Development of a callus regeneration protocol for "Masan" (*Ziziphus mauritiana*) using shoot tip culture

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Plant tissue culture is a widely used technique for the commercial-scale production of uniform, disease-free, and high-quality plants under controlled laboratory conditions. Lack of an efficient regeneration protocol for *Ziziphus mauritiana* "Local Masan", limits commercial cultivation in Sri Lanka's dry zones despite its adaptability and economic value. This study aimed to develop a tissue culture protocol by optimizing sterilization conditions and cytokinin concentrations using shoot tip explants, to enable the large-scale propagation of "Local Masan". The study was carried out in a completely randomized design (CRD), with ten replicates per treatment using Murashige and Skoog (MS) culture medium. Four 6-benzylaminopurine (BAP) levels (1.5 mg/L, 1.0 mg/L, 0.5 mg/L, and 0 mg/L) and two sterilization protocols (S1, S2) were tested. Protocol S1 consisted of 15% Clorox, 0.1% mercuric chloride and 70% alcohol. Protocol S2 had 8% Sodium hypochlorite, 0.1% mercuric chloride and 70% alcohol. Tested parameters included viability, contamination, callus number, callus width, and callus length. Protocol S1 resulted in a higher percentage of viable, green explants (90% ± 1.14; p=0.0788) and significantly lower contamination rates (45% ± 3.37; p=0.0461) compared to S2. Among the cytokinin treatments, 1.5 mg/L BAP induced the highest number of callus regeneration (1.8 ± 0.13; p=0.0006) followed by 1.0 mg/L (1.5 ± 0.22). In terms of callus morphology, the 1.5 mg/L BAP treatment resulted in the highest callus width (4.78 mm ± 0.62; p=0.008) and the highest callus length (3.47 mm ± 0.55; p=0.0107), significantly outperforming the lower cytokinin concentrations. The results indicate that higher cytokinin concentration of 1.5 mg/L boosts morphogenic responses consistently yielding the most robust callus regeneration in *Z. mauritiana* 'Local Masan'. These findings highlight the importance of optimizing hormonal levels and sterilization conditions in improving *in vitro* culture success for *Z. mauritiana*.

Keywords: *Cytokinin, Callus, Tissue culture, Sterilization, Ziziphus mauritiana*