Effective method of acclimatization for *In-vitro* propagated hybrid coffee seedlings (*C. arabica* L.) at Jimma, Southwest Ethiopia

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Introduction

Coffee Arabica L. is the only self-fertile and tetraploid species identified with 2n = 4x = 44 chromosomes. The basic genome of the genus *Coffee* comprises 11 chromosomes (Bayeta, 2001). The development of hybrid coffee varieties started in the early 1980s (Ameha and Belachew 1983). Ababuna, the first hybrid coffee variety, was released in 1997 (Behailu et al. 2008). Coffee hybrid seed development through hand pollination is possible but it is a difficult job and cannot produce enough seed to fulfill the demand. In many countries, the Coffee Research Institutes and Private Group Consortium have successfully propagated hybrid coffee through crossing, softwood stem cuttings and grafting, somatic embryogenesis, and mini cuttings (Georget et al., 2010; 2017, Opile and Agawanda, 1993). Industrial hybrid coffee multiplication in Ethiopia has not yet started even though high-yielding hybrids adaptable to mid and low-altitude coffee growing areas were developed and released since 1997. These hybrids produce about 26 q/ha of clean coffee, which has a 20-40% superior yield compared to the high-yielding parent and check materials (Bayetta et al., 1998). Variety development has value if and only if enough and quality planting materials are multiplied and distributed to farmers.

The production of healthy and vigorous seedlings is the first step toward the formation of a productive coffee crop. In *Coffea arabica* L., as an autogamous plant, the formation of new crops depends on seedlings from seeds, and the variability between offspring in advanced generations is small. However, with the possibility of taking advantage of possible hybrid vigor for productivity in this species, an increase in interest in vegetative propagation of F 1 hybrids, on a commercial scale. It is known that hybrids can be more productive than their parents when they are complemented well genotypically (BUENO; MENDES; CARVALHO, 2006). One can also introduce some desirable characteristics such as resistance to diseases and pests, below size or uniformity of fruit maturation (JESUS et al., 2010), in a heterozygous condition, and exploitation of such traits guaranteed through cloning since the respective phenotypes are expressed in offspring. Thus, the cloning of F 1 hybrids of *C.arabica*, via somatic embryogenesis could represent a revolution in the cultivation of coffee in the coming years.

However, optimizing the coffee SE process requires not only laboratory operation adjustment but also greenhouse environment manipulation. Given that the country's economy relies heavily on the export of Arabica coffee, it should benefit from utilizing superior coffee varieties such as hybrids by optimizing the process in which these materials are multiplied cost-effectively. The objective of this study was to determine the optimum hardening of conditions for invitro-propagated somatic seedlings, particularly optimizing ex-vitro conditions about soil substrates to grow somatic seedlings in the initial stage of acclimatization under a greenhouse. The findings can be a significant step towards commercial coffee tissue culture development and production.

Methodology

The study was conducted from 2023 to 2024 in Jimma (Melko), located in southwestern Ethiopia. The soil in the study areas is classified as Nitosol, with pH levels above 5. The region has an elevation of 1,760 meters above sea level (masl), situated at a longitude of 36°47′E and a latitude of 7°40′N. The sites receive an annual rainfall of over 2,200 mm, with an average temperature of 26.8°C. Coffee is a major crop produced in the area, which primarily practices mixed farming alongside other annual crops. Various soil substrates were evaluated at room temperature as part of the requirements for primary and secondary acclimatization. The experiment included three treatments: topsoil, sand soil, and a mixture of topsoil, sand, and compost, with the following ratios: 100%, 100%, and 67:33:33, respectively. A randomized block design was employed, with six replications for each treatment.

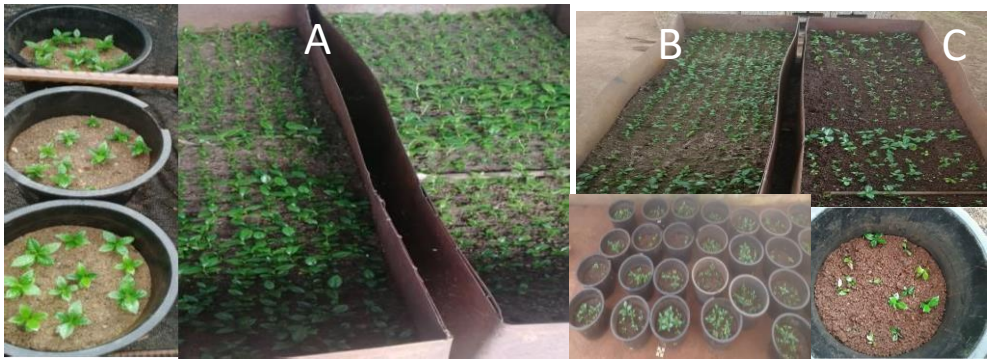

Figure 1. Performance of invitro propagated coffee under greenhouse conditions during primary acclimatization in a plastic pot and metal box; (A) using sand soils only; (B) mixing top soils, sand, and compost (C) Only top soils

Results

There were significant differences ($P < 0.05$) among the treatments regarding plant survival and overall seedling performance. Sand produced the best results across all materials tested, with survival rates of

87.6% under bricks, 89% in metal boxes, and 96% in plastic pots (Table 1). In contrast, using only topsoil resulted in a survival rate of 69%. Mixtures of substrates in a 2:1:1 ratio showed survival rates ranging from 73% to 78.5%, depending on the specific materials used for hardening off.

Table 1. Mean percent survival of hybrid coffee seedlings under primary acclimatization

	Percent survival (%)		
Treatment	Plastic pots	Brick box	Metal box
Sand soils (only)	96.33a	87.59a	89.00a
Mixtures	78.50b	73.17b	75.33b
Top soil (only)	77.17b	64.33c	66.17c
CV	4.18	4.74	4.14
F-value	55.52	64.86	78.09
Pr(>F)	1.17e-07***	4.114e-08***	1.17e-08***

Means followed by same letter within a column are not significantly different at 5% level probability

Survival rates were higher in sandy soils, likely due to their larger pore spaces that promote healthy root growth. In contrast, higher seedling mortality was noted in top forest soils with increased moisture compared to other soil types. These moist conditions favor fungal development, leading to seedling death caused by damping off.

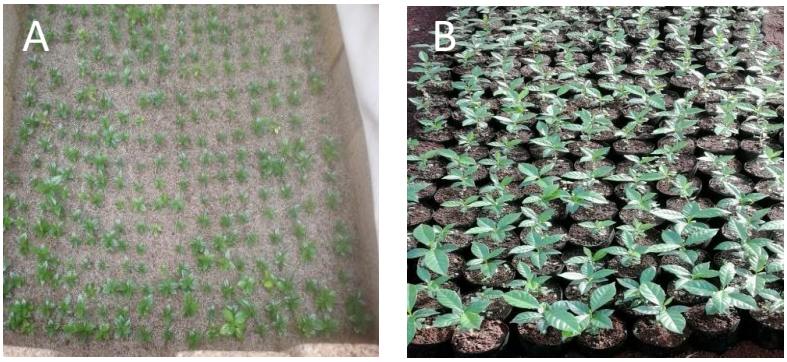

Figure 2. Performance of somatic seedlings. A) Primary acclimatization using a brick box with sandy soil. B) Uniform growth of seedlings under secondary acclimatization in polyethylene bags with soil mixtures.

Conclusion

Seedling handling at greenhouse is crucial to ensure the success of invitro propagation and distribution of elite materials. Increased efficiency of hybrid coffee multiplication achieved overtime by optimizing proper soil mixtures and growing conditions. Our target will be maximizing seedling survival, putting the annual loss just under 10%. Using sand soils gave maximum result to acclimatize highly delicate invitro produced plantlets. Over 90% plant survival was obtained under primary acclimatization using sand soils medium.

References

Behailu A., Bayetta B., Fikadu T,, Ashenafi A. and Tadesse B. (2008) Genetic Diversity and Heterosis in Arabica Coffee. In: GirmaAdugna, Bayetta Belachew, TesfayeShimber, EndaleTaye and TayeKufa (eds.). Coffee Diversity and Knowledge. Proceedings of a National Workshop Four Decades of Coffee Research and Development in Ethiopia, 14-17 August 2007, Addis Ababa, Ethiopia

BUENO, L. C. S.; MENDES, A. N. G.; CARVALHO, S.P. Plant genetic improvement: principles and procedures. 2nd ed. Lavras: Ed. UFLA, 2006.

Etienne, H., Breton, D., Breitler, J. C., Bertrand, B., Déchamp, E., Awada, R., Marraccini, P., Léran, S., Alpizar, E., Campa, C., Courtel, P., Georget, F., & Ducos, J. P. (2018). Coffee Somatic Embryogenesis: How Did Research, Experience Gained and Innovations Promote the Commercial Propagation of Elite Clones From the Two Cultivated Species?. Frontiers in plant science, 9, 1630. https://doi.org/10.3389/fpls.2018.01630

Georget, F., Courtel, P., Garcia, E. M., Hidalgo, M., Alpizar, E., Breitler, J. C., ... & Etienne, H. (2017). Somatic embryogenesis-derived coffee plantlets can be efficiently propagated by horticultural rooted mini-cuttings: a boost for somatic embryogenesis. *Scientia Horticulturae*, *216*, 177-185.

JESUS, AMS et al. Anatomical observations onCoffea arabica L. plants obtained by rooting cuttings.ceres magazine, Viçosa, v. 57, no. 2, p. 175- 180,2010.

Mesfin A. and Bayetta B. (1983). Heterosis in crosses of indigenous coffee selected for yield and resistance to coffee berry disease: II. First three years. Eth. J. Agr. Sci., V: 13 – 2