IN VITRO CONDITIONS FOR A SUCCESSFUL BIOLISTICS TRANSFORMATION SYSTEM OF PINEAPPLES (ANANAS COMOSUS MERR.)

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Abstract. In order to develop an efficient and reliable biolistics transformation system for pineapples parameters need to be optimised for growth, survival and development of explants pre- and post transformation. We have optimised in vitro conditions for culture media for the various stages of plant and callus initiation and development, and for effective selection of putative transgenic material.

Shoot multiplication and proliferation is best on medium containing MS basic nutrients and vitamins with the addition of 0.1 mg/L myo-inositol, 20 g/L sucrose, 2.5 mg/L BAP and 3 g/L Phytagel, followed by transfer to basic MS medium for further development.

Callus production on leaf base explants is best on MS nutrients and vitamins, to which 10 mg/L of BAP and NAA each was added.

Optimum explant age for bombardment is 17-35 week old callus, while a pre-bombardment osmoticum treatment in the medium is not required.

By comparing several antibiotics as selective agent, it has been established that a two-step selection of 2 fortnightly sub-cultures on 50 μg/mL of geneticin in the culture medium, followed by monthly sub-cultures on 100 μg/mL geneticin is optimal for survival of transgenic callus.

Shoot regeneration from callus cultures is optimal on medium containing MS nutrients and vitamins, 5% coconut water and 400 mg/L casein hydrolysate. Plants can be readily regenerated and multiplied from transgenic callus through organogenesis.

Rooting of shoots does not require any additional plant hormones to the medium.

A transformation efficiency of 1 – 3.5% can be achieved, depending on the gene construct applied.