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Enhancement of *in vitro* Regeneration on Several *Ocimum* Species and Varities

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Objectives

Tissue culture systems to optimize regenerated plant species of *Ocimum* spp are being evaluated as a method to micropropagate individual plants and to better study their biology *in vitro*. Species also be evaluated for the production of natural plant products in and following the regeneration process.

The goals of this project are to enhance the regeneration efficiency of basil.

Materials and Methods

1. Plant material and explant sources: Seeds from several different species and varities of basil: Sweet Dani lemon basil, African beauty, Genevese, Tree basil, Juicy fruit and Methylcinnamate basil were used for this study. Explant tissue was collected at juvenile stage of development first true leaves. Leaf tissue was surface sterilized by immersion into a solution of 20% commercial bleach (1.05% sodium hypochlorite) for 20 min and rinced 3 times with sterile deionized water.
2. Callus and shoot initiation: Explant materials were prepared in a sterile petri dish by making two longitudinal cuts along side the mid-rib and a horizontal cut to remove the outer tissue including leaf margins and the basal portion of the stem. The basal part of the desected mid-lib section was then sliced into four pieces to the size of 5 mm × 5 mm. Explants were inoculated abaxial side down on the callus and shoot induction medium. To enhance the regeneration efficiency on basil, several factors that cytokinin combination, activated charcoal, gelling agents, and different carbon sources were all examined.

The pH of all nutrient media was adjusted to 5.8 before addition of agar and autoclaved for 20 min at 121 C. Cultures plates were wrapped with parafilm and maintained in darkness at 26C for 14 days in a culture room.

Results and Discussion

1. Eeffect of combination with several cytokinins with 4 mg/L TDZ: Anthocynin spots accumulation occurred from the leaf derived callus, only 4 varieties among 6 tested. Sweet Dani showed the best results, while African beauty, Tree basil and Methylcinnamate formed only a few spots. Shoots were obtained only from Sweet Dani by addition to the 2.0 mg/L zeatin and 1.0 mg/L BAP with TDZ 4 mg/L subsequently.
2. Effect of Activated charcoal: According to the incresement of activated charcoal concentration, callus formation rate decreased respectively compare to control from all the varieties. In spite of Juicy fruit which has the most vigorous callus formation, callus growth decreased respectively by supplement of 0.1 g/L and not respond at higher concentration. However some root formation occured especially in African beauty, inducing long hairly roots in 23 to 33% of explants.
3. Effect of gelling agents: Pink color callus and shoot regeneration observed on supplemented with 9.5 g/L agar in Sweet Dany simoultenously. Methylcinnamate and Genevese were low callus formation rate and poor callus growth. Juicy fruit was just growing callus very strongly but did not form any shoots or roots.

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