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A STRATEGY FOR THE DEVELOPMENT OF DROUGHT-TOLERANT GENETICALLY MODIFIED PLANTAIN

By

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Abstract: Plantain thrives in humid environments with dry season of less than three months. Recently, plantain suffers severe water stress; an impact of climate change. There have been attempts at engineering drought tolerance in plants by successfully transferring functional genes that encode enzymes associated with the synthesis of osmotically active compounds, transporters chaperones and reactive oxygen species scavengers into crops like rice, tobacco and tomato. However, the report of attempts at transferring drought tolerance encoding gene into plantain is yet to be available. This paper therefore made an attempt at developing a hypothetical strategy that would result in a transgenic plantain that would express drought tolerance based on existing documented evidence of earlier work with other crops. The drought tolerance is expected to be constitutively expressed and as such, the possibility of using a constitutive promoter gene CaMV35SP was considered in the work. The procedure of moving the transgenic event from tissue culture to an elite experimental line on the field is briefly discussed.

Keywords: biosafety, drought tolerance, genetically modified organism, plantain.

INTRODUCTION

Plantain is a popular food crop eaten throughout Nigeria and widely grown in southern Nigeria. It is a sterile crop and as such cultivation is through vegetative perennating organs like the sucker.

Plantain is cultivated in humid environments with a relative humidity of 60 to 100% and easily overcomes dry season of less than three months (Swennen and Vuylsteke, 2001). In some plantain growing regions of Nigeria however, dry season may last more than four months. The impact of global warming has resulted in prolonged and more severe dry season in Nigeria. There is therefore, severe water stress effect on plantain. This is more so that Plantain is susceptible to wind breakage and the effect is more pronounced during the dry season. The call for plantain improvement and engineering drought tolerance is a promising option. Olaniyi and de Oliviera (2014) proposed that plantain improvement through genetic engineering could be a solution to biotic production constraints like plant parasitic nematodes and concluded that the approach they proposed offers the ability to control several nematodes attacking plantain concurrently or at different locations without any need for awareness of nematode problems at the grower level. Similarly, engineering drought tolerance into the crop would help resource poor farmers whose plantain crop is vulnerable to drought, yet he could not afford the irrigation cost of managing the drought.

Recent attempts at engineering drought tolerance in plants have successfully transferred functional genes that encode enzymes associated with the synthesis of osmotically active compounds, transporters, chaperones and reactive oxygen species scavengers (Umezawa *et al.*, 2006). To date, many genes involved in the synthesis of compatible solutes

among others, a variety of sugars and sugar alcohols like trehalose have been used. Trehalose (TPS1, TPSP, OtsA and OtsB) has been introduced into crops like rice, tobacco and tomato. Trehalose (TPS1) from the bacterium *S. cerevisiae* has already been successfully used for tomato and would be the choice in this proposed work. Therefore, the hypothesis considered engineering plantain, *Musa* spp. AAB sub-group, for drought tolerance. The drought tolerance should be constitutively expressed (throughout the plant) and as such, a constitutive promoter gene CaMV35SP would be used.

PROPOSED METHODOLOGY AND EXPECTED RESULT

Transformation Strategy, Plant Transformation Vector, and The Plant Crop Variety

Plantain cultivar Agbagba, a False Horn plantain is a common and preferred cultivar in Nigeria because of its large fruits however, it is seed sterile. A cultivar that is often used in breeding programmes is Obino l'Ewai, a French plantain because it sets seeds and is seed fertile. Therefore, the cultivar that shall be used in plant transformation is Obino l'Ewai. Transformed plants would then be crossed with cultivar Agbagba. Transformation would be done by Biolistics (particle bombardment).

The Plasmid

Plasmids pHyg-TPS1 (CaMV35Spromoter::AMVleader::TPS1::NOSpolyA + CaMV35Spromoter::*aphIV*::SpolyA) and pJIC200 (ubi-5' region::*gusA*::NOSpolyA) would be used for transformation (*aphIV* provides hygromycin resistance; *gusA* codes for b-glucuronidase). The gene construct that is expected to result is shown in figure 1. The Particle bombardment of plantain embryo cell suspension would be carried out as described by Christou *et al*. (1991).

The Gene Construct

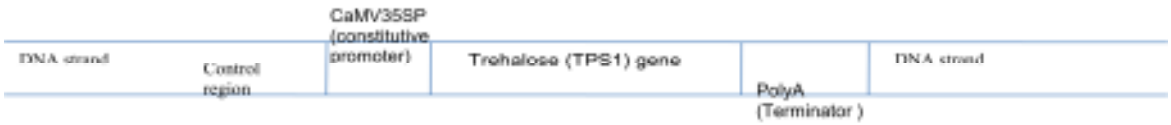

Figure 1: Drought-tolerant plantain gene construct (simulated by author)

Selection and regeneration of transformed plants

Two days after bombardment, embryo cells would be subcultured onto CCM3 medium supplemented with 50 mg/l of hygromycin (CCM3H50). After 10 days, embryogenic calli initiated from cells would be dissected into small pieces and transferred to fresh hygromycin-containing medium, with subculture at 10-day intervals. After 6-8 weeks, plants would be regenerated by transferring the hygromycin-resistant embryogenic calli onto CCM3H50 medium without 2,4-D (CCR2H50). Plantlets would first be germinated on plates, then grown in tubes on MSR6H50 medium prior to transfer to the greenhouse. The MSR6H50 medium contains 40% (v/v) MS basal salts (Murashige and Skoog, 1962), 10 g/l sucrose, 2 g/l phytagel (SIGMA P-8169), 4 g/l bactoagar (Difco), pH 5.7. Filter-sterilized B5 vitamins (Gamborg *et al.*,1968) and hygromycin (50 mg/l) would be added after autoclaving.

Polymerase Chain Reaction (PCR)

DNA would be prepared using a rapid extraction protocol. Leaf samples would be ground in a 1.5-ml Ependorf tube using a hand pestle, sand and liquid nitrogen. Two hundred microliters of extraction buffer (500 m*M* NaCl, 100 m*M* TRIS-HCl pH 8 and 50 m*M* EDTA; Yan *et al*., 1999) and 20µl 20% (w/v) SDS would be added to the powder before further grinding. After centifugation (5 min at 12 000 rpm) the supernatant would be removed and added to an equal volume of isopropanol for DNA precipitation. After centrifugation, the DNA pellet would be washed with 70% ethanol and suspended in TE buffer. PCR would be performed in a total volume of 25 µl containing 25 ng plantain genomic DNA, 10 m*M* TRIS-HCl pH 8.3, 2.5 m*M* MgCl2 , 50m*M* KCl, 0.05% Noridet p40, 200 l*M* dNTPs, 0.4 l*M* of each primer and 1 unit of *taq* polymerase. DNA would be denatured at 95^{o}C for 1 min followed by 30 cycles of amplification (30 s at 95^{o}C, 30 s at 60^{o}C, 1 min at 72^{o}C) and by 10 min at 72^{o}C.

GUS (b-glucuronidase) activity

Leaf tissue from 5-week-old rice plants would be assayed buorometrically for b-glucuronidase activity according to Jefferson *et al*. (1987).

Western blot hybridization for TPS1activity

Total protein would be isolated from roots of resultant plantain plants. Ten microgams of total root protein and standard quantities of TPS1 protein would be loaded on SDS PAGE gels using the BioRad mini system. The TPS1 protein would be recovered from an *E-coli* expression system (Urwin *et al.*, 1995). The gel would be blotted according to BioRad protocols and probed with a polyclonal antibody raised against TPS1 (Urwin *et al.*, 1995). The bands would be visualized using an image analyzer system consisting of a monochrome CCD camera (Sony) plus a frame grabber and appropriate software (Quantimet 500, Leica).

TRANSFORMANT ANALYSIS FOR THE SELECTION OF THE ELITE EVENT

Confirming the success of plant transformation

Clear differential growth between transformed (hyg+) and non-transformed (hyg-) calli during proliferation, regeneration and germination would confirm transformation. Resulting plants are thus confirmed transgenic. Southern blot analyses of regenerated plants would confirm the integration of pHyg-TPS1 plasmid into the plantain genomic DNA (Vain *et al*., 1997).

The expression of unselected gusA and TPS1 genes in plants transformed with both pHyg-TPS1 and pJIC200 (the gusA containing plasmid) would be confirmed by enzymatic assays and western blot analysis, respectively.

From Laboratory to the field: Bioassay for drought tolerance

Four-week-old transgenic plantain plants would be potted into a sand/loam mix and experiment laid out in appropriate experimental design of two treatments in the screen house. Conditions in the screen house would represent as much as possible, the field conditions with the exception of watering regimes which would be the treatments for investigation.

All plants would be watered at planting through the first week of planting to ensure proper establishment of plants. One half of the plants would be watered every day while one half would be left unwatered for five weeks. All plants would be harvested on the sixth week. Shoot biomass production would be assessed by taking the difference between fresh shoot and dry weights. Data shall be analyzed statistically to access level of resulting drought tolerance.

Field Test

The transgenic plants will then be tested in confinement at a suitable location and with standard agronomic practices. Biosafety regulation for GM field trials will be in strict adherence to the Cartagena Biosafety Protocol (CBP). Phenotypic growth habit of the transformed plant would be compared with the landrace cultivar Obino l'Ewai, the target material.

Moving the Transgene from Tissue Culture Variety to an Elite Experimental Line/Variety

On assessment of the performance of the transgenic plant and noting the length of days to flowering, the transgenic would be grown in confinement and the preferred landrace Agbagba would be grown at such a time to synchronize flowering of the two materials for the same period. At flowering, the inflorescences would be covered with polythene bags to prevent possible cross fertilization with undesired materials. The pollen shall be harvested from the Agbagba plants and mechanically fertilize the transgenic plants. Progeny seeds would be germinated in appropriate substrate to regenerate the hybrid. Resulting hybrid shall be compared with both parents (transgenic Obino l'Ewai and landrace Agbagba) for morphological characters, physiological growth responses and yield and tested for heterosis and trait inheritance.

RISK ASSESSMENT OF THE RESULTANT TRANSGENIC PLANT

Risk assessment is necessary before a genetically modified organism (GMO) is approved to be moved from containment to the field. Often, risk of a GMO to man and animals is assessed in comparison with a substantial equivalent, i.e. a standard non-modified material; while environmental risk is assessed by assessing the behaviour of the GMO in the environment. A likely risk assessment matrix for the expected transgenic plantain is given in table 1.

Table 1. Hypothetic risk assessment matrix for an engineered drought-tolerant plantain based on standard risk assessment procedure.

Identification of potential adverse effect	Estimation of likelihood	Evaluation of consequence	Estimation of risk	Consideration of risk management
Toxicity Human and animal	1. Unlikely: CaMV is not zoonotic, hence GM plant should not be toxic to man, 2. Exposure (Likely): level may be high	Biological equivalence Low risk	Negligible Low	Take no action
Contamination of animal products	Unlikely: CaMV is not transferred from plant to animals		Negligible	
Allergenicity	Unlikely	Biological equivalence	Negligible	Risk monitoring
Effects on target organisms: Resistance development	Likely: no report confirming this	None	Low	Limited approval
Indirect non-target effect: fishes and daphnia	Not likely: may not be transferred to fish through leaf litter and run-offs because viruses are generally obligate	None	Negligible	None
Gene flow: Horizontal	Not likely: CaMV is not an animal/man pathogen	None	Not known	None
Gene flow: Vertical	Not likely: plantain is often propagated vegetatively and the danger of cross fertilization is low	potato and biodiversity	Low	Remove the male inflorescence once the fruits start to fill.
Residue in soil	Not likely: Viruses are obligate parasites and may	None	Unknown	None

	not survive in non-host			
Pathogenicity of virus and other pathogens	1.Unlikely: May induce cross-protection, thus reduce susceptibility 2. Likely: May aggravate susceptibility	Further protection from pathogens conferred Increased susceptibility and crop loss	Moderate Moderate	Monitor effect to assess when such protection breaks down. Increased pesticide use, change in agricultural production system

CONCLUSIONS

Based on the above risk assessment, it is unlikely that there would be risk to man and animal health from the use of the constitutive promoter CaMV35SP in plantain; neither is the transgenic plantain expected to constitute a threat to environmental safety. However, it is recommended that there should be monitoring in order to identify any untoward reaction from animal and human health.

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Her area of focus is the management of plant diseases especially those incited by plant parasitic nematodes. She has worked on the integrated management of plant parasitic nematodes in plantain (*Musa* spp. AAB-subgroup) since 1996. Currently, she works on novel, biotechnological and conventional approaches in plant nematode management.