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The Role of Biotechnology on Coffee Plant Propagation: A Current topics paper

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Abstract
This review paper was aimed to assess the significance of biotechnology on coffee propagation. In this paper, biotechnological coffee propagation methods such as in vitro techniques (somatic embryogenesis, direct organogenesis, androgenesis and protoplast culture), genetic transformation, molecular marker techniques and germplasm conservation are reviewed. This paper revealed that explosion of plant biotechnology has led to many advances in the field of coffee quality improvement including breeding and cultivar development since conventional coffee breeding involving selection from wild populations followed by hybridization and progeny evaluation, backcrossing, and interspecific crossing, takes more than 30 years to obtain a new cultivar. Many studies showed that micro propagation of coffee is highly desirable as it can reduce the time it takes to establish high-valued coffee plantations. In most of the studies, it is presented that development of marker-assisted selection provides an alternative to overcome the limitations of conventional coffee breeding.

1. INTRODUCTION

Coffee is one of the most important traded commodities in the world. The sector's trade structure and performance have large development and poverty implications, given the high concentration of production by small-holders in poor developing countries. Coffee's global value chains are quickly transforming because of shifts in demands and an increasing emphasis on product differentiation in importing countries (Daviron and Ponte 2005).It belongs to the family Rubiacea and consist more than one hundred species(Illy and Viani, 2005) .*Coffea arabica* L. and *Coffea canephora* are the two common species of coffee (Rick and Graham, 2004). *Coffea arabica L.*, is more economical and it represent three quarters of the world coffee productions while *Coffea canephora* P. makes the rest one quarter of world coffee production (Kristina, 2011).

Coffee is an important source of income and employment in developing countries of Latin America, Africa and Asia (Anthony *et al.,* 2001).According to the current context of over production and low prices of the coffee market, improvement and valorization of coffee quality could provide the coffee chain with a new impetus. In this context, the efficiency of integration of coffee quality is the main target in breeding programmes as opposed to its previous status as a secondary selection criterion (Van der Vossen, 2001).

Coffee quality is a quantifiable characteristic which is related to tastes, smells, and personal preferences. Though Coffee quality is an aggregate outcome of genotype, environment, agronomic and postharvest attributes, human controlled factors largely contributes to the intrinsic quality which basically emanates due to difference in knowledge and attitudes of smallholder coffee farmers with the context of cultural managements which they were practicing in their farms (Petit, 2007).Ponte (2002) stated the importance of coffee quality rather than the quantity with respect to the producers of coffee. He argued that producers should keep in mind, the final consumers' preferences and the characteristics of the coffee for which consumers would be willing to pay more. Quality coffee provides more revenue to producers and it is a better strategy to earn more revenue for same quantity of coffee compared to low quality coffee.

Biotechnology is a branch of applied bioscience and technology which involves the practical application of biological organisms, or their sub-cellular components in agriculture, health, manufacturing and service industries, and in environmental management (Kasonta et al., 2002).

Objectives

To review coffee propagation technologies other than convectional breeding

2. LITRATURE REVIEW

2.1. Coffee Breeding

According to Santana-Buzzy *et al* (2007), the major challenge in coffee breeding is the very narrow genetic base of the crop. To overcome this, breeders need to come up with ways of broadening coffee's genetic variability by means of traditional and/or biotechnological methods .In addition, conventional coffee breeding involving selection from wild populations followed by hybridization and progeny evaluation, backcrossing, and interspecific crossing, takes more than 30 years to obtain a new cultivar (Carneiro, 1997). Enlarging the genetic base and improvement of Arabica cultivars, characterized by homogeneous agronomic behavior with high susceptibility to pests and diseases, have become high priorities for researchers (Lashermes *et al*., 2000b).

Many of the resistance traits to diseases and pests such as coffee leaf rust (*Hemileia vastatrix*), coffee berry disease caused by *Colletotrichum kahawae*, and root-knot nematode (*Meloidogyne* sp.) not found in

C.arabica has been found in *C. canephora* (Lashermes *et al.,* 2000b).*Coffea racemosa* also constitutes a promising source of coffee leaf miner (*Perileucoptera coffeella*) resistance (Guerreiro Filho *et al.,* 1999).

Modern coffee breeding programs need to address some of the crucial needs of the coffee industry, moving away from yield increases as the highest priority toward high cup quality and broader genetic base (Vega *et al.,* 2008).

Based on Fazuoli *et al* (2000) breeding programs should address the following objectives:
Broaden genetic base of modern varieties to combat the constant threat caused by the emergence of new strains of existing diseases;
Increase in yield without compromising coffee quality;
High bean and cup quality;
Production efficiency through easier harvesting (compact growth);
Confer host resistance for reduced disease and pest control; and
Develop environmentally friendly or organic production systems through zero or minimal pesticide use in disease- and pest-resistant varieties.Among the modern biotechnology methods, in vitro, genetic transformation and molecular marker techniques play a key role in coffee crop improvement

2.1.1. Coffee Propagation Using Modern Biotechnology

2.1.1.1. In Vitro Techniques

Propagation of coffee by seeds is associated with slow, asynchronous germination, making it hard to obtain uniform seedlings (Eira *et al*., 2006).Cuttings show relatively low multiplication rates since they can be harvested only from orthotropic branches (Kumar *et al.,* 2006). Use of cuttings in commercial quantities has been limited due to the difficulty in transporting cuttings and the risk of spread of diseases (Etienne *et al.,* 2002).These propagation challenges can be overcome by utilizing tissue culture techniques,which offer a viable alternative to traditional propagation methods in coffee (Kumar *et al.,*2006).

Many advances in in vitro techniques through the manipulation of cells at cellular and molecular levels have already been achieved in coffee, and other woody plant species (Carneiro, 1997).It was also true for the application of biotechnological breeding programs, including genetic transformation (Kumar *et al.,* 2006). Plant tissue culture is a crucial step in all plant transformation experiments and the ability of isolated cells or tissues in vitro to regenerate whole plants underpins most transformation systems. Some of the commonly used in vitro techniques used in coffee plant regeneration include: somatic embryogenesis, direct organogenesis through meristem and axillary bud culture, androgenesis and protoplast culture (Kumar *et al.,* 2006

2.1.1.1.1. Somatic embryogenesis

Numerous studies have shown the suitability of somatic embryogenesis for the multiplication of coffee, which has been tested on different explants such as leaves, stems, embryos, etc. (Berthouly and Etienne, 2000). Yasuda *et al* (1995) established somatic embryogenesis in *C. arabica* and *C. canephora* from leaf explants of mature trees using cytokinin as a sole plant hormone. Both species reacted in different ways. In *C. canephora*, somatic embryos formed from the cut edges of cultured young leaf explants in contact with cytokinin of the medium. Addition of auxin with cytokinin inhibited embryo formation. Somatic embryos were grown to young plants on the cytokinin medium. In *arabica*, embryogenic callus was induced after prolonged culture with cytokinin and then somatic embryos formed on the embryogenic callus. Leaf pieces of *arabusta* coffee trees can be induced to form embryos directly when cultured on a basal MS medium devoid of auxin and containing high levels of cytokinin.By this means, somatic embryogenesis of coffee has been obtained in single-step (Dublin, 1981). Somatic embryogenesis of *C. arabica* was induced by the nitrogen source. The optimum nitrogen concentrations were between 3.75 and 15 mmol.L-1 nitrogen with a nitrate/ ammonium molar ratio of 2:1 or 1:2 (Fuentes-Cerda *et al.,* 2001).

Berthouly and Etienne (2000) reported two types of somatic embryogenesis have been described using leaf sections as explants.

A. Low Frequency. A small number of somatic embryos (a few to 100 per explants) are generated using one medium without the production of calli. This quick process takes approximately 70 days

B. High Frequency. A large number of somatic embryos (several hundreds to thousands per gram of callus) are generated using two liquid media; an induction medium for primary callogenesis and econdary regeneration medium to generate friable embryogenic callus. This process takes about 7-8 months for *Coffea canephora* and the interspecific hybrid, Arabusta, and 9-10 months for *C. Arabica.*In coffee, somatic embryogenesis has been used for rapid multiplication of *C.canephora* genotypes, to shorten breeding cycle of *C. arabica* by true-to-type micropropagation of hybrids, and as a tool for genetic transformation (Berthouly and Etienne, 2000).

2.1.1.1.2. Direct organogenesis

Direct embryogenesis can be induced on certain explants. Direct somatic embryogenesis is the formation of somatic embryos from the explant without the formation of an intermediate callus phase (Raghavan and Sharma, 1995).In most plants, direct somatic embryogenesis is difficult to obtain. Loyola-Vargas *et al.* (1999) have reported direct somatic embryogenesis from explants of leaves in *Coffea arabica* and it was supported by histological evidence. They modified the protocol described by Yasuda et al (1985). Browning of the tissues, caused by an

excessive accumulation of phenolic compounds, is necessary for the somatic embryogenesis process in coffee (Quiroz-Figueroa *et al.,* 2002). Similar observations have been reported by other authors (Baumann, 1983). Several studies using apical or axillary meristem and nodal cultures have been reported for the micro propagation of superior coffee genotypes; though the average rate of multiplication is quite low yielding only 9 shoots per shoot explant (Carneiro, 1997). The cost per unit is very expensive with this method due to the low multiplication rate yielding limited number of cloned individuals and hence is more suited for research activities such as propagation for germplasm preservation and establishment of clonal gardens (Söndahl *et al*., 2000). Direct differentiation of shoot buds from the collar region of hypocotyl segments of *Coffea canephora* has been achieved using optimal levels of AgNO3 with 65% survival rate upon hardening and transplantation to pots (Sridevi *et al.,* 2010). Plantlets developed through this method were further used for genetic transformation by *Agrobacterium tumefaciens* (Sridevi *et al.,* 2010).

2.1.1.1.3. Androgenesis.

The first attempt to produce haploid plants from anther culture was made by Sharp *et al* (1973) in *C. arabica* (Santana-Buzzy *et al.,* 2007). Various other authors have reported successful androgenesis using various coffee cultivars and a correlation was found between the different developmental stages of anther, flower bud size and the quantity of callus produced after 90 days in culture (Carneiro, 1997; Santana-Buzzy *et al.,*2007).

2.1.1.1.4. Protoplast Culture

Acuna and de Pena (1987) isolated protoplasts from leaf callus, leaves, and cell suspensions, respectively. In all cases, cell wall formation, cell division, and callus formation was observed but in no case did regeneration of plants occur. Among the several procedures reported for the

regeneration of coffee protoplasts, there are great differences, especially with regard to the growth regulators and the cultured media used. Flexibility towards growth regulators can be seen in protoplast cultures that resulted in the regeneration of plantlets. The embryogenic tissue used for protoplast isolation can be induced either by an auxin/ cytokinin mixture (Spiral and Petiard, 1991) or by a cytokinin alone (Tahara *et al.,* 1994). Protoplasts are ideal for genetic transformation with foreign DNA and for producing interspecific and intergeneric hybrids with desirable traits. Protoplast culture and fusion offer new possibilities for genetic improvement of coffee. Various authors have reported successful protoplast isolation and culture in coffee using leaves, leaf-derived calli, embryogenic calli, somatic embryos, embryogenic suspension cultures from leaf-derived calli, cell suspension cultures from hypocotyl-derived and non-embryogenic root-derived calli (Santana-Buzzy *et al.,* 2007).

2.1.2. Genetic Transformation

To establish a genetic transformation system a competent explant is required for the transformation process, together with an *in vitro* culture system that permits a high frequency

of regeneration(Ogita *et al.,* 2004). Biotechnological advances such as genetic transformation allows the insertion of specific traits without changing the whole genome. Two main techniques used in plant transformation include: 1) direct transformation through biolistics, DNA uptake, or protoplast electroporation. The first genetic transformation of coffee cells reported was by protoplast electroporation. Barton *et al.* (1991) obtained plantlets of *Coffea arabica* genetically altered. They established suspension cultures to obtain protoplasts which were transformed with a kanamycin-resistance gene by an electroporation procedure. Embryos were formed from transformed cells and regenerated into plantlets. The regenerated embryos contained the inserted foreign DNA. and 2) indirect transformation using viruses or *Agrobacterium* sp. (Dufor *et al.*;2000).First reports of genetic transformation in coffee appeared in the 1990s (Carneiro, 1997). Transgenic plants were successfully created in 1993 by Spiral *et al.* in *C. canephora* by co-culturing somatic embryos with *Agrobacterium rhizogenes* (Dufor *et al;* 2000). Ocampo and Manzanera (1991), using wild-type *Agrobacterium* strains, observed the production of tumors on infected hypocotyls of *in vitro* germinated coffee seeds.Spiral and Pétiard (1991) obtained preliminary results using protoplast co-culture with different *Agrobacterium* strains carrying neomycine phosphotransferase (NPTII) and β-glucuronidase (GUS) marker genes under control of the CaMV35S promoter. They observed transient expression by GUS histochemical assay on callus tissue derived from the treated protoplast.Vega *et al* (2008) reported that advances in genetic transformation techniques will be beneficial in coffee crop improvement by targeting specific traits.

A few examples of transformation programs that could benefit coffee crop production include

Incorporation of *Bt* genes of *Bacillus thuringienses* to introduce resistance to pests such as coffee leaf miner, coffee berry borer and nematodes.

Incorporation of the bacterial gene with enzyme resistant to glyphosate herbicide toconfer plants with resistance to the herbicide.

Modification of ethylene biosynthesis to impart uniform fruit ripening.

Transfer of genes involved in traits such as drought tolerance, low temperature tolerance and flooding adaptation.

Modification of caffeine biosynthesis to produce caffeine-deficient coffee plants using the RNA anti-sense technology.

2.1.3. Molecular Marker Techniques

During the last three decades, molecular techniques based on polymorphisms in proteins or DNA have played a key role in the evaluation of genetic variability, catalyzing research in a variety of disciplines such as phylogenetics, taxonomy, ecology, genetics, and breeding .Properties making a specific molecular marker desirable include: 1) moderately to highly polymorphic; 2) co-dominant inheritance; 3) unambiguous assignment of alleles; 4) frequent occurrence in the genome; 5) even distribution throughout the genome; 6) selectively neutral; 7) easy access; 8) easy and fast assay; 9) high reproducibility; 10) easy exchange of data between laboratories; and 11) low cost for both marker development and assay (Weising et al., 2005).

Though no single marker will fulfill all of these criteria, based on the particular application, there are many marker systems to choose from, combining many of the desirable characteristics. Molecular marker techniques have been used in coffee to assess genetic diversity of the species, construct genetic maps, and identify quantitative trait loci (QTLs) (De Kochko *et al*., 2010).The development of marker-assisted selection (MAS) provides an alternative to overcome the limitations of conventional coffee breeding (Lashermes *et al.,* 2000b).The general principle of MAS is the use and selection of an identified molecular marker linked to a gene for a specific trait rather than selection for the trait itself and reduces the number of backcrosses required (Lashermes *et al.,* 2000b).Molecular markers have been used in coffee for introgression assessment, determination of mode of inheritance of disease and pest resistance, assessment of beverage quality, and analysis of quantitative trait loci (QTLs), all of which have great implications for future breeding. Using markers, introgressed genotypes derived from the Timor Hybrid were evaluated and compared to parental genotypes of *C. Arabica* and *C. canephora* to estimate the amount of introgression present to gain insights into the mechanism of introgression in *C. arabica* (Lashermes *et al.,* 2000a). These researchers concluded that AFLP is an extremely efficient technique for DNA marker generation in coffee and offers an efficient way of distinguishing and fingerprinting coffee germplasm collections. In early breeding programs in India, S.26, a putative natural hybrid between *C. arabica* and a diploid species has been used as a main source of rust resistance (Prakash et al., 2002.progeny derived from a cross between a root-knot nematode (*Meloidogyne exigua*) resistant introgression line T2296 and a susceptible accession Et6, segregation data analysis was performed showing that resistance to *M. exigua* is controlled by a simply inherited major gene designated as the *Mex*-1 locus with 14 AFLP markers associated with the resistance (Noir *et al.,* 2003). The study to identify the genetic basis and host resistance and identification of molecular markers associated with coffee berry disease caused by *Colletotrichum kahawae*, eight AFLP and two microsatellite markers were identified to be tightly linked to the resistant phenotypes, which were mapped to one unique chromosomal fragment introgressed from *C. canephora* (Gichuru *et al.,* 2008).Three RAPD markers were also found to be closely associated with resistance to coffee berry disease in Arabica coffee controlled by the *T* gene found in the varieties Hibrido de Timor and Catimor (Agwanda *et al.,* 1997).

2.1.4. Germplasm Conservation

Due to the narrow genetic base of cultivated coffee (*C. arabica*), utilization of wild species of coffee in future breeding programs for crop improvement is imperative. With many wild species being lost due to habitat destruction, there is an urgent need for the conservation of these valuable genetic resources. Coffee seeds are recalcitrant or exhibit intermediate storage behavior, making preservation of germplasm through seed banking problematic. With forests being lost at a fast pace, conserving these species in ex situ germplasm becomes vital. To meaningfully conserve the genetic diversity of a taxon, knowing the genetic structure of the population is essential and hence this should become one of the principal strategies in the conservation efforts of species to ensure success (Shapcott *et al.,* 2007). To make progress in coffee improvement, detecting and quantifying genetic diversity becomes key for effective conservation of coffee genetic resources (Moncada and McCouch, 2004).Using microsatellite markers, Moncada and McCouch (2004) analyzed allelic diversity of five diploid species and 23 wild and cultivated accessions of *C. arabica* from Africa, Indonesia, and South America, with the five diploid species exhibiting more allelic diversity than the 23 tetraploid genotypes. The wild tetraploids on average exhibited 55% of the alleles not shared with cultivated genotypes, supporting the importance of utilizing wild tetraploid ancestors from Ethiopia as a source of novel genetic variation for crop improvement and expansion of the gene pool of *C. Arabica* germplasm. Utilization of wild coffee species with significantly lower natural levels of caffeine than *C. arabica* and *C. canephora* in breeding programs will be critical in meeting the increasing demand for decaffeinated coffee (Mazzafera *et al.,* 1991).Ex situ field gene banks offer an alternative to conserve genetic resources of crop plants for preserving germplasm of taxa that are difficult to conserve as seed (Duloo *et al*.,1998). One of the big drawbacks of plants held in ex situ collections is that they are grown in monoculture leading to susceptibility to pests and diseases and the growing of plants in ecological conditions not suitable for their growth, leading to strong selection pressure and genetic erosion (Duloo *et al.,* 1998).In situ conservation of wild species and landraces should also be emphasized. Molecular tools utilizing DNA markers should be utilized to increase our understanding of coffee genetic diversity and to develop strategies for conservation of coffee genetic resources with wide genetic representation. Due to the non-orthodox nature of seeds and difficulty of long-term storage,*Coffea* species have been traditionally conserved as living plants ex situ in field collections (Santana-Buzzy

et al., 2007). Alternative methods for long-term preservation of *Coffea* germplasm include cryopreservation and in vitro slow growth methods.

2.1.4.1. Cryopreservation

Cryopreservation in liquid nitrogen (-196°C or -320°F) is the best technique described so far for the long-term storage of coffee germplasm (Santana-Buzzy *et al.,*2007). IRD (Institut de Recherche pour le Development) in Montpellier, France have made considerable efforts in coffee seed cryopreservation research since 1997 leading to development of procedures with satisfactory survival rates (Dussert *et al.,* 2007).Two cryopreservation strategies have been routinely used in coffee genebanks, each with its own advantages and drawbacks the advantages of Strategy 1 are that the frozen seeds can be transferred to greenhouse directly without going through a tissue culture process. The two main drawbacks of this strategy are that the mean survival rate is moderate at about 52% and the requirement of a programmable freezer. The advantages of Strategy 2 are the high average survival rate of about 74% and the elimination of a programmable freezer since the seeds are immersed directly in liquid nitrogen. The main disadvantage of this strategy is the laborious nature of tissue culture compared to direct germination. Additionally, loss of plantlets due to the risk of contamination and the acclimatization of in vitro plantlets recovered from frozen embryos pose .Considerable progress has been made in understanding the mechanisms of coffee seed sensitivity to desiccation and exposure to liquid nitrogen and refining the rewarming and rehydration protocols allowing the achievement of 100% survival of frozen seeds. These improvements will enable in applying cryopreservation techniques for future long-term conservation of coffee germplasm (Dussert *et al.,* 2007).

2.4.4.1. In Vitro Slow Growth

Alternative storage techniques such as in vitro culture techniques have been developed for coffee germplasm storage to overcome the problems associated with traditional ex situ conservation techniques (Duloo *et al*;1998).The main aim of slow growth in vitro conservation is to reduce the number of transfers required of the plant material onto fresh medium, which is achieved by manipulating storage temperature, growth regulator levels, sugar, mineral salts, addition of growth retardant, reduction of oxygen tension levels, etc. (Duloo *et al*., 1998). Medium-term conservation based on slow growth has been achieved for *C. arabica* at 20°C and for *C. canephora* at 23°C (Santana-Buzzy *et al*;2007). Slow growth technique in coffee has been performed on explants such as shoot apex, orthotropic nodes, and zygotic embryos (Santana-Buzzy *et al.,* 2007).

3. SUMMARY

Coffee is one of the most important beverages in the world. Coffee quality is a quantifiable characteristic which is related to tastes, smells, and personal preferences. Biotechnology is a branch of applied bioscience and technology which involves the practical application of biological organisms, or their sub-cellular components in agriculture, health, manufacturing and service industries, and in environmental management. The major challenge in coffee breeding is the very narrow genetic base of the crop. C. *canephora* is disease and pest resistant than C.*arabica.* Propagation of coffee by seeds is associated with slow, asynchronous germination, making it hard to obtain uniform seedlings. Many advances in in vitro techniques through the manipulation of cells at cellular and molecular levels have already been achieved in coffee, and other woody plant species.). Plant tissue culture is a crucial step in all plant transformation experiments and the ability of isolated cells or tissues in vitro to regenerate whole plants underpins most transformation systems. Alternative storage techniques such as in vitro culture techniques have been developed for coffee germplasm storage to overcome the problems associated with traditional ex situ conservation techniques

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