Strategies For Enhancing Flowering In Cassava Using Molecular Tools: Towards A More Efficient Breeding Programme

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INTRODUCTION

Cassava plays a significant role as an edible source of carbohydrates in many tropical countries as the fifth most important source of food energy and the most important staple food in half of sub-Sahara Africa (FAO 2001).It's tolerance to water stress and poor fertility soils makes it an important food security and famine-reserve crop. Introducing improved cultivars through traditional breeding programmes have been successful, (Hershey and Jennings, 1992), however, the high degree of heterozygosity in cassava, irregular flowering in some cultivars, low seed set, and variable germination rates have impeded faster progress via classical breeding.

Flowering in Cassava (*Manihot esculenta*) is an important agronomic trait whose control is not optimal due to cassava's recalcitrant flowering nature. Induction of flowering through hormone application, or photoperiod manipulation is difficult, cumbersome and sometimes expensive. Development of a low-cost alternative means of inducing flowering at will in cassava, would greatly overcome the problems of flowering synchronization being faced by breeders and enhance the genetic improvement of the crop. Thus make the many excellent cassava genotypes, with otherwise locked-up desirable traits, readily accessible for conventional breeding.

Flowering genes (Flowering Locus T(FT), Apetala 1(AP1), Suppressor of Over-expression of Constans (SOC1) and Constans (CO)) from *Arabidopsis thaliana* were introduced to cassava genotype 60444 through agrobacterium mediated transfer using cassava friable embryogenic callus.The flowering genes are under the control of an ethanol inducible system. Putative transgenic lines regenerated were genotyped using conventional PCRs and Real Time PCR.

This study shows how modern molecular techniques is used to modify flowering in cassava for enhanced genetic improvement of the crop and it represents the first report on the introduction of flowering genes into Cassava.

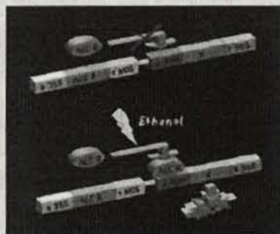
MATERIALS AND METHODS

Plasmid construction

Constructs were generated by insertion of the complementary DNA (cDNA) clones of CO, AP1, LFY, SOC1 and FT into the 10.1kb transformation vector pNew1-Mik1-alcR-antisense by gateway cloning techniques. The expression vector system generated can be induced by spraying small doses of ethanol. The switch is formed by two components: (i) a transcriptional factor AlcR that upon ethanol induction binds to and (ii) activates the transcription of a target promoter AlcA (Odell et al,1985). (Fig 1)

The constitutive expression of the flowering genes are driven by the Cauliflower Mosaic Virus 35S promoter (CaMV35S) upstream of the alcR transcription factor. This activates the transcription of the target promoter alcA controlling the expression of the flowering gene in an antisense orientation. (Fig 2).

Fig 1.The Ethanol inducible system



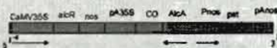


Fig 2: Schematic representation of the orientation of the construct.

nos, pA35S and pAnos are transcriptional terminators and pat is the selectable marker coding for phosphinothricin,herbicide resistance.

Arabidopsis Transformation

Arabidopsis mutant genotypes FLC:FRI, FLC:fri, flc:FRI and flc:fri were planted and vernalized for five weeks after which were transferred to long day growth conditions.
FLC is a Flowering Locus C gene and a floral repressor while FRIgida causes up-regulation of FLC expression. FLC is in the active state and flc is the repressed state.

The floral dip transformation procedure of Clough et al 1998 was used to transform these lines,by dipping the developing secondary floral tissues into a solution containing *Agrobacterium tumefaciens* (ABI) containing the constructs, 5% sucrose and 500 µl/L surfactant Silwet L-77.

Cassava Transformation

The constructs were introduced to cassava genotype 60444 through agrobacterium mediated transfer using the protocol described by Schopke et al 1996 with some modifications as developed by CIAT (CIAT 2002). (Figs.3-8)

Fig 3: Washing the agrobacterium off the FEC.

Fig 4: Proliferation of the transformed FEC in growth medium.

Fig 5: Cassava Friable Embryogenic Callus being selected in Maturation medium.

Fig 6: Cassava Friable Embryogenic Callus Differentiating into somatic embryos.

Fig 7:RITA system for Multiple organ development

Fig 8:Cassava Putative Transgenic Line in rooting medium.

RESULTS

PCR amplification of DNA samples from the F_2 generation of the shy flowering Arabidopsis independent transgenic lines showed the presence of the insert. Gene expression of the transgenes monitored by quantitative Reverse Transcriptase-PCR after the application of varying ethanol dosage showed induction and expression of the flowering genes.(Fig 11). However, the time of flowering has not yet been determined.

Fifteen independent transgenic plantlets were generated from Cassava FECs transformed with FT constructs, nine out of which have been genotyped positive to the pat and FT genes. The genotyping of the transgenic lines by amplification of the 444bp fragment from the selectable marker pat is indicated by the melting curve.(Fig 10).The peaks signified by the arrow shows the presence of the transgene. The red peak shows the plasmid positive control, while the other peaks are those from the transgenic cassava lines, and the negative controls have no peaks.

Expression studies were conducted by measuring the molecular and morphological changes after the induction of the ethanol inducible system by small doses of ethanol vapour. Branching was noticed shortly after. (Fig 12)

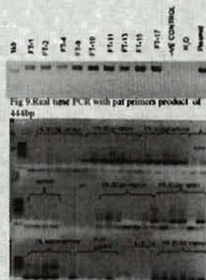


Fig 11. Gene expression results in Arabidopsis

Fig 10. Real time PCR melting curve

Fig 12. Cassava transgenic line Branching after application of ethanol

CONCLUSIONS AND PERSPECTIVES

In Arabidopsis, flowering time induction kinetics will be conducted to determine the time of flowering in shy Arabidopsis transgenic lines and this will be compared with Cassava.

The initiation of branching after ethanol induction is a good cue, since flowering in cassava is characterized by branching (Alves, 2002).The Real Time PCR technique is a very accurate and efficient method of transgene detection, however, Southern Blot Hybridization technique will be used to confirm the transgenicity of these cassava lines and they will be transferred to the field for gene expression for induction of flowering after the application of ethanol.

The success of these transgenic cassava genotypes flowering at will, will "open up" to conventional breeding the many excellent genotypes that are recalcitrant to flowering and thus synchronize flowering of genotypes that currently flower at different times in the breeding cycle, which will ultimately lead to increased income and improved livelihoods of rural communities who will eventually grow the high yielding disease resistant cassava varieties developed as a result of improved ability to make desired crosses.

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