GENETIC ENGINEERING FOR COCOA PLANT IMPROVEMENT FOR PEST AND DISEASE RESISTANCE AND QUALITY

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Introduction

Malaysia is the fifth largest producer of cocoa in the world but the yield of cocoa is slowly declining and dried fermented cocoa seeds are slightly acidic properties compared with cocoa from Ghana. Major pests and fungal diseases particularly vascular streak dieback (VSD) and black pod (PD) diseases are an endemic in cocoa growing countries in the Far East and directly affecting production of good quality cocoa. Genetic engineering such as transformation of genes mediated by *Agrobacterium spp* or mediated by protoplast chemical or electro-fusion and bombardment of embryogenic cells or tissues by a ballistic gun offers solution to this problem (Yang and Christou, 1994). In order to studies the pathogenicity of fungal pathogens such as VSD and BP diseases, dual culture technique (host tissue/cell with absence and presence of pathogen – spore or mycelium suspensions of the isolated fungal pathogen). Another alternative tool to distinguish between clones that are resistant or susceptible to VSD is by using AFLP technique (Kasran et al. 1998a). The similarity index and cluster analysis were used to classify the cocoa clones as being resistant or susceptible to VSD.

Materials and Methods

Mature pods of *Theobroma cacao*, L. from resistant and susceptible clones (52clones) obtained from MARDI Research Station, Hilir Perak, Teluk Intan, Perak were regularly used to initiate callus and cell-suspension cultures. This research were used plant biotechnology, biochemistry and molecular biology techniques such as DNA isolation, RNA determination, protoplast isolation, protoplasts chemical or electro-fusion and micro-injection, gene transformation mediated by *Agrobacterium spp* and biolistic bombardment techniques (if possible). Cocoa callus and cell-suspension cultures were also initiated to form stable somatic embryogenesis tissues and cells by manipulating of PGRs, tissue culture media and supplements with amino acids (rich proteins compounds) and silver nitrates. In order to study the pathogenicity of fungal pathogens such as VSD and BP diseases, the dual culture technique may be used such as host tissue/cell with absence and presence of pathogen - spore or mycelium suspensions of the isolated fungal pathogen (Ingram & Helgeson, 1983). Another alternative tool to distinguish between clones that are resistant or susceptible to VSD is by using AFLP technique. The similarity index and cluster analysis were used to classify the cocoa clones as being resistant or susceptible to VSD.

Results and Discussion

The yield of protoplasts was around 2.5 x 10^6 to 15.0 x 10^6 per g.fresh weight tissue and their size: 0.5 to 2.0 µm diameter for cocoa leaf protoplast and around 2.5 x 10^6 to 23.0 x 10^6 per g. fresh weight of filtered cells and their size: 2.0 to 10.0 µm diameter for cocoa cell-suspension protoplasts. This closely agrees with the previous findings of Muse et al. (1988b) working with protoplasts of *Theobroma cacao*, L. (cocoa). Calli inoculated with *O. theobromae* pathogen was extensivley colonized by intercellular hyphae and caused browning responses but resistant calli were associated with slow colonization and limited pathogen mycelial growth. Culture filtrates (CFs) of *O. theobromae* pathogen also contained some growth inhibitor compounds or phytotoxins, which inhibited the growth of cocoa leaf disc/callus /cell-suspensions more in for susceptible cocoa clones than the resistant clones. Then resolution of polymorphism using AFLP was demonstrated for genomic DNA of resistant and susceptible clones to VSD disease. Extra one band of amplified DNA was observed in resistant clones (ICS95 & KKM25) compared to susceptible clones (NA32 & PA7) for all primer combinations tested. The MW of these extra bands was estimated 350bp and was expected as a resistant gene in controlling VSD disease; assumed that low percentage of repeated sequences. This showed cocoa tree has a small genome of 0.4 pg per haploid cell as a similar report by Kasran et al. (1998b). The cDNA was successfully cloned into PCR-TRAP ^{TM} vector and cDNA sequence was in progress.

Conclusions

The present results indicated that plant biotechnology and molecular cell biology using cocoa tissue cultures (callus, cell-suspension & protoplasts) were established in our laboratory since in 1990 until now. Monoculture and dual-culture techniques of investigating the resistant and susceptible cocoa clones were much useful and easy in a controlling environment system from any contaminants. The best and rapid techniques of cocoa protoplast isolation were discovered and chemical or electro-fusion of cocoa protoplasts would be not difficult to cocoa researchers. Then, cocoa callus and cell-suspensions were also successfully formed embryogenic cells by manipulating the PTC medium with 0.0295 mM Ag NO_3 or 15.0 mM NH_4NO_3 and the peroxidase & PPOxidase would be the best biochemical markers. An expected resistant gene for VSD disease was sucessfully isolated from *Theobroma cacao*, L., genome using AFLP method. Its MW was estimated to be 350bp. The cDNA was cloned for PCR-TRAP ^{TM} vector as blue script.

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