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Optimization of *in vitro* propagation protocols via somatic embryogenesis to multiply hybrid coffee (*C. arabica* L.) cultivars of Ethiopia

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Introduction

Coffee is an important global commodity and the most consumed beverage. It has social and cultural importance for Ethiopia, as the livelihood of about one-fourth of the population directly or indirectly depends on the coffee sector. It plays the leading role in the economy of Ethiopia, accounting for 30% of the total export earnings, 15-20% of which is said to be derived from primary and secondary forests that contribute to about 40–45% of the total coffee production of the country, while the remaining 45% and 10–15% come from garden stands and plantation coffee, respectively (ECTDMA, 2017/18), indicating that over 90% of the total production comes from the small holdings. Nevertheless, the productivity of those stands is very low, primarily because of a lack of improved coffee technologies and a very low rate of adoption of improved technologies (only less than 5% of the small holders apply) (CSA, 2016).

Jimma Agricultural Research Centre, in collaboration with national and regional research centres, has conducted coffee research for more than fifty years and developed improved technologies, including the release of 35 pure lines and nine coffee hybrids that are suitable for different coffee agro-ecologies in the country. Despite the existence of improved varieties, the national average yield is very low (around 0.7 tonnes per ha). In many coffee-producing areas of the country, coffee production is hampered due to several factors, including climate change, biotic factors such as coffee wilt and berry disease, leaf rust, and old, unproductive trees. Contrary to popular belief, the potential of hybrid coffee is yet to be exploited. As indicated, the crop yield ranges from 2.2-2.68 t/ha on station. So, the demand for hybrid seed and seedlings from coffee farmers as well as from coffee plantation owners is tremendously high. Thus, introducing hybrids in coffee-growing regions can significantly increase productivity, as the hybrid coffee varieties give up to a 40% yield advantage over the pure line varieties (Bertrand et al., 2011). The hybrids are also efficient in agroforestry systems, which ensure sustainable production by protecting the coffee from extreme temperatures.

However, supplying hybrid coffee planting materials is not easy compared to the pure line varieties, as the former require special attention to produce seeds or seedlings. Hybrid seed can be produced by hand pollination—a process that requires tedious work beginning with emasculation, pollination, and continuous removal of the newly growing flower buds. The techniques are not only labour-intensive but also inefficient, often characterised by low seed production that is not sufficient to cover a wider area. Alternatively, hybrid seedlings can be propagated through cutting and tissue culture techniques. Through tissue culture techniques that employ an invitro culture of leaf in artificial media, in aseptic conditions, and in controlled environments, a large number of planting materials like hybrid coffee are often yielded. Several methods of in vitro regeneration and propagation in coffee have already been optimized successfully, including somatic embryogenesis and scale-up of somatic embryogenesis using bioreactors (van Boxtel and Berthouly (1996); Texeira et al. 2004; Etienne et al. 2013; Aguilar et al. 2018). However, the available protocols vary with the composition of media, concentration and combination of plant

growth hormones, genotype, physiological condition of the explant or mother tree, laboratory conditions, etc. As a result, published protocols are often difficult to repeat, and plant tissue culture laboratories need to optimize protocols for their targeted genotypes before launching large-scale multiplication of elite genotypes. To this effect, a research project has been conducted since 2006 to establish protocols for hybrid coffee mass propagation.

Material and Methods

The study was conducted in the Jimma agricultural research centre plant tissue culture laboratory between 2019 and 2023. Green house evaluation or acclimatisation was conducted at Melko, Jimma, and the plant performance was tested at Goma and Gera woreda, Jimma Zone. The coffee micropropagation through somatic embryogenesis method employs the following major steps (**procedures**).

i. Explant collection, sterilization and in vitro conditioning

Leaf explants are harvested from 2 to 3 paired positions from the orthotropic branch of greenhouse-maintained plants. The explant is washed with running water using detergent, soaked in a 0.3% fungicide solution containing the active ingredient mancozeb 80% for 15-20 minutes, and rinsed with distilled water to clean up any fungicide remaining. To further disinfect the leaves, the treated leaves are aseptically surface sterilized by soaking in Sodium Hypochlorite 2.4% following three drops of Tween 20 for 15 minutes in a laminar flow cabinet and rinsed three times in sterile distilled water. Then, properly sterilized leaf explants are cut into small pieces (1 cm^2 leave discs) by avoiding the mid vein, margins, and apical and basal portions of the leaf and inoculated in the abaxial position in a jar containing semi-solid culture medium.

ii. Embryogenic callus induction and multiplication

The leaf discs are transferred to a semi-solid MS medium containing various combinations of PGRs by modifying the protocol described in van Boxtel and Berthouly (1996). The callus will be sub-cultured on the induction medium containing a half-strength of MS supplemented with macro- and micro-salts, thiamine (1 mg L-1), glycine (1 mg L-1), pyrodixine (1 mg L-1), myoinositol (100 mg L-1), L-cysteine (25 mg L-1), casein hydrolysate (100 mg L-1), malt extract (400 mg L-1), sugar (30 g L-1), as well as agar (7 g L-1) to solidify the medium. Leaf discs with pre-embryos (direct embryogenesis) are transferred to hormone-free medium to multiply the embryogenic tissues and then transferred to embryo induction medium.

iii. Regenerations- Somatic embryos formations

The induced embryogenic calli are selected based on morphology and cultured on a regeneration medium containing a half-strength of MS supplemented with macro- and micro-salts, thiamine (100 mg L-1), myoinositol (200 mg L-1), sugar (30 g L-1), as well as agar (7 g L-1), to solidify the medium. MS basal medium will be supplemented with different concentrations of plant growth regulators, cytokinin (BA/Kinetin), auxin (2,4-D/IBA), and embryogenesis-promoting agents.

iv. Embryo germination, plantlet conversion and acclimatization

Somatic embryo maturation and germination take place both in the jar and in Rita using half-strength MS basal medium and a small quantity of cytokinin hormone. This enables mature embryonic (cotyledonary stage) conversion into a plantlet, and the plant eventually produces roots before transferring to an external (ex-vitro) condition. Morphologically well-developed plantlets with four pairs of leaves (Figure 1E) are taken to the greenhouse. The coffee seedling successfully completes the acclimatization stage and is ready for field planting after six months outside the laboratory.

Results and Discussion

The number of somatic embryos significantly varies from genotype to genotype, ranging from 252–401 per leaf disc (table 1). The results are consistent with a previous study on coffee arabica, which classified

somatic embryogenesis greater than 120 somatic embryos per explant as high-frequency somatic embryogenesis. (van Boxtel and Berthouly, 1996). We examined three different regeneration protocols and came up with the best conditions that gave the maximum number of somatic embryos (Figure 2). The germination percentage was also ranging from 45–67%. From a single fully expanded leaf, it could be possible to produce up to 7,500 somatic seedlings, which is estimated to cover a 3 ha area. Regarding acclimatization efficiency, we obtained plant survival rates of more than 90% during primary and secondary acclimatization. We also evaluated the agronomic performance of TC-raised seedlings along with coffee hybrid seedlings from seeds and cuttings and obtained non-significant results in yield and cup quality at Melko and Agaro locations(unpublished). The findings thus highlight the significance of developing protocols for micropropagation of F1 hybrid coffee.

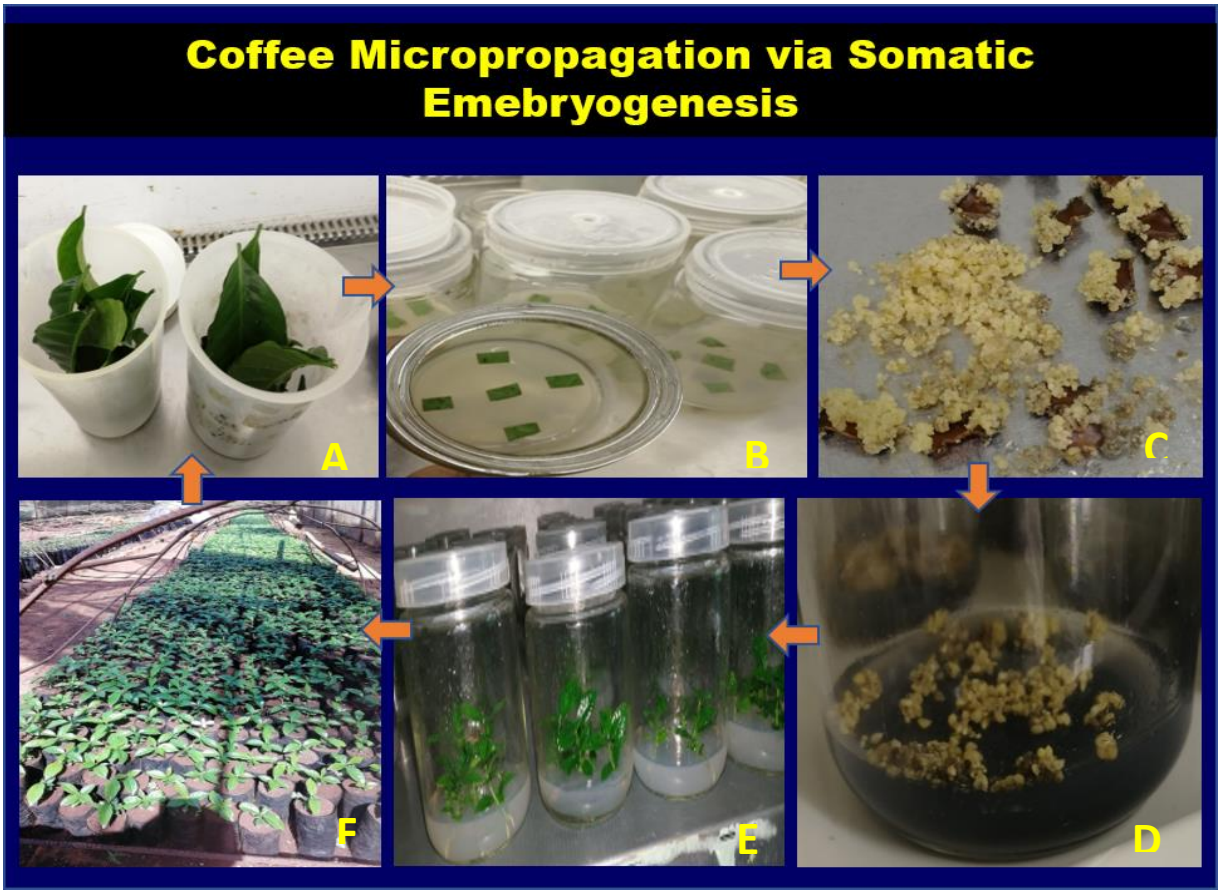

Figure 1. Coffee somatic embryogenesis process: A. Leaf sample preparation B. Explant inoculation/initiation C. Embryogenic callus formation D. Somatic embryo regeneration E. Plantlet conversion (shoot and root development) F. Acclimatizing coffee seedlings under natural conditions using different ratio soil mixtures

Table 1. Somatic embryogenesis potential of coffee hybrids

S.no.	Hybrid varieties	Number of somatic embryos per explant	Replication
1	Melko Ch2	401.64[a]	18
2	Gawe	367.38[a]	20
3	EIAR-50 Ch	316.15[ab]	22
4	Tepi HC5*	252.31[b]	18
Mean		329.5	
LSD (5%)		72.24	
CV		9.42	

Data represent mean ± SD. Different letters represent statistical differences among treatments by Tukey test at 5% of significance ($P < 0.05$)

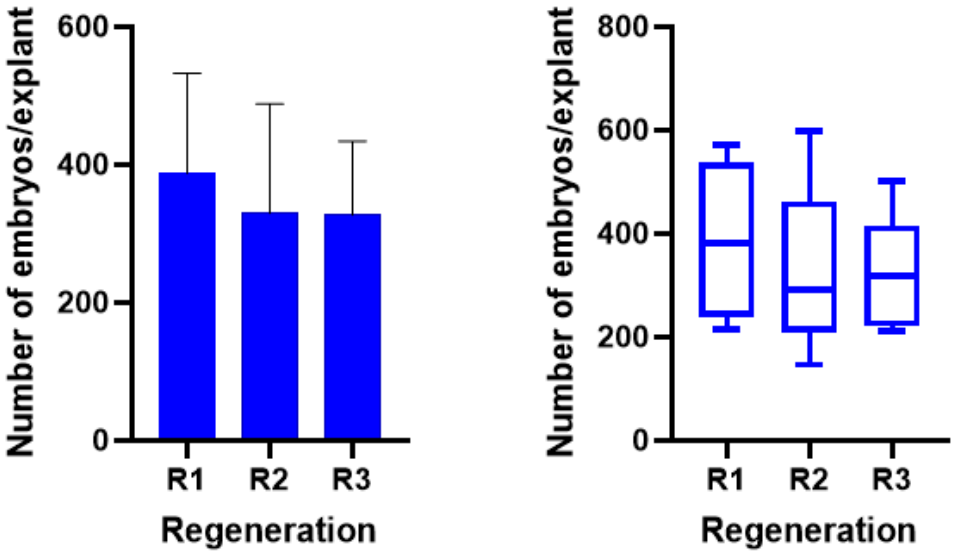

Figure 2. Regeneration results

Conclusions and Outlook

The coffee tissue culture project was successfully conducted by optimising an efficient and reproducible tissue culture protocol for mass propagation of selected hybrid coffee varieties. Large-scale hybrid coffee seedlings can be multiplied via SE methods. Future efforts could concentrate on mass-producing F1 hybrid coffee clones through adaptation and improving the processes for newly released hybrid varieties. To fully utilise the potential of hybrid coffee, it should be a priority to strengthen the local laboratory capacities through engaging commercial producers.

References

Aguilar M. E., Ortiz J. L., Mesén F., Jiménez L. D., Altmann F. (2018). "Cafe arabica (Coffee arabica l.)," in *Step wise protocols for somatic embryogenesis of important woody plants*. Eds. Jain S., Gupta P. (Gewerbestrasse, C: Springer [Switzerland]), 39–62. doi: 10.1007/978-3-319-79087-9-3

Bertrand, B., Alpizar, E., Lara, L. et al. (2011). Performance of Coffea arabica F1 hybrids in agroforestry and full-sun cropping systems in comparison with American pure line cultivars. Euphytica 181, 147–158 https://doi.org/10.1007/s10681-011-0372-7

CSA (Central Statistical Agency). 2016. Agricultural Sample Survey 2014/2015. Report on area and production of major crops in Ethiopia. Central Statistical Agency, Addis Ababa, Ethiopia

ECTDMA. 2017and 2018. (Ethiopian Coffee and Tea Development and Marketing Authority) (2016/17) Addis Abeba, Ethiopia

Etienne H., Bertrand B., Georget F., Lartaud M., Montes F., Dechamp E., et al. (2013). Development of coffee somatic and zygotic embryos to plants differs in the morphological, histochemical and hydration aspects. Tree Physiol. 33 640–653. 10.1093/treephys/tpt034

Teixeira J. B., Junqueira C. S., Pereira A. J. P., Mello R. I. S., Silva A. P. D., Mundim D. A. (2004). Multiplicação Clonal de Café (Coffea arabica L.) via Embriogenêse Somática, 1st Edn. Brasília: EMBRAPA.

Van Boxtel J., Berthouly M. (1996). High frequency somatic embryogenesis from coffee leaves. *Plant Cell Tissue Organ Cult.* **(44)**7-17. 10.1007/BF00045907.