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Optimized *in vitro* seed culture for disease free propagation of curry leaf

W.M.A.C. Premasiri[1], D.L.C.K. Fonseka[2*], S. Terensan[1], and N. Nanayakkara[2]
[1]Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Sri Lanka.
[2]Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka.

Abstract:
Curry leaf (*Murraya koenigii*) is a valuable plant for both culinary and medicinal purposes, preliminary due to its unique aroma, flavour and bioactive compounds. This semi-hardwood species in tropical and subtropical regions, however faces challenges in conventional propagation due to bacterial, fungal and viral infections. These pathogens reduce yield and disrupt genetic fidelity, affecting desirable traits such as aroma, disease resistance and overall vigor. Although, seed propagation is preferable to vegetative methods, it remains susceptible to contamination. *In vitro* seed culture presents a viable solution by enabling seed sterilization and cultivation in a controlled, aseptic environment, thereby eliminating external and some internal contaminants. This approach supports the propagation of disease-free, true-to-type seedlings and helps conserve elite genotypes. To develop a standardized *in vitro* seed culture protocol, seeds were collected from fully ripened berries of premium, export-grade curry leaf plants in Jaffna. A total of 108 replicates were used in the experiment applying 10% and 15% Clorox concentrations with three exposure times: 10, 15 and 20 minutes. The most effective treatment was 15% Clorox for 20 minutes, yielding the highest survival and contamination-free rate of 78% ($P < 0.05$). Germinated seedlings were then cultured for shoot proliferation under various hormonal treatments, including 2.0 mg/L, 2.5 mg/L, and 3.0 mg/L 6-Benzylaminopurine (BAP) combined with 0.5 mg/L Naphthaleneacetic acid (NAA). Among them, 2.5 mg/L BAP with 0.5 mg/L NAA produced the maximum number of shoots per explant (6) after eight weeks, significantly outperforming other treatments ($P < 0.05$). These findings, validated through SAS software using ANOVA and Duncan Multiple Range Test (DMRT) analysis, demonstrate that *in vitro* seed culture with optimized protocols enables large-scale propagation of genetically stable, disease-free curry leaf plants, boosting both commercial viability and export potential.

Keywords: Curry leaf; *In vitro* propagation; *Murraya koenigii*; Seeds sterilization; Shoot proliferation

[*]***Corresponding Author:*** *dlckumari@crop.ruh.ac.lk*