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Standardization of the Somatic Embryogenesis Protocol for Micropropagation of the improved N39-2 *Coffea arabica* Hybrid

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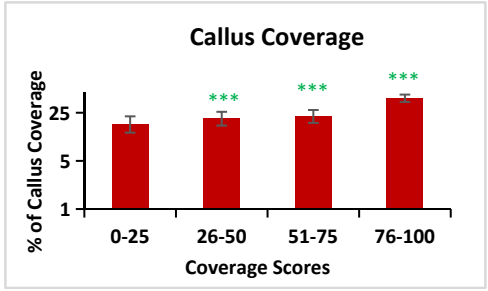
Introduction

The Tanzania Coffee Research Institute (TaCRI) has developed 19 improved *Coffea arabica* hybrids to enhance coffee productivity and sustainability [1]. Among these, the improved N39-2 hybrid has demonstrated favourable agronomic traits such as high productivity, good cup quality, and resistant to coffee leaf rust and coffee berry disease. However, despite TaCRI's development of somatic embryogenesis procedures with proven regeneration potential in other coffee varieties [2], an efficient *in vitro* micropropagation method for large-scale multiplication of N39-2 is not yet established. Therefore, this study aimed to standardize and develop an indirect somatic embryogenesis (ISE) protocol to enable micropropagation of the N39-2 hybrid.

Methodology

N39-2 leaves were surface sterilized using 70% ethanol and 50% of 3.4% sodium hypochlorite before culturing on MS medium with 1 mg/L 2iP and 1 mg/L 2,4-D (N1) for callus induction. Callus formation was evaluated in four coverage categories ranging from 0–25, 26-50, 51-75, and 76–100. Explants producing callus were transferred to MS medium containing 4 mg/L BAP and 1.5 mg/L 2,4-D (N2) to induce embryogenic callus. After six months, friable callus was moved to MS media with 1.5 mg/L BAP (N3) for embryo maturation and subsequently to 1.2 mg/L BAP with 0.1 mg/L biotin (N4) for embryo germination.



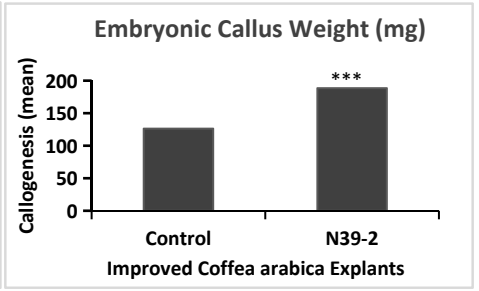


Fig 1: Callus Coverage Distribution on N39-2 explants 30 Days after initiation on N1 media.*** **p ≤ 0.0001**

Fig 3: Indirect somatic embryogenesis of *N39-2*. A.Friable Embryonic Callus **B.** Somatic Embryos **C.** Embryos germiation stages **D.** Complete N39-2-formed plantlets

Fig 2: Embryonic callus formation of N39-2 explants 90 days after initiation on N2 media and Control; N39-2 on N1 for 30 days.
*** **p ≤ 0.0001**

Results/Discussion

30 days after initiation, callus induction in improved N39-2 *Coffea arabica* was successfully achieved on N1 medium, with the highest callus coverage (40.6%) recorded in the 76–100 category **(Fig 1).**Embryogenic callus induction reached 60% on N2 medium **(Fig 2 and 3A)**, where the friable calli further developed into globular and torpedo-stage embryos on N3 medium **(Fig 3B)**. Remarkably, 70% of the embryos regenerated into viable plantlets when transferred to N4 medium as seen in germination stages and plantlets formation on N39-2 **(Fig 3C and D)** respectively.

Conclusion/Perspectives

These findings demonstrate that indirect somatic embryogenesis is a viable alternative for large-scale propagation of the N39-2 hybrid, offering a scalable solution under climate stress conditions. Further evaluation is needed to enhance somatic embryo maturation and assess the environmental adaptability of these N39-2 plantlets.

References:

[1].Kilambo, D. L., et al. "A decade of contributing to a profitable and sustainable coffee industry in Tanzania: the arabica and robusta improvement programmes." American Journal of Research Communication 3.1 (2015): 31-35.
[2] Ramadhani, F. J., et al. "Promotion of Indirect Somatic Embryogenesis of Coffea Arabica var Geisha and Improved N39-6 Hybrid through Auxin-Cytokinin Coordination." J of Agri Earth & Environ-mental Sciences, 4 (1), 01 8 (2025).