OPTIMISATION OF THE PRODUCTION CONDITIONS OF FLAVOUR COMPOUNDS FROM 'KARI' *MURRAYA KOENIGII* (L.) SPRENG.

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Introduction

Curry tree or *Murraya koenigii* (L.) Spreng. belongs to the family of Rutaceae and is found in South East Asia, Tropical Australia and New Caledonia (Salikutty and Peter, 1985). Curry leaves are used extensively in Indian and also Malay cooking and form one of the major leafy spices. Research shows that volatile component such as cyrophylene, pinene and pinene are found in the leaves. Other compounds that have been identified include mahamnimbine from the stems, koenoline methoxy-3-hydroxy-methyl-carbazole from the root and bark and mukonicine, antibacterial agents and antioxidants from the leaves. Plant metabolites such as the flavour compounds are generally produced in highly differentiated tissues under specific growth conditions. Since plant calli are undifferentiated cells, the production of these metabolites is naturally more difficult. Several studies have shown that synthesis of similar or greater quantities of volatiles in the calli as in the original plant materials is possible (Bhuyan et al. 1997; Wee et al. 1997). It has also been shown that some of the compounds synthesised were different or absent from the original plants. The objective of this project was to optimise the production of curry flavour by callus culture of *Murraya koenigii*.

Materials and Methods

Various explants from the curry plant including seeds were used to induce callus formation. The seeds were excised from curry fruits that have been sterilised. Also used as explants were parts of plantlets generated from curry seeds germinated under sterile condition on solid Murashige and Skoog's medium supplemented with 3% sucrose and 1 mg/L BAP under a 16-hour photoperiod growth regime. Where necessary, the explants were first sterilised before their culture on solid media to which phytohormones at known concentrations and combinations were added. Stem and petiole explants were cultured in both dark and dark-light. The hormones used were Picloram, Dicamba, NAA, IAA, 2,4-D and BAP. Callus obtained from suitable 'kari' explants were propagated, and was maintained by regular sub-culture on fresh medium. Various growth regimes including liquid suspension culture are being evaluated for the ability of the curry callus to produce flavour compounds.

Results and Discussion

Results show that stem explant was the best in terms of ease and frequency of callusing when cultured on MS medium supplemented with 3% sucrose, 2-4 mg/L Dicamba and 4 mg/L NAA. Higher concentrations of hormones slowed callus growth, and may cause the callus to become brown and die. Cotyledon and embryo explants took similar time as the stem explant to produce callus. However, the frequency of callus formed was lower (50-80%) and especially when lower (2 mg/L) of Dicamba was used compared to 4 mg/L. Leaf explant had a very low (1%) success rate of callus formation. It failed to respond to most phytohormones either added singly or in combination to the growth media, and conditions of culture. The flowers failed to produce any callus under the conditions of this study. Generally, callus that were formed were friable and white/green and white/brown in colour. Combinations of BAP and 2,4-D were less effective, and using BAP alone was inadequate to induce callus. MS medium was better than LS medium. Dark incubation was more effective in inducing callus from both stem and petiole compared to dark-light incubation. Addition of charcoal to the growth media improved induction of callus. Callus that developed was sub-cultured and maintained on fresh growth medium. To date, callus grown on solid medium has failed to produce flavour compounds similar to those present in *Murraya koenigii* leaves. Induction of hairy root or crown gall formation through infection with *Agrobacterium tumefaciens* was unsuccessful. Currently, studies are being carried out to induce flavour compound synthesis using callus suspension culture.

Conclusion

While callus of *Murraya koenigii* could be formed by using the appropriate explants, induction of flavour compound formation by the callus is difficult. More experiments will have to be conducted to find the right combination and concentrations of hormones, culture conditions to achieve success.

References

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