Gros Michel germinated plantlets were transferred to the basal medium.

DNA extraction and Polymerized Chain Reaction (PCR) analysis of the regenerated plants

Using the DNeasy plant micro kit, genomic DNA was extracted from thirty lines of Gros Michel and 24 lines of Sukali Ndizzi putative altered plantlets (Qiagen, GmbH, Germany). The presence of the ES-*Pflp* gene in the genomes of randomly selected putatively transgenic lines was confirmed using Polymerized Chain Reaction (PCR) analysis using gene-specific primers having a predicted band size of 450 base pairs. *Pflp* gene primers were 50 CAAGAAAACCAGCTGTGACAAGCCTTAAAC 30 and 50 CGAGTTCTGCCTCTTTGTGAGTCTCAATAG 30; nptII gene primers were 50 CCTATCCGCAACTTCTTTACCTA 30 and reverse 50 ACACCCAGCCGGCCACAGTCG 30. PCR reactions were carried out using an Eppendorf Master Cycler (EPAG 5341 012727, H Hamburg, Germany). 2l plant DNA, 1mM MgCl2, 0.4M primers, 2.5X PCR buffer, 0.75M dNTPs, and 0.001 units Taq Hot star in a 25l reaction volume. This reaction volume was initially denaturated at 94ºC for 10 minutes, followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 58ºC for 1.3 minutes, extension at 72ºC for 1 minute, and final extension at 72ºC for 7 minutes. The positive control was pBI-ES-*Pflp* plasmid DNA, while the negative was non-transgenic plant DNA and water. Electrophoresis of the PCR products in a 0.8 % l agarose gel in tris base acetic acid EDTA buffer containing 2l gel red was performed. The results were seen using a UV transilluminator.

Multiplication, rooting and acclimatization of transgenic plants

Each of the PCR-positive transgenic lines were doubled to get at least six duplicates. Two duplicates of each line were retained as the mother plant culture, while the remainders were planted in the rooting medium. After washing the rooted plants to remove any remaining media, they were placed in plastic cups containing sterile soil in a 1:1 ratio with manure. They were hardened off for a month in a plastic chamber in the glasshouse and then transferred to larger pots (15 cm diameter) for continued growth. Three-month-old plants were utilized to screen for BXW resistance.

Screening of transgenic lines for resistance to BXW disease

An Xcm culture was grown at 28ºC on YPGA-CC media with cephalexin (50 mg/L) and cycloheximide (200 mg/L) for two days. The culture's OD was determined and centrifuged to settle the cells. Then the media was drained out. The pellet was re-suspended in sterile water and the optical density (OD600nm) was set to 1.0. The suspension was prepared for inoculation by placing it in 1.5 mL tubes. The second open leaf of each three-month-old line in the glasshouse was infected with 100 µl of Xcm culture using a hypodermic needle. For 60 days, the inoculated plants were housed in a glasshouse and monitored for disease symptoms. The disease severity of the inoculated plants was determined using a 0-5 scale: 0 no symptoms, 1 only the inoculated leaf wilted, 2-2 to 3 leaves wilted, 3-4 to 5 leaves wilted, 4 all leaves wilted, but the plant remained alive, and 5 the entire plant died. The percentage of resistance was estimated as (Reduction in wilting in comparison to control plants/Total number of wilted leaves in control plants) X 100.

Genomic DNA isolation and Southern blot analysis

Southern hybridization was used to examine the ES-*Pflp* gene's incorporation into the banana genome. In this study, it was done utilizing the DIG labeling and detection system.

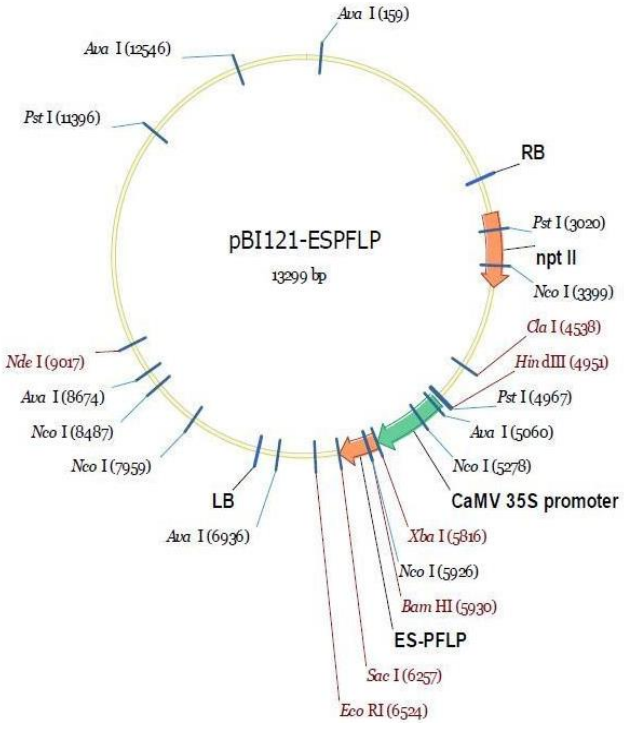

Figure 1. Map of pBI 121-ES-*Pflp* plasmid vector (Lin et al. 2011).

Genomic DNA isolation

Using the hexadecyltrimethylammonium bromide (CTAB) technique, genomic DNA was extracted from leaf tissue of six Sukali Ndizzi ES-*Pflp* transgenic lines and six Gros Michel ES-*Pflp* transgenic lines that demonstrated significant resistance during glasshouse screening and a non-transgenic plant (Gawel and Jarret 1991). Around 1 gram of leaf tissue was crushed in liquid nitrogen and incubated for 30 minutes at 65 °C in 700 µl of extraction buffer (2% CTAB, 200 mM Tris-HCl [pH 8], 0.14 M NaCl, 0.1% mercaptoethanol, and 20 mM EDTA).

Total DNA was extracted with 700 µl chloroform-isoamylalcohol (24:1) v/v and precipitated with the same amount of isopropanol. After washing with 1 mL of 70% cold ethanol, the DNA was treated with RNAse A, re-extracted, re-precipitated, washed, and re-suspended in sterile water. Southern analysis was performed on the measured DNA.

Preparation of probe

The PCR DIG probe synthesis kit was used to synthesize the DIG-labeled probe. one µl of Taq polymerase, 5 µl dNTP mix, 1 µl of plasmid DNA, and 0.5 µM of forward and reverse primers each were used in the 50 µl PCR reactions. Denaturation at 95ºC for 2 minutes was followed by 30 cycles of 94ºC for 30 seconds, followed by temperatures of 55ºC for 30 seconds, 72ºC for 2 minutes, and a final extension at 72ºC for 5 minutes.

Restriction analysis of genomic DNA

According to Table 1, the Hind *III* restriction enzyme was used to digest the plant genome.

After overnight incubation at 37ºC, the mixture was lyophilized to concentrate to a volume of 30 µL. On a 0.8% gel, the limited DNA was resolved. Positive control was plasmid DNA, and negative control was a non-transgenic plant. The gel was first operated at 60V for 30 minutes and then at 50V for 6 hours. GeneSys gel documentation system was used to capture the image. The gel was then divided into 10 cm by 10 cm squares, making tiny gels more manageable and processable.

Preparation of the gel for Southern blotting

Depurination. Restricted DNA on the gel was broken to aid membrane transfer, as DNA more extended than 10 kb does not transfer. This was accomplished through acid depurination. The gel was immersed in a 0.25 M HCl solution and gently shook for 15 minutes. Bromophenol blue's color should change from blue to yellow to indicate that the gel has been saturated with acid. After that, the gel was gently rinsed with sterile distilled water.

Table 1. DNA restriction reaction.

DNA (10 µg)	40 µL
Restriction enzyme (Hind *III)*	5 µL
Sterile water	45 µL
Buffer 10X	10 µL
Final volume	100 µL

Denaturation. The double-stranded DNA was denatured by incubating the gel in a denaturation buffer (200 mL) for 30 minutes at 50 rpm while shaking it constantly. By the end of the incubation period, the bromophenol blue in the gel had transformed from a yellow to a blue color.

Neutralization. The denaturation buffer was discarded, and the gel was immersed in neutralizing buffer for 30 minutes while gently shaking throughout the procedure.

Blotting of gel. The gel was wrapped in parafilm and placed more extensively than the 10X saline sodium citrate tray. Two lengths of Whatman 3 MM paper were cut more comprehensive than the gel and quickly soaked in 10X SSC, before being placed in a glass plate. Air bubbles were eliminated between the paper and support by rolling the pipette back and forth across the surface many times. One sheet of blotting paper and four sheets of Whatman 3 MM paper were cut to be approximately 1mm more prominent on each side than the gel. The membrane was handled with gloves and with blunt-end forceps at the edges. On the platform, the prepared gel was placed upside down. Rolling a 1 mL pipette tip back and forth over the gel freed trapped air bubbles. The gel was then encased in plastic wrap to ensure that the 10X SSC flows through it rather than around it. Next, the precut nylon membrane was placed on the gel and air bubbles were eliminated between the gel and the blotting paper. Four pre-cut sheets of Whatman 3MM paper were moistened in 2X SSC, placed on the nylon membrane, and then trapped air bubbles were removed. On top of the Whatman filter sheets, 20 cm of dry paper towels were laid. This ensures that the plastic wrap enclosing the gel prevents contact between the paper towels and the 10X SSC and moist filter paper behind the gel. It was assured that the towels did not fall over the side, as this could result in the liquid flowing around the gel rather than through it. On top of the paper towels, a second glass top was installed, and a weight was placed on top of the plate. The transfer was permitted to continue for twenty hours, while wet paper towels were replaced with dry ones to guarantee efficiency.

Fixing the DNA to the blot. Following completion of the transfer, the weight, paper towels, and four sheets of filter paper were removed. Next, the gel was overturned with the nylon membrane and laid them gel side up on moist filter paper. To ensure that the gel lanes could be identified afterwards, the positions of the gel lanes on the membrane were marked with a soft lead pencil. After that, the gel was peeled away from the membrane. After staining the gel with gel red, the efficiency of DNA transfer was determined. Finally, the DNA was fixed to the membrane using UV cross linking at a power of 12X 1000V. This led to increased accuracy and sensitivity.

Pre-hybridization and hybridization. Dig hybridization buffer (20 mL) was added to a clean hybridization bottle with the membrane facing the buffer rather than the bottle wall. This was incubated at 42°C for 3 hours at a speed of 60 rotations per minute. After replacing the solution with 15 mL DIG hybridization buffer containing PCR labeled probe, the mixture was incubated overnight at 42°C and 60 rpm.

Stringent washes and detection. For the high salt wash, the membrane was washed twice in 200 mL sterile water containing 2X SSC and 0.1 percent sodium dodecyl sulfate (w/v) for 5 minutes and shaken on an orbital shaker. This was followed by a low salt wash at 65ºC in a preheated 0.5 X SSC and 0.1 % SDS (w/v) buffer for 15 minutes twice. Each wash contained 200 mL buffer. For 5 minutes, the membrane was normalized in 20 mL washing buffer (100 mM maleic acid and 0.3% tween 20). After that, the membrane was immersed in 70 mL blocking solution. Three µL of antibodies (AP conjugate) in a 1: 10000 dilution was added to 30 mL of the remaining blocking solution and used to wash the membrane at 60 rpm. After that, the membrane was washed twice with 100 mL washing buffer at 60 rpm. For 5 minutes, the membrane was incubated with 20 mL detection buffer. After that, the membrane was placed on cling film with the DNA side facing up and prepared for usage. Add disodium 3-(4-methoxyspiro 1,2-dioxetane-3,2'-(5'-chloro) tricyclo[3.3.1.13,7] decan-4-yl) phenyl phosphate (CSPD) and cover with cling film. Excess CSPD was eliminated after 5 minutes. In the dark, an X-ray film was inserted in an X-ray film holder and the membrane was transferred to it. The X-ray film was produced as follows: 5 minutes in developer solution, 30 seconds in distilled water, and 5 minutes in fixer solution to visualize the X-ray film. This was done in a dimly lit room.

RNA extraction and RT- PCR

The lines that demonstrated resistance to BXW disease screening in the glasshouse (six of Gros Michel and six of Sukali Ndizzi) were Reverse Transcriptase PCR (RT-PCR) tested for expression of the desired gene. A leaf tissue sample (100 mg) was pounded in liquid nitrogen using a motor and pestle treated with Diethylpyrocarbonate, and total RNA was extracted using the Qiagen RNeasy Kit (Qiagen, Maryland, USA) (Qiagen 2010). To avoid DNA contamination, the isolated RNA was processed with DNase. Quantification and normalization of the isolated RNA were performed. The whole RNA template (1 g) was utilized to synthesize cDNA in a 20:l reaction mix, using the first-strand cDNA synthesis procedure from thermo scientific. This reaction contained 13 liters of RNA, 1 liter of oligo (dT) 18 primer, 1 liter of 10 mM dNTP mix, 4 liters of 5X RT Buffer, 1 liter of Maxima H Minus Enzyme Mix, and nuclease-free water. The reaction mixture was gently stirred and then incubated at 50ºC for 30 minutes. The reaction was brought to a halt by heating to 85ºC for 5 minutes. The cDNA generated was utilized for RT-PCR.

The PCR reaction was carried out using 100 mg of cDNA synthesized. In a 25 µL reaction volume, this reaction contained 1 mM MgCl2, 0.4 µM of each primer, 2.5X PCR buffer, 0.75 µM dNTPs, and 0.001 units Taq Hot star. This reaction volume was initially denatured for 10 minutes at 94ºC, followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 55ºC for 1 minute 30 seconds, extension at 72ºC for 1 minute, and final extension at 72ºC for 7 minutes.

Positive controls included pBI-ES-*Pflp* plasmid DNA, whereas negative controls included non-transgenic plant DNA and water. After electrophoresis, , the PCR products were separated and observed using a UV transilluminator in a 0.8 percent agarose (w/v) gel stained with 2l gel red.

Measurement of plant growth

The growth of the transgenic lines for each cultivar was measured at 90 days post-weaning and compared to the growth of the control non-transgenic plant at the same time. The physiological characteristics that were measured were leaf length, leaf number, pseudostem base diameter, and plant height, amongst others.

Data analysis

The studies were carried out in a completely randomized design, and each experiment was carried out in three different replicates to ensure accuracy. As a proportion of the number of independent lines per milliliter of settling cells volume, transformation efficiency was measured for each experiment. One-way ANOVA (software GenStat 14 edition) was used to evaluate the data, and Duncan's Multiple Range Test was used to compare the differences between the groups.

RESULTS AND DISCUSSION

Transformation of cell suspension and regeneration of transgenic plants

Gros Michel and Sukali Ndizzi embryogenic cell suspensions transformed with ES-*Pflp* were effectively selected for embryo initiation, maturation, and development on 100 mg/L Kanamycin-containing MA3, RD1, and MA4 medium, respectively. Every two weeks, cells or embryos were transferred to fresh media with 100 mg/L Kanamycin and 300 mg/L Cefotaxime for selection. Sukali Ndizzi ECSs were first implanted in MA3 medium with proper antibiotics until tiny white embryos were regenerated in three weeks and Gros Michel ECSs in four weeks. Sukali Ndizzi embryos were then placed in RD1 medium with suitable antibiotics for one month and Gros Michel embryos were placed in RD1 medium for six weeks for embryo development. The developing embryos were then placed in MA4 media supplemented with antibiotics to mature into plantlets. All these procedures were carried out in complete darkness. Following plantlet production, plates of MA4 (Figures 2 and 3) were exposed to light for one week before being placed in appropriate proliferation conditions for growth and multiplication. For both cultivars, approximately one hundred putatively altered lines were created.

As selection progressed, a substantial difference between the transformed and non-transformed cells became apparent, with the latter becoming brown to black and eventually dying. In contrast, the transgenic cells continued to multiply (Figure 2).

Transformation efficiency

For each cultivar, the efficiency of embryo regeneration to plantlets following transformation was determined by doing three transformation experiments with a settling cell

volume of 1 mL each. As shown in Figure 4, Sukali Ndizzi had greater embryo regeneration efficiency than Gros Michel. This demonstrates that the Sukali Ndizzi cultivar was more efficient at transformation than the Gros Michel cultivar.

Molecular characterization of transgenic plants

Polymerized Chain Reaction

The PCR analysis employing *Pflp* specific primers on randomly selected putative transgenic plantlets (31 lines of Gros Michel and 24 lines of Sukali Ndizzi) confirmed the presence of a 450 bp band in the PCR profiles (Figure 5 A, B, C and D). In addition, the existence of the banana housekeeping gene was confirmed using the 25S housekeeping gene in PCR positive lines of both cultivars, and an expected band size of 110 bp was detected (Figure 5).

RT-PCR analysis

In this study, PCR positive lines of both cultivars Gros Michel and Sukali Ndizzi were randomly selected and subjected to reverse transcription polymerized chain reaction analysis. The ES-*Pflp* primers with an anticipated band size of 178 bp were used to achieve this result, as depicted in Figure 6. This was done to evaluate the transgene expression in the lines that had been chosen.

Southern blot analysis

Selected Sukali Ndizzi ES-*Pflp* lines were assessed for stable integration of the ES-*Pflp* transgene using *Pflp* gene-specific probe, as shown in Figure 7.

Acclimatization of transgenic plants

A total of 18 Sukali Ndizzi lines and 30 Gros Michel lines were rooted and weaned in the soil after PCR positive. All the lines were toughened and then potted for a total of two months before being screened (Figure 8).

Growth analysis of banana plants to be screened

Growth parameters of Gros Michel ES-Pflp lines

When comparing the 90-day old potted transgenic lines for each cultivar to the control line, growth parameters such as leaf number, leaf length, pseudostem width, and plant height were measured on the transgenic lines (Figure 9).

When compared to the control line, the pseudostem width of all line metrics revealed no significant difference; $p=0.0847642$, where $\alpha=0.05$. However, the height of the plants on line numbers 72, 27 and 77 differed significantly from the height of the control line. Lines 72 and 77 had considerably higher plant heights when compared to the control line, while line 27 had substantially lower plant heights when compared to the control line; $p=0.0295013$ when 0.05 was used as the significance threshold. This is seen in Figure 10.

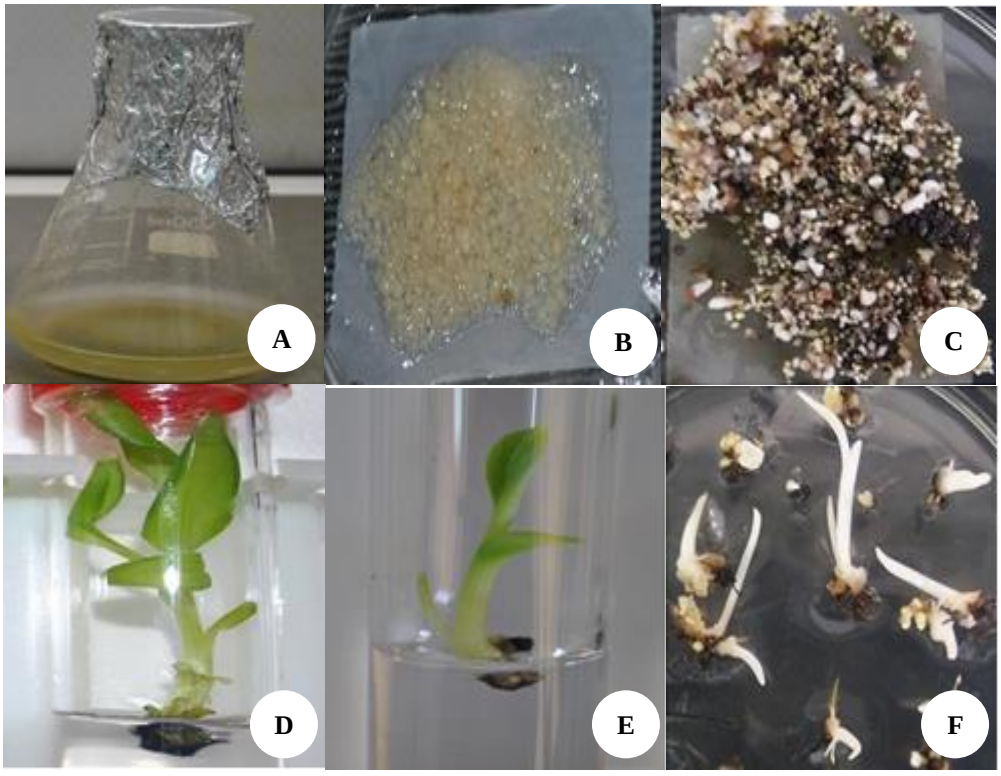

Figure 2. Transformation, selection, and regeneration of transgenic plants of Sukali Ndizzi. A: Embryogenic cell suspension, B: Agro-infected cells, C: Selection of cells on embryo development media (MA3), D: Embryos on selection on RD1 medium, E and F: Shoots in proliferation media.

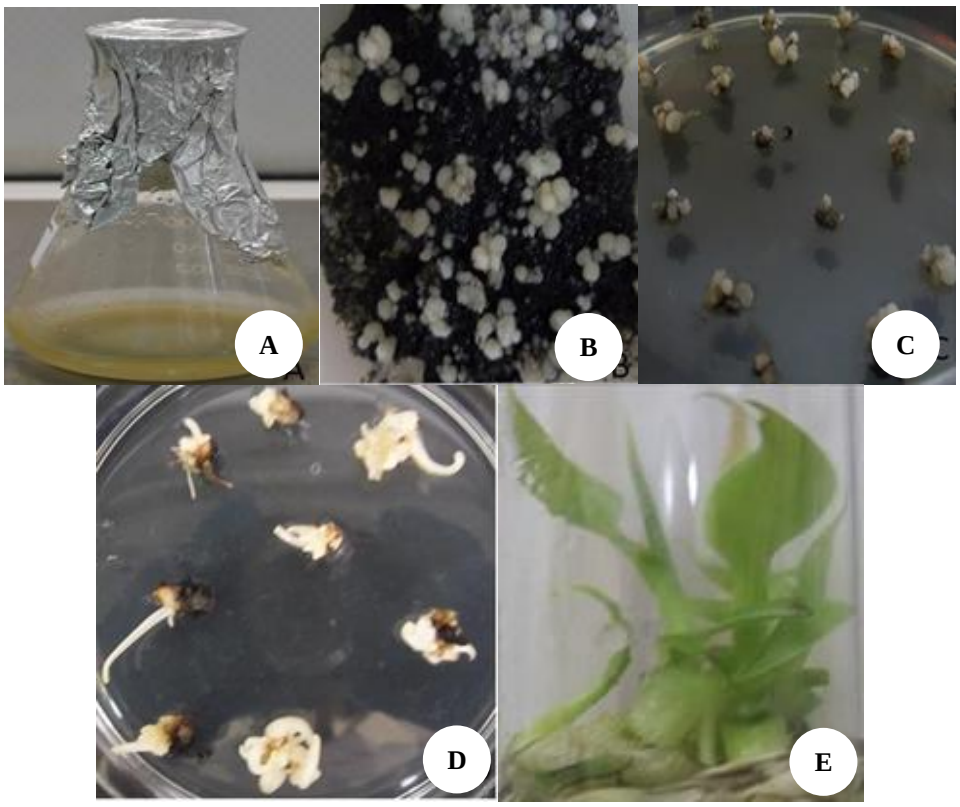

Figure 3. Transformation, selection, and regeneration of transgenic plants of *Gros Michel*. A: Embryogenic cell suspension, B: Selection of cells on embryo development media (MA3), C: Embryos on selection in development media (RD1), D: Embryos on selection in maturation media (MA4), E: Shoot inproliferation media.

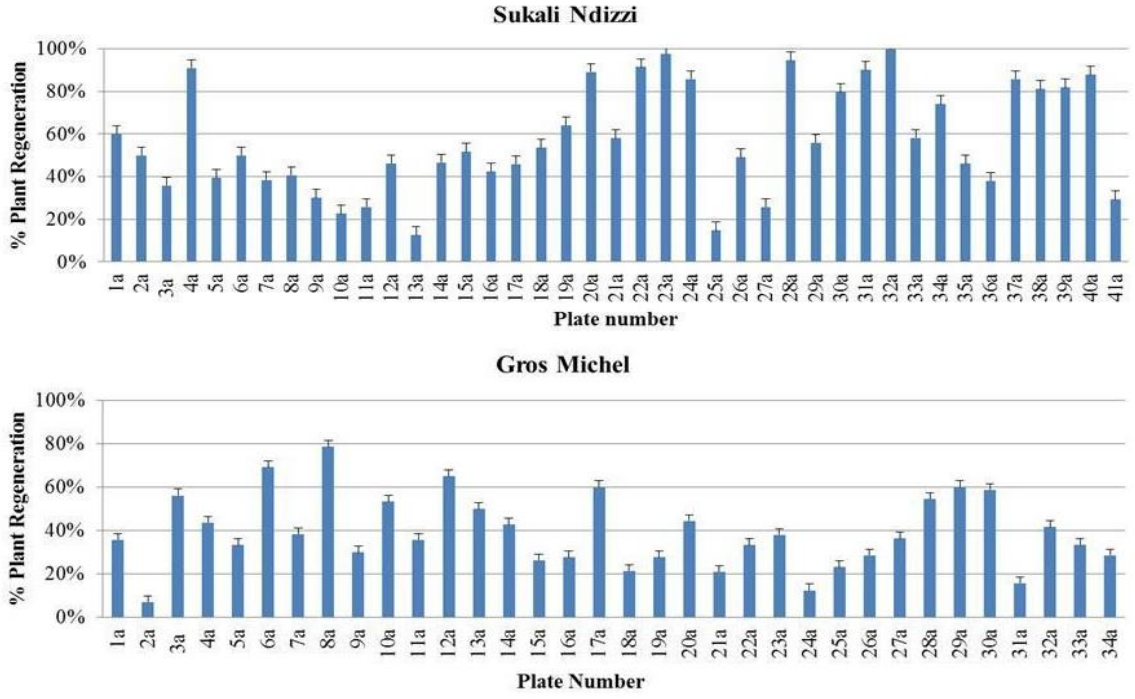

Figure 4. Embryo regeneration efficiency. Graph showing Gros Michel ES-*Pflp* embryo regeneration efficiency and Sukali Ndizzi ES-*Pflp* embryo regeneration efficiency.

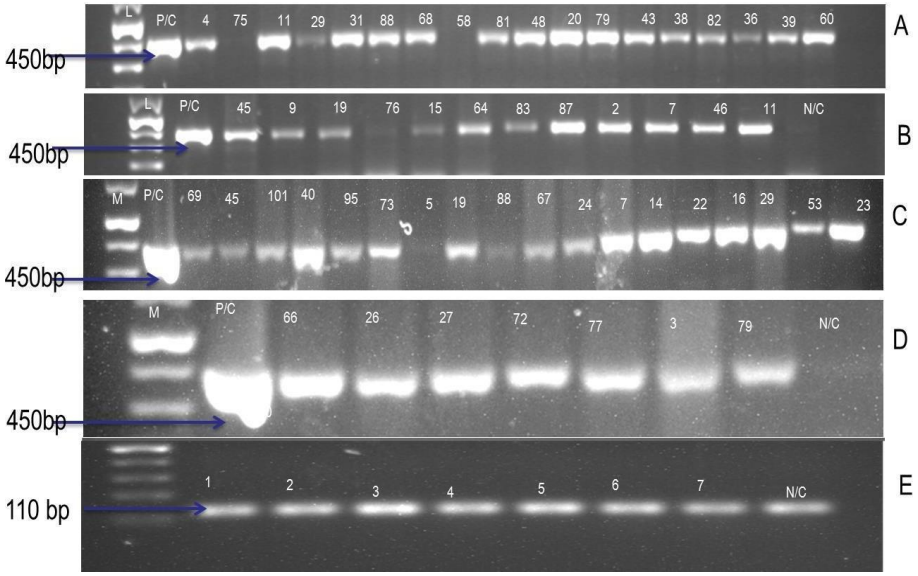

Figure 5. Representative PCR analyses of Sukali Ndizzi and Gros Michel transformed lines. A and B: PCR of Gros Michel Es-*Pflp* lines with *Pflp* specific primers, C and D: PCR of Sukali Ndizzi lines with Pflp primers, E: Sukali Ndizzi ES-*Pflp* and Gros Michel ES-*Pflp* lines with housekeepingprimers (25s).

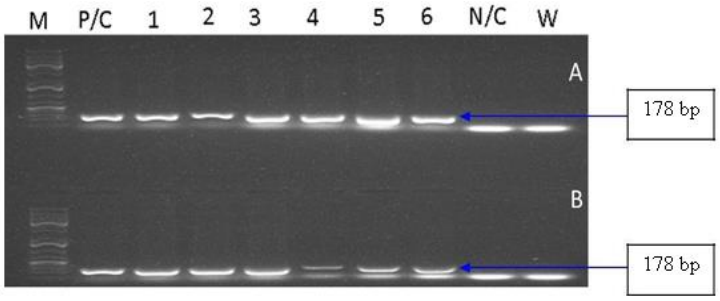

Figure 6. Representative RT-PCR picture of lines transformed with ES-*Pflp.* A: Sukali Ndizzi lines with ES-*Pflp*, B: Gros Michel lines with ES-*Pflp.*

*Growth parameters of Sukali Ndizzi ES-*Pflp *lines*

Table 2 displays growth measurements for various Sukali Ndizzi ES-*Pflp* transgenic banana lines, before they were subjected to disease testing. It appears that, as compared to non-transformed plants, most transgenic lines (line 19, 65, 80, 43.47, 9, 101.88.87, 31, 15.62, and 82) had a lower average number of leaves. In addition, when compared to the control plants, the Sukali Ndizzi ES-*Pflp* transgenic lines 64, 7 and 81 showed significantly greater mean leaf numbers. However, the analysis of variance (ANOVA) revealed that there were no statistically significant changes (p=0.27708) in the number of leaves between the transgenic and non-transformed plants in terms of leaf number between the two groups.

In terms of leaf length, the bulk of the Sukali Ndizzi ES-*Pflp* transgenic lines had significantly longer average leaf lengths than the non-transformed plants, when compared to the control plants (Table 3). Although the observed differences in leaf length between transgenic and non-transgenic plants were statistically significant (p=0.274748), the results of the ANOVA revealed that they were not statistically significant (p=0.274748). Comparing the leaf diameter of Sukali Ndizzi ES-*Pflp* lines to the control line showed superiority, with no statistically significant differences (p=0.190951).

As indicated by ANOVA values of p (p= 0.287434), the pseudostem width of Sukali Ndizzi ES-*Pflp* transgenic lines examined varied with no statistically significant difference. The most significant values were found in lines 43, 47, and 7. It was found that there was no statistically significant difference between the Sukali Ndizzi ES-*Pflp* transgenic lines evaluated and the control, with plant height values ranging from 45±1.2cm to as high as 64.670±0.633cm for the transgenic lines screened (p=0.297481).

Growth parameters of Gros Michel Pflp *lines*

Compared to the control line, there was no significant variation in leaf number, leaf length, leaf diameter, or pseudostem width. There was a difference in plant height between the lines and the control line, but the post ANOVA test revealed that the difference was not significant.

Screening of transgenic lines for resistance to BXW disease

It was decided to inoculate the potted plants of Gros Michel and Sukali Ndizzi with Xcm and observe for the development of disease symptoms 60 days after the inoculation. The development of necrosis at the site of inoculation in control plants occurred roughly one week after inoculation in the experimental plants. The first symptoms of illness were detected in the control group 10 days after the vaccination. As illustrated in Figure 11, the transgenic line had a delayed onset of illness symptoms, with some exhibiting no symptoms at all.

Resistance of different Sukali Ndizzi ES-Pflp *transgenic banana lines toBXW disease*

For the Sukali Ndizzi ES-*Pflp* transgenic lines tested, BXW disease resistance was found to be comparable to that of non-transgenic plants when grown in an unstressed environment. Eight days after inoculation in the control line and twenty-four days after inoculation in line 82, which eventually succumbed to the disease after the screening period, the onset of symptoms occurred. Three lines were sensitive to BXW sickness, even though the symptoms of line number 47 and number 82 began later than those of line number 65. Six transgenic lines showed complete resistance to *Xanthomonas campestris* pv. *Musacearum*, namely 24, 101, 87, 2, 31 and 81. In the study, these were the lines that displayed no signs of sickness. Line screenings yielded p=0.034 and α=0.05., a statistically significant difference in resistance between the two lines.

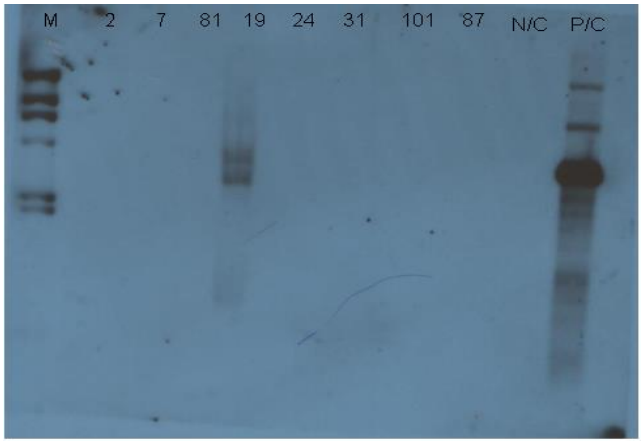

Figure 7. Southern blot analysis of selected lines. M is the molecular weight marker. 2, 7, 81, 19, 24, 31, 101, 87, are the Sukali Ndizzi transgenic lines transformed with ES-*Pflp* gene; NC is the non-transformed control; PC is the plasmid (pBI121 *Pflp*).

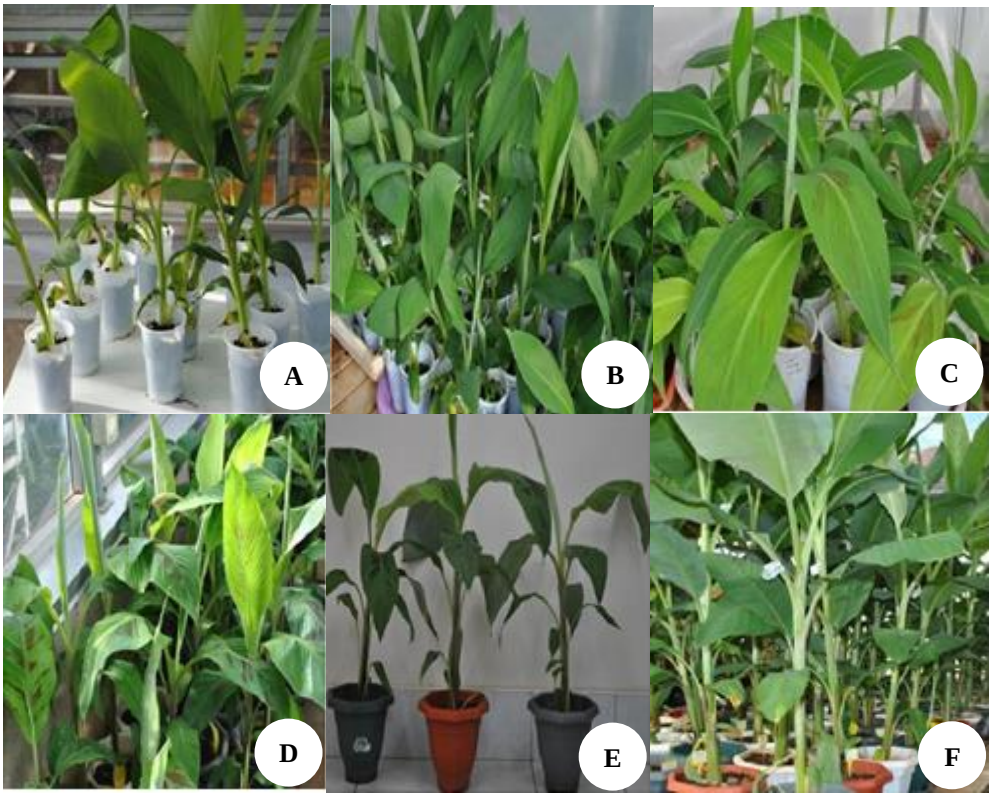

Figure 8. Hardening and potting of Sukali Ndizzi and Gros Michel lines. A and B: One month old Sukali Ndizzi lines in small pots under weaning chamber, C: One month old Gros Michel lines in small pots under weaning chamber, D and E: Three-month-old Gros Michel lines in bigger pots, F: Three-month-old Sukali Ndizzi lines in bigger pots.

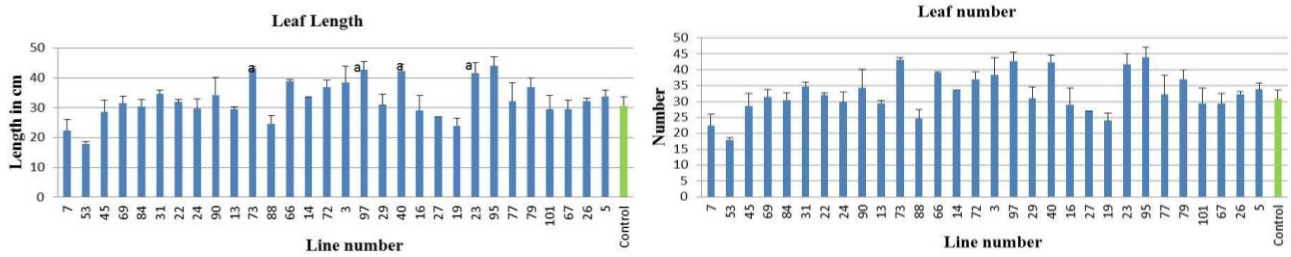

Figure 9. Agronomic traits of Gros Michel ES-*Pflp* in comparison with the controlline. The leaf length of line numbers 73, 97, 40 and 23 showed significant variation with the control line; p=0.00 where α=0.05. There was no significant variation in leaf number of the lines measured in comparison withcontrol line; p=0.054334 where α=0.05.

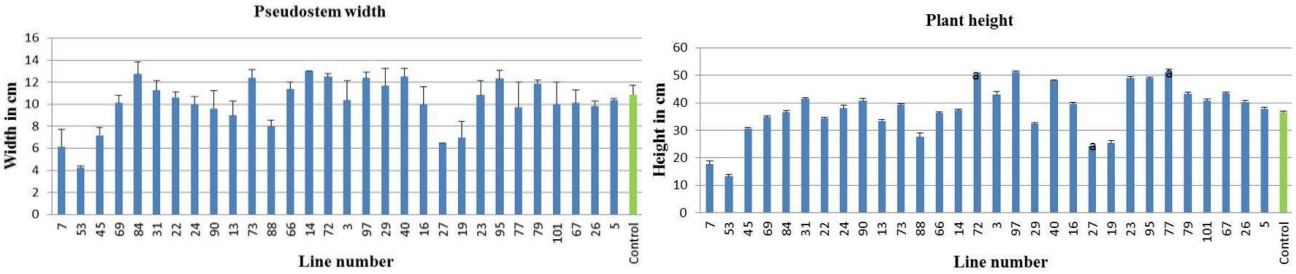

Figure 10. Agronomic traits of Gros Michel ES-*Pflp* in comparison with the controlline.

Figure 11. Representative picture of the screening process. A and B: Onset of disease symptom showing necrosis at the point of inoculation in Sukali Ndizzi transgenic lines, C: Gross Michel under BXW screening in the glasshouse, D: Plant with inoculated leaf with symptom, E: Plant with one leaf without symptom, F: Completely wilted plant.

Table 2. Growth parameters for different Sukali Ndizzi ES-*Pflp* transgenic and non-transformed banana plants grown underunstressed conditions.

Line no.	19	24	65	80	64	43	47	9	101	88	87	2	31	15	7	62	82	81	Control
No. of leaves	8.75±0.478a	9.0±0.0a	8.67±0.33a	7.67±0.67a	9.33±0.33a	8.5±0.5a	8±0a	8±0.82a	8.5±0.5a	8.5±1.5a	8.5±0.5a	9±0a	7.5±0.5a	8.5±0.5a	10±0a	8.33±0.67a	8±0a	10±0a	9±0.41a
Leaf length	35±3.63a	39.25±2.32a	39.67±3.33a	33.67±5.81a	43.67±0.33a	40.5±1.5a	40±3.9a	22.75±1.44a	31±1a	33±0.9a	40.5±2.5a	41±2a	28.5±0.65a	39±1a	42±0a	38±2a	35±0.8a	42.5±0.5a	33.75±0.63a
Leaf diameter	14.5±1.32	15±1	15.67±0.88	13.67±2.7	18±1.15	15.5±0.5	16±2	9.5±3.5	11.5±3.5	13±3	16±0	17±1	11.5±3.5	15.5±0.5	18±0	15.75±1.03	14±2	18±0	14.5±0.5
PS width	6.25±0.144	7.625±0.125	7±0.5	6±1	7.33±0.33	8±1	8±0	5.5±1.5	6.5±1.5	5.5±1.5	7.25±0.75	6.75±0.3	6±1	7±0	8±0	6.5±0.29	6.5±1	7.25±0.25	7.25±0.353
Plant height	45.38±1.8	62.75±4.48	61.67±4.48	52.67±0.56	60.67±0.67	61.5±2.5	66±3	45±2.2	45±1.2	46±1.1	61.5±0.5	62±3	51±1.3	63.5±0.5	63±0	64.670.6	57±0	61±1	57.5±0.417

Table 3. Growth parameters for different Gros Michel *Pflp* transgenic and non-transformed banana plants grown underunstressed conditions.

Line no.	1	4	5	7	9	12	13	21	29	32	35	36	Control
No. of leaves	7±0	7±0.40	6.67±0.33	6±0.41	6.75±0.25	7.25±0.25	6.25±0.25	5.75±0.63	7.25±0.48	6.75±0.25	6.33±0.67	7.25±0.75	7.33
Leaf length	30.5±2.5	27.5±2.02	31.33 ±0.41	27.75±0.85	30.25±2.39	33±1.08	26.75±2.02	33±2.12	31±1.91	33.25±1.701	23.33±0.67	30.25±2.39	29.67±1.45
Leaf diameter	12.75±1.03	11.25±0.85	12.67 ±1.33	9.75±1.25	10.5±0.5	11.75±1.31	11.5±1.19	14.25 ±0.75	12±1.08	12.5±0.29	11.33±0.88	10.5±1.19	10.33±1.2
PS width	7.63±0.24	5.63±0.38	6±0.5	5.88±0.13	6.25±0.25	7.88±0.66	6.5±0.456	6.75±0.32	6.88±0.13	6.63±0.314	7.17±0.44	6.5±0.5	6.13±0.554
Plant height	33.75±1.18	23.5±1.84	30±0.6	28.75±1.7	29.25±0.29	37.5±3.3	31.75±1.37	33.5±2.9	36.75±1.84	38±1.63	31±1.73	28±1.53	25.75±4.1

Table 4. Assessment of different Sukali Ndizzi ES-*Pflp* transgenic banana lines for superior resistance to *Xanthomonascampestris* pv. *musacearum* under glasshouse conditions.

Transgenic line number	19 α	24 α	65α	80 α	64 α	62α	43α	47α	9 α	101α	88α	87α	2α	31α	15α	82α	81α	7α	Control
Number of days for appearance of the first symptom	10.25±4.11	0.00	9.33±3.2	11±2.1	17.67±1.12	9±2	12.5±1.44	22.5±2.5	14±3.40	0.00	19.5±3	0.00	0.00	0.00	25±3	24.5±2.5	13.5±2.157	33.00	8±2.97
Number of days for complete wilting	0.00	0.00	20±4.3	0.00	13.67±3	13.33±4	21±3.9	36±2.6	28.5±4.4	0.00	30±1.437	0.00	0.00	0.00	23±0.67	47±1	0.00	0.00	11.75±2.35
Disease severity	4.00	0.00	5.00	3.00	2.00	1.00	2.00	5.00	2.00	0.00	3.00	0.00	0.00	0.00	3.00	5.00	1.00	3.00	5.00
Average resistance (%)	0.00	100.00	66.67±1.4	88.89±1.11	59.259±3.03	66.67±2.1	50±2	0.00	50±1.2	100.00	50±1.3	100.00	100.00	100.00	37.5±2.91	0.00	100.00	70.00	0.00
Rating	PR	R	S	PR	PR	PR	PR	S	PR	R	PR	R	R	R	PR	S	R	PR	S

Note: PR: Partial resistance, R: Resistance, S: Susceptible, α: Resistant lines

Table 5. Assessment of different Gros Michel *Pflp* transgenic banana lines for superior resistance to *Xanthomonas campestris* pv. *musacearum* under glasshouse conditions.

Transgenic lines	Mean number ofdays for appearance of the first symptom	Mean number of days for complete wilting	Average resistance (%)	Degree of wilting	Rating
1	5.5±2.25	11±1.1	69.097±2.3	3	PR
4	11.5±2.22	20.5±4.88	50±2.23	4	PR
5	17.33±0.866	20±2	38.095±3.12	4	PR
7	9±3.43	15±1.5	70.83±2.39	3	PR
9	12±3.48	18±1.052	50±2.88	4	PR
12	12±2.27	22.5±1.315	50±2.88	4	PR
13	19±3.34	32.33±1.06	33.33±2.1	4	PR
21	18.5±3.43	32.5±1.19	25±2.5	4	PR
29	4.75±0.475	9.75±0.975	75±2.5	2	PR
32	23.5±1.012	0	72.32±1.95	3	PR
35	14.33±1.43	0	96.67±3.33	1	PR
36	20.75±4.043	35.25±1.263	20±2	4	PR
Control	5.5±0.55	11.75±1.175	0	5	S

Note: PR: Partial resistance; R: Resistance; S: Susceptible

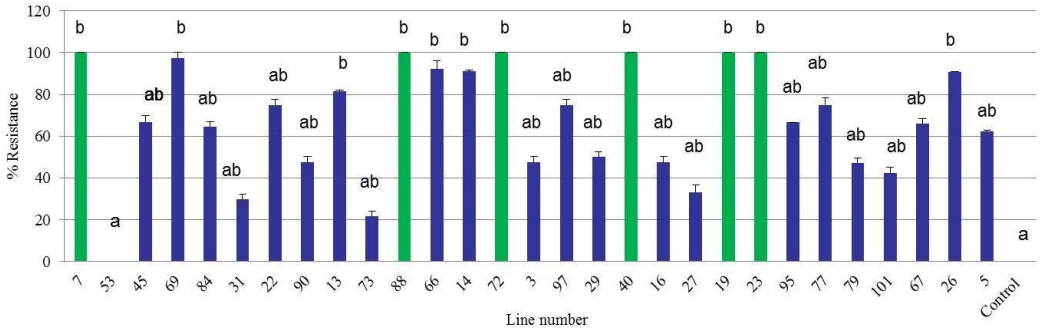

Figure 12. Screening results of Gros Michel ES-*Pflp* lines against BXW disease.

*Assessment of different Gros Michel ES-*Pflp *transgenic banana lines forsuperior resistance to* Xanthomonas campestris *pv.* musacearum *under glasshouse conditions*

A variety of Gros Michel ES-*Pflp* strains were tested for their ability to resist the BXW pathogen. The resistance of each screening line to Xcm is depicted in Figure 12. BXW could infect both the transgenic line 53 and the control line. Numbers 7 through 73 completely resisted the attack of BXW. There was a significant statistical difference in the resistance between the lines screened; p=0.009334 where α=0.05, post-Duncan multiple range tests grouped the lines as indicated above, where any groups that are not significantly different from one another will have the same letter in the grouping column.

Gros Michel Pflp *transgenic banana lines evaluated for superiorresistance to* Xanthomonas campestris pv. musacearum *under glasshouseconditions*

Table 5 compares the beginning of disease symptoms to total wilting and resistance of the different lines examined. For the control line and line number 1, symptoms appeared at 5 days post-inoculation; for line number 32, symptoms appeared at 23 days. None of the BXW-resistant lines tested were utterly resistant. According to the p-value of 0.0335708 for the screening lines, there was a significant difference in resistance among them.

Distribution

With the world's population predicted to quadruple by 2050, there is a pressing need to improve food production to satisfy this growing demand. It is critical to employ methods capable of overcoming cumulative biotic restrictions to maintain food production. These restrictions hamper the ideal genetic production potential for food and fruit crops. Wheat, maize, cassava, and bananas are among of Africa's most important food crops. Despite its immense potential as an export crop, pests and diseases have hampered banana production in recent decades (Jones 2009).

In *Arabidopsis*, extracellular secreted *Pflp* improved harpin-mediated HR disease resistance to *R. solanacearum* and Pectobacterium carotovorum (Lin et al. 2010). In this research, *Pflp* outside the chloroplast had a greater disease resistance. In addition, sea anemone secretion protein is employed in the ES-*Pflp* gene (Lin et al. 2010).

Tissue culture and *Agrobacterium*-mediated transformation were recognized to be difficult for bananas, like any other monocotyledonous plant. In recent years, strategies for transforming banana somatic embryogenic callus and apical meristems using *Agrobacterium*-mediated approaches or particle bombardment have been devised (May et al. 1995; Becker et al. 2000; Ganapathi et al. 2001; Khanna et al. 2004).

Agrobacterium-mediated approaches are chosen because of their ease of use, low cost, capacity to transfer long DNA strands without rearrangement, and low copy number (Huang et al. 2004).

This study employed ECSs from the cultivars Sukali Ndizzi and Gros Michel to create over 100 transgenic lines for each cultivar. Sukali Ndizzi had a greater transformation efficiency (441) than Gros Michel (97.5), indicating that the latter was easier to transform than the former in the two experiments. The embryo regeneration effectiveness of Sukali Ndizzi was higher than that of Gros Michel in both experiments conducted in this study. According to these findings, Sukali Ndizzi is quickly changed and regenerates more embryos than Gros Michel.

Individual regenerated transgenic lines were clonally reproduced, and a few lines were randomly chosen for PCR analysis. Around 97 % of lines carried the ES-*Pflp* gene, indicating that the selection procedure was effective, and few escapes were recovered. The transgene expression patterns were investigated using RT-PCR, which found the transcripts in all transgenic lines tested with no expression in the control line. This demonstrated that the transgene was expressed in the transgenic lines examined, making them appropriate for screening for BXW disease.

Compared to the control line, Southern blot analysis of transgenic lines of Sukali Ndizzi line 19 revealed effective incorporation of two transgene copies. However, other transgenic lines included in this investigation did not demonstrate integration or transgene copy number, which could have been due to technological constraints. Based on these molecular characterization results, Gros Michel and Sukali Ndizzi cultivars were effectively converted using the ES-*Pflp* gene.

The agronomic parameters of the transgenic lines were compared to the control line for each cultivar and revealed no variations in morphology. As a result, transformation with the ES-*Pflp* gene resists BXW disease without altering the plant's physiological state (Tripathi et al. 2014).

Randomly selected transgenic lines of Sukali Ndizzi ES-*Pflp* (19, 54, 65, 80, 64, 62,43, 47, 9, 101, 88, 87, 2, 31, 15, 82, 81 and 7) and Gros Michel ES-*Pflp* (7, 53, 45, 69, 84,31, 22, 24, 90, 13, 73, 88, 66, 14, 72, 3, 29, 40, 16, 27, 19, 23, 95, 77, 79, 101, 67, 26 and 5) were tested for Xcm resistance. Three replicates of 90-day-old potted transgenic lines were intentionally infected with Xcm.

Sukali Ndizzi ES-*Pflp* (18 lines) exhibited variable resistance to BXW, indicating non-targeted gene insertion, a feature of *Agrobacterium*-based transformation. This was validated by statistical analysis, which revealed a significant difference in the resistance to BXW disease between the lines examined. Within 10 days post-inoculation (dpi), non-transgenic control plants displayed symptoms (chlorosis, necrosis, and wilting) and were entirely wilted by 19 dpi. Six of these lines exhibited no symptoms at 60 dpi and were categorized as resistant. Two of the symptomatic transgenic lines wilted, but the remaining 1-3 leaves had symptoms but never entirely wilted, indicating that they possessed some resistance.

Additionally, the Gros Michel ES-*Pflp* screening lines exhibited variable resistance to BXW disease, indicating non-targeted gene insertion, a feature of *Agrobacterium*-based transformation. This was also supported statistically, since substantial differences in BXW disease resistance were observed between the lines evaluated. By 60 dpi, three transgenic lines and three non-transgenic control lines were entirely wilted. Six transgenic lines demonstrated total resistance, while the remaining lines showed partial resistance. Sukali Ndizzi ES-*Pflp* lines demonstrated 33% resistance, while Gros Michel ES-*Pflp* lines demonstrated 20% resistance.

Additionally, twelve Gros Michel transgenic lines (1, 4, 5, 7, 9, 12, 13, 21, 29, 32, 35, 36) transformed with the *Pflp* gene were tested. The non-transgenic lines developed symptoms at 11 days post-inoculation and were fully wilted at 60 days post-inoculation. All screened transgenic lines demonstrated partial resistance, but none showed complete resistance. These results suggest that transgenic Gros Michel banana plants transformed with ES-*Pflp* exhibit increased resistance to BXW than Gros Michel transgenic banana lines transformed with the *Pflp* gene. Previous studies on the ectopic expression of *Pflp* in *Arabidopsis* yielded comparable results (Lin et al. 2010).

In conclusion, the results of this study reveal that the ES-*Pflp* gene can be successfully converted into the Gros Michel and Sukali Ndizzi cultivars. The resistance of the transgenic lines tested against BXW disease varied significantly between the two cultivars, with six lines of each cultivar demonstrating total resistance to the disease. Compared to Gross Michel *Pflp* lines, Gross Michel ES-*Pflp* lines showed more excellent resistance to BXW disease.

REFERENCES

Abele S, Pillay M. 2007. Bacterial wilt and drought stresses in banana production and their impact on economic welfare in Uganda: Implications for banana research in East African Highlands. J Crop Improv 19: 173-191. DOI: 10.1300/J411v19n01_09.

Becker DK, Dugdale B, Smith MK, Harding RM, Dale JL. 2000. Genetic transformation of cavendish banana (*Musa* spp. AAA group) cv. grand nain via microprojectile bombardment. Plant Cell Rep 19: 229-234. DOI: 10.1007/s002990050004.

Dangl JL, Dietrich RA, Richberg MH. 1996. Death don"t have no mercy: Cell death programs in plant-microbe interactions. Plant Cell 8: 1793-1807. DOI: 10.1105/tpc.8.10.1793.

De Langhe E, Hribová E, Carpentier S, Doležel J, Swennen R. 2010. Did backcrossing contribute to the origin of hybrid edible bananas? Ann Bot 106: 849-857. DOI: 10.1093/aob/mcq187.

FAO Statistical (FAOSTAT). 2012. Food and Agriculture Organization of the United Nations. http://faostat.fao.org/

Freeman S. 2003. Plant Defense Systems. Biological Science, Upper Saddle River, PrenticeHall, New Jersey.

Ganapathi TR, Higgs NS, Balint-Kurti PJ, Arntzen CJ, May GD, Van Eck JM. 2001. *Agrobacterium*-mediated transformation ofthe embryogenic cell suspensions of the banana cultivars Rasthali (AAB). Plant Cell Rep 20: 157-162. DOI: 10.1007/s002990000287.

Goodman RN, Novacky AJ. 1994. The Hypersensitive Reaction in Plants to Pathogens: A Resistance Phenomenon. American Phytopathological Society, Minnesota.

Gawel NJ, Jarret RL. 1991. A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Mol Biol Rep 9: 262-266. DOI: 10.1007/BF02672076.

Hadiwiyono. 2011. Blood bacterial wilt disease of banana: The distribution of pathogen in infected plant, symptoms, and potentiality of diseased tissues as source of infective inoculums. Nusantara Biosci 3: 112-117. DOI: 10.13057/nusbiosci/n030302.

Hasanah R, Daningsih E, Titin.2017. The analysis of nutrient and fiber content of banana (*Musa paradisiaca*) sold in Pontianak, Indonesia. Biofarmasi (Rumphius J Nat Prod Biochem) 15: 21-25. DOI: 10.13057/biofar/f150104.

Huang SN, Chen, CH, Lin HJ, Ger MJ, Chen ZI, Feng TY. 2004. *Plant ferredoxin-like protein* AP1 enhances Erwinia-induced hypersensitive response of tobacco. Physiol Mol Plant Pathol 64: 103-110. DOI: 10.1016/j.pmpp.2004.05.005.

Jones DR. 2000. Diseases of Banana, Abacá and Enset. CABI Publishing, Wallingford, UK.

Jones DR. 2009. Diseases and pest constraints to banana production. Acta Horticulturae 828: 21-36. DOI: 10.17660/ActaHortic.2009.828.1.

Karamura DA. 1998. Numerical Taxonomic Studies of the East African Highland Bananas (Musa AAA-East Africa) in Uganda. [Unpublished Thesis]. University of Reading, Reading, UK.

Khanna H, Becker D, Kleidon J, Dale J. 2004. Centrifugation Assisted *Agrobacterium tumefaciens*-mediated Transformation (CAAT) of embryogenic cell suspensions of banana (*Musa* spp. Cavendish AAA and Lady finger AAB). Mol Breed 14: 239-252. DOI: 10.1023/B:MOLB.0000047771.34186.e8.

Lin Y-H, Hsiang-En H, Yen-Ru C, Pei-Luan L, Ching-Lian C, Teng-Yung F. 2011. C-Terminal region of *Plant ferredoxin-like protein* (*Pflp*) is required to enhance resistance to bacterial disease in *Arabidopsis thaliana*. Phytopathology 101 (6) :741-749. DOI: 10.1094/PHYTO-08-10-0220.

Lin Y, Huang H, Feng T. 2010. Extracellular Plant *Ferredoxine-Like Protein* and Uses Thereof. International Application Published under the Patent Cooperation Treaty (PCT), world Intellectual property organization, International Publication Number W0 2010/135728A2.

May GD, Rownak A, Mason H, Wiecko A, Novak FJ, Arntzen CJ. 1995. Generation of transgenic banana (*Musa acuminata*) plants via *Agrobacterium*-mediated transformation. Nat Biotechnol 13: 486-492. DOI: 10.1038/nbt0595-486.

Namuddu A, Kiggundu A, Mukasa SB, Kurnet K, Karamura E, Tushemereirwe W. 2012. *Agrobacterium* mediated transformation of banana (*Musa* sp.) cv. *Sukali Ndiizi* (ABB) with a modified *Carica papaya* cystatin (CpCYS) gene. Afr J Biotechnol 12 (15): 1811-1819. DOI: 10.5897/AJB12.2478.

Namukwaya B, Tripathi L, Tripathi JN, Arinaitwe G, Mukasa SB, Tushemereirwe WK. 2012. Transgenic banana expressing *Pflp* gene confers enhanced resistance to *Xanthomonas* wilt disease. Transgenic Res 12: 855- 865. DOI: 10.1007/s11248-011-9574-y.

Novak FJ, Afza R, Van Duren M, Perea-Dallos M, Conger BV, Xiaolang T. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.). Nat Biotechnol 7: 154-159. DOI: 10.1038/nbt0289-154.

Pachuau L, Atom AD, Thangjam R. 2014. Genome classification of *Musa* cultivars from Northeast India as revealed by ITS and IRAP markers. Appl Biochem Biotechnol J 172: 3939-3948. DOI: 10.1007/s12010-014-0827-0.

Qiagen. 2010. QIAamp® RNA Mini and Blood Mini Handbook. 3rd Edition, 27-29.

Roux N, Baurens FC, Doležel J, Hřibová E, Heslop-Harrison P, Town C, Sasaki T, Matsumoto T, Aert R, Remy S, Souza M, Lagoda P. 2008. Genomics of banana and plantain (*Musa* spp.), major staple crops in the tropics. In: Moore PH, Ming R (eds). Plant Genetics and Genomics: Crops and Models Genomics of Tropical Crop Plants, Springer, Berlin.

Ssekiwoko F, Taligoola HK, Tushemereirwe WK. 2006. *Xanthomonas campestris* pv. *musacearum* host range in Uganda. Afr Crop Sci J 14: 111-120. DOI: 10.4314/acsj.v14i2.27917.

Tripathi L, Odipio J, Tripathi JN, Tusiime G. 2008. A rapid technique for screening banana cultivars for resistance to *Xanthomonas* wilt. Eur J Plant Pathol 121: 9-19. DOI: 10.1007/s10658-007-9235-4.

Tripathi L, Mwangi M, Abele S, Aritua V, Tushemereirwe WK, Bandyopadhyay R. 2009. *Xanthomonas* wilt: A threat to banana production in east and central Africa. Plant Dis 93: 440-451. DOI: 10.1094/PDIS-93-5-0440.

Tripathi L, Mwaka H, Tripathi JN, Tushemereirwe WK. 2010. Expression of sweet pepper hrap gene in banana enhances resistance to *Xanthomonas campestris* pv. *musacearum*. Mol Plant Pathol 11: 721-731. DOI: 10.1111/j.1364-3703.2010.00639.x.

Tripathi JN, Muwonge A, Tripathi L. 2012. Efficient regeneration and transformation of plantain cv. "Gonjamanjaya" (*Musa* spp. AAB) using embryogenic cell suspensions. In Vitro Cell Dev Biol Plant 40: 216-224. DOI: 10.1007/s11627-011-9422-z.

Tripathi L, Tripathi JN, Kiggundu A, Korie S, Shotkoski F, Tushemereirwe WK. 2014. Field trial of *Xanthomonas* Wilt disease-resistant bananas in East Africa. Nat Biotechnol 32: 868-870. DOI: 10.1038/nbt.3007.

Tushemereirwe W, Kangire A, Smith J, Ssekiwoko F, Nakyanzi M, Kataama D, Musiitwa C, Karyaija R. 2003. An outbreak of bacterial wilt on banana in Uganda. Infomusa 12: 6-8. DOI: 10.4314/acsj.v12i1.27658.

Tushemereirwe W, Kangire A, Ssekiwoko F, Offord LC, Crozier J, Ba E, Rutherford M, Smith JJ. 2004. First report of *Xanthomonas campestris* pv. *musacearumon* banana in Uganda. Plant Pathol 53: 802. DOI: 10.1111/j.1365-3059.2004.01090.x.

Vuylsteke D. 2000. Breeding bananas and plantains, from intractability to feasibility. Acta Holticulturae 540: 149-156. DOI: 10.17660/ActaHortic.2000.540.16.