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COFFEE MICROPROPAGATION IN ETHIOPIA: A REVIEW

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Abstract: **Economically, Coffee is one of the most significant commodities in the world. Coffee is grown in about 80 different species around the world, but only two of them are economically significant: *Coffea Arabica L.* and *Coffea Canephora Pierre*. Arabica accounts for 70% of global coffee trade, while Robusta accounts for the remaining 30%. Ethiopia is one of the Arabica coffee's birthplaces. In the third world (such as Ethiopia), population growth combined with climate change has posed a new obstacle to the goal of achieving long-term economic sustainability. Traditional coffee vegetative propagation methods are genetically stable, but the process of producing large coffee plantlets required by farmers and investors is laborious and time consuming. Direct and indirect somatic embryogenesis are the most used strategies for propagating coffee Arabica. One of the promising ways for growing a large number of coffee plantlets in a short period of time is indirect somatic embryogenesis. However, there are other restrictions that occur throughout the somatic embryogenesis of coffee. The most plausible reason is because embryos to plantlet conversions are inefficient. This article presents an overview of the existing stages of micropropagation, their challenges, and recent findings on coffee somatic embryogenesis.**

Keywords: **Direct somatic embryogenesis, Micropropagation, indirect Somatic embryogenesis, explant.**

1. INTRODUCTION

Coffee is one of the world's most frequently planted major economic crops, being grown in over 80 nations (FAOSTAT, 2014). Ethiopia is one of the origins of Arabica coffee. It is blessed with a varied range of coffee varieties and origins (www.etbuna.com, 2016). Coffee is one of Ethiopia's earliest export commodities and a source of income, particularly in rural areas. Arabica coffee is the most extensively farmed species, and higher-quality, higher-value coffee is grown at elevations of 3300 feet (1000 meters) or more in the tropics and subtropics. In the roast and ground coffee business, Arabica is employed (FAO, 2005). There are over 80 different types of coffee, but only two are economically significant (*Coffea Arabica L.* and *Coffea Canephora Pierre*) (Robusta). Arabica coffee accounts for 70% of global coffee trade, while Robusta accounts for the remaining 30%. (Rena *et al.*, 1994). It takes at least 20 years to generate a new cultivar for both species through convectional breeding (Campos *et al.*, 2017). As a result, the quickest expansion of population balances with coffee consumption is increasing from time to time. As a result of various technical issues, such as explant sterilization, excessive phenol concentration in the explant, apical dormancy, and poor rate of shoot multiplication, organogenesis for arabica coffee multiplication was ineffective (Raghramuhu *et al.*, 1989; Ribeiro and Carneiro 1989). Somatic embryogenesis, on the other hand, is a promising technology since it has the ability to create a high number of seedlings at a low cost (Dehayes, 2000; Etienne, 2005; Kumar *et al.*, 2006; Ibrahima *et al.*, 2013). The goal of this study is to describe the research on coffee micropropagation via somatic embryogenesis, somatic embryo growth, maturation, and germination, as well as the main elements that influence each step and the path forward. Somatic embryogenesis, on the other hand, is a promising technology since it has the ability to create a high number of seedlings at a low cost (Dehayes, 2000; Etienne, 2005; Kumar *et al.*, 2006; Ibrahima *et al.*, 2013).

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2. LITERATURE REVIEW

2.1 DISINFECTIONS AND PLANT MATERIALS

Leaf is the most valued source of explant in coffee somatic embryogenesis. Several investigations on the creation of successful methods for coffee somatic embryogenesis employing leaves as an explant source have been carried out (Maciel *et al.*, 2016, Arimarsetiowati 2017 and Bartos *et al.*, 2018). Others, on the other hand, employ seeds to grow coffee plantlets (Shatnawi *et al.*, 2007; Gatica- Arias *et al*., 2008; Ebrahim *et al.*, 2007).

Leaf disinfection has been done in a variety of ways, with sodium hypochlorite being the most commonly employed disinfectant chemical. Some studies, however, utilize mercuric chloride as a disinfectant. Furthermore, ethanol was used as a disinfectant before and after the sodium hypochlorite. Finally, distilled water was used to rinse the leaves or seeds three times.

Table 1: Coffee leaf disinfection procedure

No.	Chemicals used	Explant	Concentrations (%)	Duration	Reference
1	sodium hypochlorite and Ethanol	leaf	3 and 70	15 minutes and 20–30 second.	Ahmed *et al.*, 2013
2	Ethanol and sodium hypochlorite	Leaf	50 and 2.4	1 second and 15 min.	Rezende *et al.*, 2012
3	Ethanol and sodium hypochlorite	leaf	70 and 10	3 second and 15 min	Ibrahima *et al.*, 2013
4	Sodium hypochlorite and mercuric chloride	leaf	1% and 0.1%	10 and 5 min	Giridhar *et al.*, 2004

2.2 SOMATICEMBRYOGENESIS

Somatic embryogenesis is a form of vegetative propagation strategy based on the totipotency of plant cells that provides a powerful alternative to other vegetative propagation methods (Ducos *et al.*, 2007). It is an extremely useful instrument for attaining a wide range of goals, ranging from basic biochemical, physiological, and morphological studies to the development of highly applicable technologies (Jiménez, 2001). It can allow the rapid proliferation of selected clones of the self-incompatible species *C. canephora* (Robusta) and the Arabusta hybrid in the case of coffee, one of the most important crops (*C. canephora* X *C. arabica*). Its primary application in the autogamous C. arabica species is for F1 hybrid propagation, which avoids the costly and time-consuming manual hybrid seed production and cuttings that are required in Arabica (Ducos *et al.*, 2007). Numerous research have demonstrated the usefulness of somatic embryogenesis for the multiplication of coffee explants such as leaves, stems, embryos, and so on (Berthouly and Etienne, 2000). Using leaf sections as explants, two kinds of somatic embryogenesis have been characterized (Berthouly and Etienne, 2000; Etienne *et al.*, 2002): Low Frequency is a term used to describe a frequency that is not very high. Without the generation of calli, a small number of somatic embryos (a few to 100 per explant) are created utilizing one medium. This is a short process that takes about 70 days (Berthouly and Etienne, 2000; Etienne *et al.*, 2002). b. Extremely High Frequency. Two liquid media are used to generate a significant number of somatic embryos (thousands to thousands per gram of callus): an induction medium for primary callogenesis and a secondary regeneration medium for generating friable embryogenic callus. For Coffea canephora and the interspecific hybrid Arabusta, the process takes about 7-8 months, while for C. arabica, it takes around 9-10 months (Boxtel, 1996 and Etienne *et al.*, 2002).

2.2.1 DIRECT SOMATIC EMBRYOGENESIS

On calli induction medium, explants that had produced pre-embryos (direct embryogenesis) were moved to the regeneration medium. The regeneration media has the same composition as the indirect somatic embryogenesis medium reported previously. The number of globular and torpedo somatic embryos were found in both indirect and direct somatic embryogenesis (Ibrahima *et al.*, 2013).

2.2.2 INDIRECT SOMATICEMBRYOGENESIS

Small, yellowish, dense cytoplasm with large nuclei and vacuoles structures containing grains starch characterized the development of embryos through indirect somatic embryogenesis on coffee tissues (Williams and Maheswaran, 1986). After two months of growth on regeneration media, the embryogenic calli changed color from yellow to brownish black. The three-month-old calli appeared blackish brown and white pre-embryo when examined under an electron microscope. The pre-embryo became globular after four months in regeneration medium and was torpedo after eight months. Indirect embryogenesis created more globular and torpedo embryos, according to the study (Ibrahima *et al.*, 2013).

2.3 EMBRYOGENIC CALLUS INDUCTION AND SOMATIC EMBRYO FORMATION

Because their structures can form the radical and plumule axis, which can advance into the root and shoot in a single step, somatic embryos is the most common micropropagule employed for artificial seed generation. Plant lines derived from somatic embryos can maintain their regeneration capacity for a long time, resulting in uniform plant production and consistent genetic structure production by avoiding the dedifferentiation callus stage (Chandra K. *et al.*, 2018).

2.4 SOMATIC EMBRYO GROWTH, MATURATION AND GERMINATION

The composition medium, stage of sub-culture, and embryo size all influence the effective germination of somatic embryos (Arimarsetiowati, 2017). Culturing of Somatic embryos on a semi-solid or liquid media utilizing BAP alone or in combination with GA3 was used to germinate Coffee Arabica Plantlets (Ahmed *et al.*, 2013). The hormone 2, 4-D was the most commonly utilized auxin (49 percent), followed by naphthalene acetic acid (27 percent), indole3-acetic acid (IAA) (6%), indole-3-butyric acid (6%), Picloram (5%), and Dicamba (5%). N-benzylaminopurine was the most commonly utilized cytokinin (57 percent), followed by kinetin (37 percent), zeatin (Z) (3 percent), and thidiazuron (3 percent) (Ibrahima *et al.*, 2013).

2.5 PLANTLET ACCLIMATIZATION

According to Ahmed *et al.* (2013), with the plantlets actively growing in the green house, 96.3 percent of survival rates were recorded. During acclimation, no morphologically distinct plantlets are observed. It appears to be mother plants. This is caused by the leaves' totpotency.

2.6 FACTORS AFFECTING SOMATIC EMBRYOGENESIS (SE) OF COFFEE

Many factors in coffee somatic embryogenesis reduce the multiplication of coffee seedlings, including the type of explant, nutrition, plant growth regulators, growing conditions, and so on (Gatica-Arias *et al.*, 2008). Coffee Arabica L. genotypes have a lower reaction to somatic embryogenesis than other genotypes (Boxtel, 1996).

Table 2: Some factors of somatic embryogenesis

No.	Factor of SE	Reference
1	Type of explant, Culture medium, Type and concentration of growth regulator, Nitrogen source and *in vitro* environmental conditions	Ibrahima *et al.*, 2013
2	Release of organic molecules by the explant	Bartos *et al.*, 2001, Ebrahim *et al.*, 2007, Ducos *et al.*, 2007
3	Secondary metabolite	Nic-Can *et al.*, 2015

3. CONCLUSION

Different restrictions still exist in coffee somatic embryogenesis, particularly in Ethiopia, the home country of coffee Arabica: Poor laboratory setup and equipment, low quality somatic embryos produced, and a low embryo-to-plantlet conversion rate were all factors. Some researchers identified two important bottlenecks in *Arabica coffee* somatic embryogenesis. These include an embryo-to-plantlet conversion rate that is too low and excessive plantlet losses in the nursery at each stage of the acclimatization process (Etienne *et al.*, 2013). Technologies were developed in order to reduce the aforementioned limits. RITA (50%) and huge Twin-Flask bioreactors, which result in a higher plant conversion rate after planting (70%), are two of them (Etienne *et al.*, 2018). Others, meanwhile, have had success with direct sowing of embryos into soil and a rooted mini-cuttings propagation technique employing somatic seedlings (Georget *et al.*, 2017).

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