Development of a Simple Genetic Engineering Procedure to Study Root Physiology and Pathogens in Cotton

COTTON INCORPORATED

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ABSTRACT

A simple root genetic engineering procedure is being produced that will not require the cumbersome and time consuming procedures typically associated with tissue culturing in sterile conditions. To accomplish this goal, a genetic engineering plasmid vector has been developed that uses the enhanced green fluorescent protein (eGFP) as a visual beacon. By using the eGFP, roots can be screened by the use of a fluorescent lamp that excites the eGFP, resulting in the observed green roots. Within the plasmid vector, a Gateway® compatible cassette has been ligated into the vector. This allows for the directional cloning of genes for that can be used in a variety of studies to investigate the molecular biology of root physiology and root-pathogen interaction studies.

Rotylenchulus reniformis life cycle

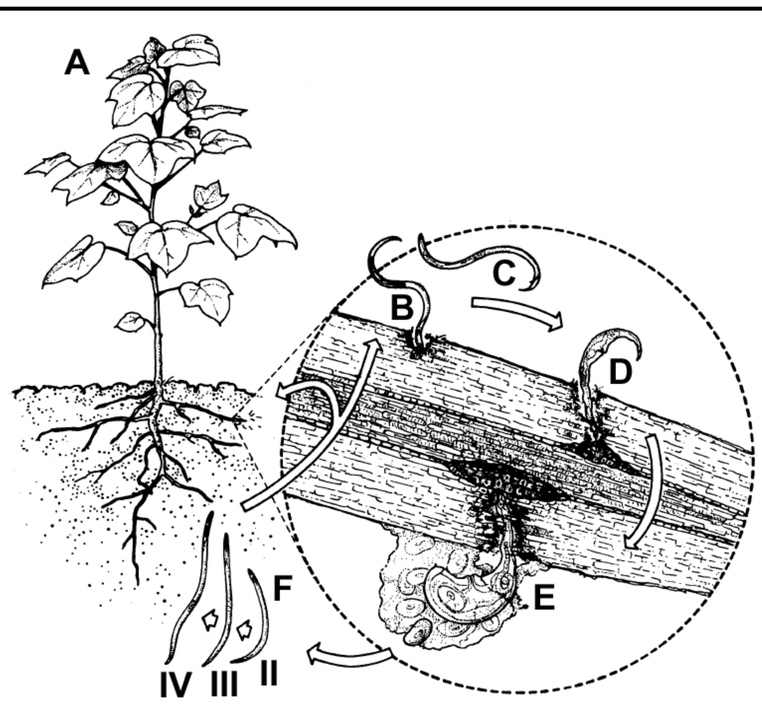

Fig. 1. ***R. reniformis*** **life cycle on** ***Gossypium hirsuta*****. (A) field grown plant in soil infested with** ***R. reniformis*****. B. Immature vermiform female burrowing into a lateral root. C. Non-parasitic male. D. Maturing female with developing gonads is establishing a nurse cell known a s a syncytium in the stele of the root. E. Mature female is feeding on a stelar syncytium and is producing eggs in a gelatenous matrix, surrounding the swollen posterior region of the body that is protruding from the root surface. F. Second, third and fourth stage juveniles (II, III, IV) in the soil. Figure is from Robison et al. (1997).**

MEDTHODOLOGY

The Genetic Engineering Vector

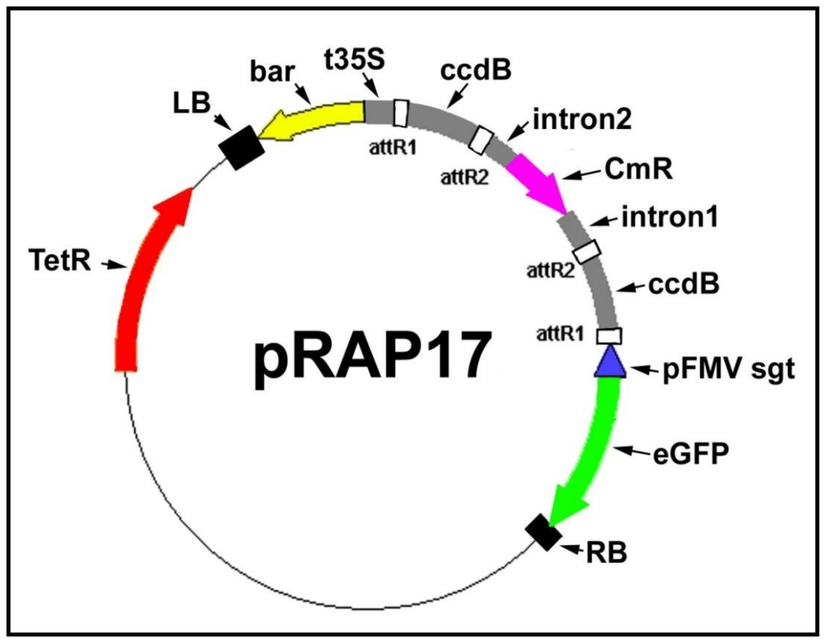

Fig. 2. The pRAP17 vector. TetR tetracycline resistance gene (red), LB left border, bar Basta resistance gene (yellow), t35S 35S terminator, ccdB lethality gene (Bernard and Couturier 1991; Salmon et al. 1994); intron 2, CmR chloramphenicol resistance gene (pink); intron 1, FMV sgt figwort mosaic virus sub-genomic transcript promoter (blue), eGFP enhanced green fluorescent protein cassette containing the ***rol*****D promoter and the 35S terminator (green), RB right border,** ***att*****R1 LR bacteriophage k-derived recombination site #1,** ***att*****R2 LR bacteriophage λ-derived recombination site #2**

Culturing Cotton Seedlings

Fig. 3. Cotton seeds were germinated and grown for two weeks. Afterwards, the roots were cut off and the plants were co-cultivated with ***A. rhizogenes.*** **The plants were then grown as shown for approximately a month. After growing the plants for a month, the plants were inspected to see if they were transgenic, using the visual screening apparatus shown in the next figure.**

The Visual Screening Apparatus

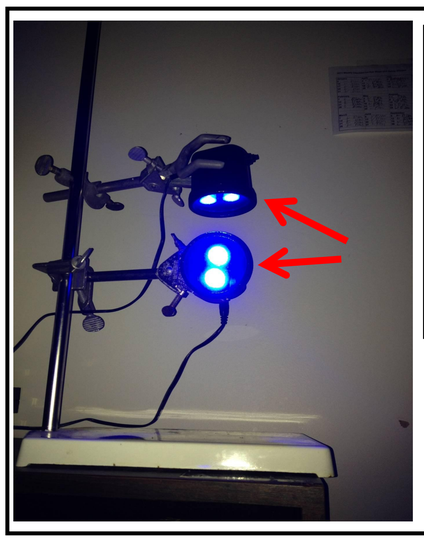

Fig. 4. The dual lamp eGFP detection apparatus. The red arrows are pointing to the 2 lamps that are secured to a ring stand.

Genetically Engineered Roots

Fig. 5. Transgenic cotton roots are expressing the eGFP gene that is made visible with the visual screening apparatus.

Conclusion: A simple genetic engineering procedure is being produced that will replace the need for tissue culture methods that rely on chemical markers. The procedure is relatively rapid and easy to perform, allowing for the testing of the function of many genes simultaneously in greenhouse conditions for the identification of gene function of genes in biological processes such as root physiology and pathogen attack. The procedure allows for the testing the function of hundreds of genes simultaneously because the engineering process is reliable, rapid and simple so that minimal training is required for its use.

Acknowledgements: The authors gratefully thank Dr. Robert Nichols and Cotton Incorporated for funding this project and their continued support.