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Biotechnology in Coffee Research

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ABSTRACT

Coffee is the second most important commodity traded worldwide and its cultivation and processing constitutes a major economic and social activity in many countries. The main limitation in coffee breeding is the very narrow genetic base of the species *Coffea arabica*. Most *C. arabica* cultivars exhibit homogeneous agronomic behaviour combined with high pest and disease susceptibility. Enlarging the genetic diversity of Arabica varieties and improving their resistance to pathogens or environmental factors are high priorities in the effort to make coffee production economically and socially sustainable. Conventional coffee breeding methods require time. Selections are made in wild populations, hybridised and the progeny evaluated with back-crossing. The resulting seed production and distribution are insufficient to meet coffee grower needs. Developing reliable, rapid tools for improving selection methods is urgent. Major biotechnological advances in coffee research include successful *in vitro* manipulation and multiplication of coffee, development of gene transfer protocols, generation of transgenic coffee plants with specific traits (high yield and good quality) and description of the metabolic pathways involved in the response mechanism to environmental factors. These advances have opened new perspectives and generated a steady flow of coffee biotechnology data focused on improving agronomic and processing qualities. Genome research in coffee promises to add new dimensions to coffee research. This review focuses on the basic and applied aspects of biotechnology in coffee research and how it will advance genetic improvement of coffee.

Keywords: *Coffea*, *in vitro* tissue culture, phospholipids, signal transduction, aluminium

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INTRODUCTION

Since the advent of agriculture, human well-being has been closely associated with the ability to productively cultivate plants. A vital aspect of this relationship is the progressive genetic improvement of plant species to adapt them to extant conditions such as the environment, diseases and human preferences. Total worldwide crop losses are largely due to environmental factors and plant diseases (Zhenjia Chen *et al*. 2005; Barnabás 2007), and, ironically, many of these are the direct or indirect results of human activity.

In the mid-1990s, about $33 billion annually were spent worldwide on agricultural research, distributed about equally between industrialized and developing countries (Pardey *et al.* 2001), with approximately two thirds allocated by public agencies. Substantial research effort continues in development of transgenic varieties without the anti-nutritive or allergenic factors found naturally in some foods (e.g. peanuts, soybean and wheat), and which provide health benefits. Specific targets include plants containing greater quantities of nutritionally desirable components (e.g. lysine, methionine, zinc, iron and vitamin A) or lesser quantities of undesirable components, for example, trans-fats (Lambrecht 2001; Ye *et al*. 2000; Zhang 2001; Chiaiese 2004; Amira 2005; Bassie 2008). Other potential applications include removal of heavy metals from contaminated soils, and enhanced sugar content for use in ethanol production (Cockburn 1994). The latter is increasingly important in a world where as much as 15 % of energy needs are met by plant-derived biomass. Industrial conversion of lignocellulose to

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alcohols is a typical use of plant biomass (Shengde *et al*. 2000; Hazell *et al*. 2006; Vertès *et al*. 2008) and ethanol is commonly combined with petroleum to produce fuel in countries such as Brazil and Kenya (Howard 1985; Jackson 2004; BBC NEWS 2006).

Biotechnology refers to a myriad biological processes used to create products, mostly from vegetal raw materials, and includes ancient processes such as beer and wine fermentation, and cheese cultures (Coombs *et al.* 1992; Zaid *et al.* 1999). Agricultural biotechnology research investment is currently focused on establishing biological models to answer questions about crop behaviour under adverse environmental conditions. Breeders and biotechnologists are using conventional genetic improvement programmes and recombinant DNA technology to enhance plant traits such as biomass yield, herbicide resistance, drought tolerance, plant disease resistance, prolonged shelf-life in end-products and increased nutritional value. Plant biotechnology offers several, real possibilities for increasing productivity, diversification and production in important crops. This vital technology includes plant tissue culture techniques, plant transformation through advanced molecular biology techniques, biochemical and physiological tools, genomic analysis coupled with breeding, and plant-disease diagnosis.

Coffee (*Coffea arabica*) is a major cash crop worldwide and the most heavily traded commodity apart from oil, accounting for 4% of total world food trade (www.ico.org) (ICO Annual Review 2005/2006). The coffee industry generates US$80 million annually, with Brazil contributing about 30% of worldwide production (www.ico.org) (ICO Annual Review 2005/2006) (**Table 1**). Indeed, world coffee consumption is second only to tea. Despite this, basic biological aspects of coffee have not received the attention they deserve. Continued production of a high-quality coffee will require more research on harvest, processing, pest and disease control, pathogen interaction and abiotic stress, as well as production-limiting factors such as disease susceptibility, photosynthetic efficiency, water utilization and tolerance to soil acidity and aluminium content. However, interest in coffee as a research model has grown in recent years, especially in challenging areas like plant transformation and regeneration. Building knowledge and understanding of the biochemical and molecular foundations of coffee is one of the most compelling approaches to improving yield and quality and will bring enormous benefits to researchers, producers and consumers. The last twenty years have seen major developments and advances in *in vitro* cell culture, somatic embryogenesis, plant regeneration and transformation, an increased emphasis on biochemical and molecular studies, and adoption of biodiversity maintenance and protection as a vital factor.

Coffee belongs to the Rubiaceae family, which encompasses some 500 genera and over 6,000 species. Most are tropical trees and shrubs that grow in lowland forests, although other members include the gardenias and the species from which quinine is extracted. The *Coffea* genus, however, is by far the most commercially significant member of the family. Since *Coffea* was first correctly described by Linnaeus in the mid-18th century, botanists have failed to agree on a precise classification system. Over one hundred species have been described within *Coffea* L. (Rubiaceae) and there are probably at least 25 major species, all indigenous to tropical Africa and certain islands in the Indian Ocean, notably Madagascar. All *Coffea* species are woody, but range in size from small shrubs to large trees over 10 metres tall; their leaves can be yellowish, dark green, bronze or tinged with purple.

Despite this diversity, only two are grown commercially on a large scale: *C. arabica* L. (Arabica type coffee) and *C. canephora* Pierre ex Froehner (Canephora or Robusta type coffee), both from Africa (Clifford M.N. and Willson K.C. 1985, Wrigley G. 1988). *C. arabica* is the more heavily traded of these two species, representing 70% of worldwide commercial coffee trade and almost 99% of all production in Latin America (www.ico.org). Its central and primary origin of diversity is Ethiopia, but it is currently grown in the tropical and subtropical regions of over 80 countries, mainly in highland regions. This species is also is quite sensitive to pests such as fungi, nematodes, insects, etc. *C. canephora* has its centre of diversity in central and eastern equatorial Africa, and Madagascar. It is cultivated in tropical lowland regions, produces a lower quality coffee and is more sensitive to pests. There are some differences between Arabica and Robusta coffee (**Table 2**).

The best known *C. arabica* varieties are Typica and Bourbon, but different strains and cultivars have been developed from these varieties, including Caturra (Brazil, Colombia), Mundo Novo (Brazil), Tico (Central America), San

Table 1 Exports by exporting countries to all destinations.

(60-kilo bags)

	Apr 08	Oct 07 to Apr 08	May 07 to Apr 08
TOTAL	8 755 122	54 900 163	95 282 773
Colombian milds	**1 074 173**	**8 028 200**	**13 218 897**
Other milds	**2 295 209**	**11 738 210**	**21 388 690**
Brazilian naturals	**2 487 005**	**16 471 047**	**28 263 331**
Robustas	**2 898 734**	**18 662 706**	**32 411 855**
Angola	0	3 170	4 746
Benin	0	0	0
Bolivia	4 000	49 600	77 600
Brazil	2 295 751	16 658 876	28 024 478
Burundi	20 000	113 000	172 798
Cameroon	64 794	236 588	548 548
Central African Republic	15 000	37 167	52 667
Colombia	914 173	7 273 559	11 818 485
Congo, Dem. Rep. of	20 000	128 249	204 506
Congo, Rep. of	0	0	0
Costa Rica	241 866	861 040	1 443 960
Côte d'Ivoire	198 890	1 132 986	2 372 406
Cuba	450	2 050	7 190
Dominican Republic	6 588	24 293	66 791
Ecuador	65 000	533 672	961 633
El Salvador	214 696	787 631	1 334 194
Ethiopia	417 455	1 298 082	2 743 172
Gabon	0	0	403
Ghana	1 000	7 000	30 299
Guatemala	528 845	2 022 164	3 957 566
Guinea	35 000	202 000	333 342
Haiti	2 000	10 000	20 565
Honduras	461 017	1 929 608	3 510 707
India	314 446	1 767 320	3 194 129
Indonesia	370 000	2 601 678	4 577 211
Jamaica	2 000	14 367	25 379
Kenya	50 000	311 267	761 259
Madagascar	5 000	69 000	116 000
Malawi	0	16 471	18 807
Mexico	300 803	1 410 563	2 751 221
Nicaragua	174 646	848 799	1 417 473
Nigeria	0	5 000	5 181
Panama	14 000	61 000	100 630
Papua New Guinea	68 946	538 679	1 004 996
Paraguay	500	7 000	14 529
Peru	50 000	1 284 697	2 735 928
Philippines	1 000	6 171	20 526
Rwanda	7 000	163 000	255 167
Sierra Leone	4 000	28 000	35 000
Sri Lanka	0	517	1 529
Tanzania	120 000	539 231	787 344
Thailand	30 000	100 800	224 200
Togo	16 000	62 995	123 648
Trinidad and Tobago	0	0	4
Uganda	237 256	1 839 353	2 947 799
Venezuela	2 000	38 000	55 620
Vietnam	1 475 000	9 816 522	16 326 617
Zambia	3 000	39 000	59 957
Zimbabwe	3 000	20 000	36 561

Source: ICO Annual Review 2005/2006 (www.ico.org)

Table 2 Some differences between Arabica and Robusta coffee.

	Arabica	Robusta
Year species described	1753	1895
Chromosomes (2n)	44	22
Time from flower to ripe berry	9 months	10-11 months
Flowering	after rain	irregular
Ripe berries	fall	stay
Yield (kg beans/ha)	1500-3000	2300-4000
Root system	deep	shallow
Optimum temperature (yearly average)	15-24°C	24-30°C
Optimum rainfall	1500-2000 mm	2000-3000 mm
Optimum altitude	1000-2000 m	0-700 m
Hemileia vastatrix	susceptible	resistant
Koleroga	susceptible	tolerant
Nematodes	susceptible	resistant
Tracheomycosis	resistant	susceptible
Coffee berry disease	susceptible	resistant
Caffeine content of beans	0.8-1.4%	1.7-4.0%
Shape of bean	flat	oval
Typical brew characteristics	acidic	bitter, full
Body	average 1.2%	average 2.0%

Ramon Dwarf and Jamaican Blue Mountain. The average *C. arabica* plant is a large bush with dark-green oval leaves, and is genetically distinct from other species in that it has four sets of chromosomes rather than two. Its berries are oval in shape, ripen in 7 to 9 months, and usually contain two flat seeds (beans). When only one bean develops it is called a pea berry. Arabica coffee is grown throughout Latin America, in Central and East Africa, in India and to some extent in Indonesia.

C. canephora is a robust shrub or small tree growing up to 10 metres in height, but with a shallow root system. Its berries are rounded, take up to 11 months to ripen, and contain oval-shaped seeds smaller than those of *C. arabica*. Robusta coffee is grown in West and Central Africa, throughout Southeast Asia and to some extent in Brazil, where it is known as Conillon.

The species *C. liberica* (Liberica coffee) and *C. dewevrei* (Excelsa coffee) are grown on a much smaller scale. *C. liberica* grows as a large tree up to 18 metres in height, with large leathery leaves and large berries and beans. Liberica coffee is grown in Malaysia and West Africa, but its very distinctive flavour keeps demand for it quite low. *C. dewevrei* grows as a bush and its berries ripen at different times.

A principal objective of the International Coffee Agreement of 2001 is to encourage members to develop a sustainable coffee economy that considers social and environmental contexts. Coffee can have positive impacts on the environment and social issues. It is an evergreen shrub and therefore contributes to carbon sequestration and effectively stabilizes soils. Its cultivation also permits preservation of much of a planted area's original bio-diversity. The main environmental impact of coffee cultivation is water pollution caused by wet processing of the beans, although this method is not generalized. Socially, coffee supports rural employment and helps to keep communities stable. Indeed, in many coffee-producing regions and countries there are no other alternative economic activities that could have such a positive impact

THE SUSTAINABILITY CHALLENGE

Since the 2000-2004 coffee market crisis, economic sustainability has been the main focus in the coffee industry. Farmers cannot continue to produce coffee indefinitely at a loss and challenges such as debt, plantation abandonment, and reductions in cash income led to a series of social problems during the crisis period. In response, producing countries in the ICO have begun to emphasize the urgency of attaining economic sustainability

Another aspect of the coffee industry that requires wider attention is its positive environmental and social contributions. The public needs to understand the very real contribution it makes to the environment, in stark contrast to activities such as livestock raising, annual crops or mining, to give just a few examples. Promotion of sustainability initiatives needs to prevent any public perception that coffee creates environmental and social problems. The ICO distributes information on sustainability issues and initiatives to allow its members to analyse and assess these initiatives' potential contributions. It is also promoting awareness of the need for a sustainable coffee economy by educating coffee industry stakeholders about the threat to sustainability posed by negative economic conditions for producers, and proposing measures in areas such as quality, promotion and diversification in an effort to restore balance to the international coffee market.

The ICO is working towards physical sustainability by promoting production and processing with minimal environmental impacts, for instance through use of environmentally-friendly technologies throughout the production and processing chain (e.g. integrated biological pest control; improved washing process technology). Quality maintenance and improvement is being promoted through development projects; for instance, improvement and diversification of old coffee plantations to cater to dynamic market segments such as organic and gourmet coffee. Initiatives are also in progress to increase demand and therefore balance the market. As these goals are attained, stability should increase in the economic environment and help to restore reasonable living standards to those dependent on the coffee industry.

COFFEE BREEDING

Ideally, traditional breeding techniques could be combined with genetic methods to improve coffee species and generate desirable agronomical (disease resistance), phenotypical (bean shape) and sensory traits (flavour, colour). A number of factors have prevented traditional techniques from having broader success; for instance, *C. arabica* is tetrahaploid, and *C. canephora* and other non-arabica species are self-sterile (Grassias and Kammacher 1975; Lashermes *et al*. 2000b).

The basic chromosome number for the *Coffea* genus is n=11, which is typical of most Rubiaceae family members. Virtually all *Coffea* species are diploid (2n=2x=22) and self-incompatible, although *C. arabica* L. is polyploid (2n= 2x=44) and self-compatible. Early chromosome counts for *C. arabica* and other *Coffea* species were not extremely accurate and usually formed part of general *Rubiaceae* studies. The earliest citation for *C. arabica* is Faber (1912), who reported a somatic chromosome count of 2n=16. In later studies of the *Rubiaceae*, Homeyer (1933) and Krug (1934) reported 2n=44 chromosomes for *C. arabica*, and established n=11 as the basic number for the *Coffea* genus. In a study of cytology in *Rubiaceae*, Fagerlind (1937) confirmed Homeyer's count of 2n=44 for *C. arabica*, and determined the chromosome number for many other *Coffea* species. During the 1930s, a series of studies included chromosome counts for most *Coffea* species, as well as some *Psilanthus* species, a genus taxonomically very closely related to *Coffea* (Sybenga 1960).

Coffea arabica

C. arabica is tetraploid (44 chromosomes) and self-pollinating. Originally, the Arabica or Typica variety was cultivated in Latin America and Asia, while the Bourbon variety arrived in South America and, later, East Africa via the French colony of Bourbon (Reunion). Because *C. arabica* is self-pollinating, these varieties tended to remain genetically stable. However, spontaneous mutations manifesting desirable characteristics have been cultivated and exploited for cross-breeding. Mutants include Caturra, a compact form of var. Bourbon; Maragogipe, a mutant Typica with large beans; San Ramon, a dwarf Typica; and Purpurascens, with purple leaf forms. These mutants have been used to develop

Table 3 Characteristics of *C. arabica* cultivars.

Cultivar	Characteristics
Blue Mountain	Grown in Jamaica and Kenya.
Mundo Novo	A cross between Typica and Bourbon, originally grown in Brazil.
Kent	Originally developed in India, shows some disease resistance.
Catuai	Developed as a hybrid of Mundo Novo and Caturra, characterized by either yellow or red cherries: Catuai-amarelo and Catuai-vermelho respectively.

cultivars intended to provide maximum return under specific regional conditions such as climate, soil, cultivation methods and pest and disease prevalence (**Table 3**).

Coffea canephora

C. canephora is diploid and self-sterile, with many different wild forms and varieties. Cultivar identification remains unclear although two main forms are recognised: Robusta, with upright forms; and Nganda with spreading forms.

Arabica / Robusta hybrids

Selective breeding of coffee has focused on improving growth and flowering, yield, bean size and shape, cup quality, caffeine content, disease resistance and drought resistance, among others. Crosses between Arabica and Robusta normally aim to improve Arabica by conferring disease resistance and vigour, or to improve the cup quality of Robusta. The Timor Hybrid is a natural Arabica / Robusta hybrid resembling Arabica in that it has 44 chromosomes. Catimor is a cross between the Caturra and Timor hybrids that is resistant to coffee leaf rust (*Hemileia vastatrix*). A new dwarf hybrid called Ruiru 11 was developed at the Coffee Research Station at Ruiru in Kenya. Launched in 1985, Ruiru 11 is resistant to coffee berry disease and coffee leaf rust, is high yielding and suitable for planting at twice the normal density. The Icatu hybrids were produced by repeated backcrossing of interspecific Arabica and Robusta hybrids to the Mundo Novo and Caturra Arabica cultivars. Arabusta hybrids are fertile inter-specific Fl hybrids from crosses between Arabica and induced auto-tetraploid Robusta coffee.

The main objective of most coffee breeding and selection programs is to develop cultivars with high yield and excellent bean quality that are adapted to specific growing conditions. Disease resistance and mechanical harvest capability are also important criteria. Coffee growing in many subtropical areas is characterized by poorly synchronized floral development and a prolonged hand-harvesting period. Fruit development in coffee can be synchronized by treatments designed to synchronize anthesis, fruit ripening or both. Plant growth regulators also help to produce more dependable and well-timed floral development and fruit ripening.

Coffee berry quality appears to be largely dependent on climatic conditions (Decasy *et al*. 2003) and slower ripening. Both high elevation (due to lower air temperatures) and shady conditions slow ripening and allow more time for complete bean filling (Vaast *et al*. 2006). Indeed, elevation has a more significant effect on bean biochemical composition than soil water availability (Silva *et al*. 2005), with chlorogenic acid and lipids concentrations increasing with elevation (Bertrand *et al*. 2006).

There are several techniques used in coffee breeding:

1. Controlled pollination and seed multiplication
Conventional coffee breeding methods involve a long process with several different steps: selection from wild populations; hybridisation; progeny evaluation; back-crossing; and inter-specific crosses. Using these methods, it takes over 30 years to produce a new cultivar, which proves expensive and does not produce enough seed to meet grower demands.

2. Vegetative (clonal) propagation
Horticultural vegetative propagation has not yet been made to function adequately with *C. arabica*, probably because it is extremely difficult to attain satisfactory multiplication rates (van der Vossen 1985).

3. Grafting, cuttings
Besides the logistical challenge of transporting cuttings, the risk of disease propagation has made use of grafts and cuttings unfeasible at the commercial level.

4. Tissue culture methods: micropropagation, somatic embryogenesis, genetic manipulation
Micropropagation techniques may allow mass production of selected *C. canephora* clones and of inter-specific hybrids such as Arabusta, but are of particular interest for *C. arabica*, for which F1 hybrid superiority over the varieties has been demonstrated in Kenya (Van der Vossen and Walyaro 1981), Ethiopia (Ameha 1983) and Central America (Bertrand *et al*. 1997). Adventitious shoot development is an alternative method of coffee micropropagation in which shoots originating in tissues from areas other than the leaf axil or shoot tips are subjected to one phase of differentiation followed by differentiation and morphogenesis (Carneiro 1999; Ganesh and Sreenath 1999).

Of these techniques, embryogenesis has the highest potential for multiplication because it involves numerous technical simplifications, and should consequently have the lowest production costs (Berthouly and Ettienne 1999; Etienne *et al*. 1999).

Plant tissue culture allows for mass production of plants, thus increasing the capacity to multiply plants with specific agronomical traits. It is also a major tool for basic and practical agricultural research through clone propagation, cell suspension culture, anther/pollen culture, embryo culture, protoplast isolation and regeneration, and *in vitro* development of specific plantlets. These *in vitro* techniques have facilitated interdisciplinary studies at the cellular and molecular levels, making coffee an optimal model with high potential in physiology, molecular biology and biochemical research with woody species. These will be discussed below.

Coffee is a perennial plant with limited results in productivity improvement using traditional methods, mainly due to sexual incompatibility between commercial species and the long time periods needed to produce new generations. However, recent advances in biotechnology have opened the possibilities for modifying the specific qualities of coffee, be they agronomic traits or end-product properties. Researchers consider coffee to be an ideal *in vitro* model because it can be improved through tissue culture and genetic transformation, among other biotechnological tools (Sondhal and Loh 1988).

The first step in regenerating large numbers of plants is to obtain a large cell population. This is begun by removing a small portion of the plant (explants), and placing it in a liquid or on a solid nutrient medium. This medium needs to contain enough nutrients and hormones to allow the cells to divide and reproduce until forming a large collection of cells (suspension if liquid medium, callus if solid medium). When this primary culture is large enough, it can be subdivided and/or transferred to another medium, depending on the type of regeneration required.

The first reports of coffee tissue culture were by Staritsky (1970), who obtained fast-growing callus using internode segments of young orthotropic shoots as explants. Sharp *et al.* (1973) then established callus from *C. arabica* seeds, shoots, leaves and anthers. Herman (1975) clonally propagated *C. arabica* from callus cultures through organoid formation. In the late 1970s Sondahl (1977, 1979) established the conditions needed for somatic embryo formation from auxin-induced *C. arabica* leaf callus using media

with 2,4-D and cytokinins.

Somatic embryogenesis is a highly promising option for coffee propagation. Plant propagation has mainly been done by inducing development of pre-existing meristematic apical or axial tissue. However, plant propagation through adventitious bud (organogenesis) and somatic embryogenesis is becoming more common. Compared to organogenesis, somatic embryogenesis is more efficient, produces more material in a shorter time and is lower cost (Gatica *et al*. 2008).

Plant embryogenesis is the process of plant embryo development as part of sexual or asexual reproduction of new plants. When it occurs naturally, embryogenesis results from sexual fertilization and the embryos are called zygotic embryos. These develop into seeds, which can germinate and produce seedlings. Plant cells can also be induced to form embryos in plant tissue culture, in which case they are called somatic embryos. An embryo consists of actively growing cells and the term is normally used to describe early tissue formation in the first growth stages. It can also refer to different stages of sporophyte and gametophyte plants. Somatic embryos are formed from plant cells that are not normally involved in embryo development (i.e. ordinary plant tissue) and as a result no endosperm or seed coat forms around them. Applications of this process include clonal propagation of genetically uniform plant material; virus elimination; production of source tissue for genetic transformation; generation of whole plants from single cells called protoplasts; and development of synthetic seed technology. Cells derived from competent source tissue are cultured to form an undifferentiated mass of cells called a callus. Plant growth regulators in the tissue culture medium can be manipulated to induce callus formation and subsequently modified to induce embryos to form from the callus. The ratio of different plant growth regulators required to induce callus or embryo formation varies by plant type.

Somatic embryogenesis is an *in vitro* tissue culture technique used to clone plants and trees. It involves the use of young tissue to generate small cell masses from which new, genetically identical plants and trees are grown. These small masses of cells are the somatic embryo and represent an early developmental stage in plant growth. In this technique, a plant or tree embryo is removed from a seed and grown on a nutrient-rich food source (medium) until it forms a fluffy white cell mass called embryogenic tissue. This embryogenic tissue is transferred to a hormone-containing medium that induces formation of somatic embryos on the surface of the embryogenic tissue. These embryos are then separated from the embryogenic tissue and transferred to another medium containing nutrient specific to plant growth. In this medium they develop into seedlings and are allowed to grow until ready for transfer into soil. Mature somatic embryos are called somatic seedlings, and these are planted in soil and grown in a greenhouse until ready for transplanting into a field. Somatic embryogenesis is an important cloning technique and biotechnology application, and is widely used to generate tree embryos that will become genetically-identical trees.

Genetic engineering is a biotechnology used to modify plant and tree genes to develop or enhance specific, desired traits. In this technology the vegetal tissue is exposed to radiation to change its genetic material, and then selection made of progeny with desired traits. Genes can also be introduced directly into a genome using techniques such as microinjection, electroporation and microprojectile bombardment.

A number of studies have been done on modification of embryogenic response in *Coffea* spp. Fuentes-Cerda *et al.* (2001) induced somatic embryogenesis in *C. arabica* by nitrogen source with optimum concentrations of 3.75 to 15 $mmol.L^{-1}$ nitrogen and a nitrate/ammonium molar ratio of 2:1 or 1:2. Salicylic acid has also been shown to have a positive effect on cellular growth and somatic embryogenesis in *Coffea* (Quiroz-Figueroa *et al.* 2001).

Broad dissemination of vegetal genetic material has been given high priority in agricultural research for many years (Berthouly 1997), with particular emphasis on mass production of somatic embryos using bioreactors. Using *Coffea* cells, Zamarripa *et al.* (1991) reported somatic embryo production and development using a method highly dependent on inoculum density. In a bioreactor, production reached up to 4,000 embryos/l/day. Later studies showed production efficiency could be greatly improved by using a stirred bioreactor, with production of up to 600,000 coffee somatic embryos/l (Ducos *et al.* 1999; Ducos *et al.* 2007). Zamarripa (1993) also described a scale-up of somatic embryogenesis in *C. canephora* and Arabusta with reported yields of 400-500 $\times 10^3$ embryos. Use of bioreactors in coffee somatic embryogenesis has been reviewed extensively by Ducos *et al*. (2007).

Somatic embryogenesis in coffee can be achieved either by direct somatic embryogenesis (DSE) from pro-embryogenic cells in the absence of embryogenic callus, or by indirect somatic embryogenesis (ISE) via callus formation (Molina *et al.* 2002). In *C. arabica* this process has been confirmed using histology (Sondhal *et al*. 1979, Michaux-Ferriérre *et al*. 1989; Quiroz-Figueroa *et al*. 2002). In comparison to ISE, DSE has the advantage of reducing somaclonal variation (van Boxtel and Berthouly 1996), and produces somatic embryos in a much shorter time (Yasuda *et al*. 1985; Hatanaka *et al*. 1991; Bieysse *et al*. 1993; Loyola *et al*. 1999; Giridhar *et al*. 2004). DSE can also be adapted to different coffee genotypes if the factors affecting the process are well controlled (Santana *et al*. 2004), although the influence of genotype, explant source and the *in vitro* protocol must all be considered. Gatica *et al*. (2007) demonstrated the influence of genotype by observing higher somatic embryo production per explant in the Caturra variety than in the Catuaí rojo variety. They also reported that explant source influenced DSE in that more somatic embryos were produced using explants from *in vitro*-plants (50% somatic embryos) than with 3 month-old explants (41%) or 1 year-old plants (0%). This was confirmed by the number of somatic embryos produced per explant: 3.3 embryos per explant from *in vitro*-plants; 1.5 embryos per explant from 3 month-old plants.

A NOBLE MODEL

The main goal of research in *Coffea* has been improvement of the crop aimed at higher yields. However, the key components of the physiological and biochemical processes of development in coffee have yet to be fully understood. Metabolite biosynthesis still requires extensive research, particularly in areas like the biosynthetic pathways of theobromine and caffeine. Recent research has also been done in *in vitro* coffee cultures on resistance to environmental factors such as metals toxicity in soils.

Coffee cell suspension culture is a noble and acknowledged model in plant culture tissue research because it allows culture manipulation under controlled *in vitro* conditions. The earliest applications of coffee suspension cultures occurred in studies of aromatic compound production in cells (Townsley 1974). It has also been used as a model in analysis of caffeine and chlorogenic acid contents (Buckland 1975), and of caffeine synthesis and purine alkaloids biodegradation (Frischknecht 1980; Baumann *et al.* 1983).

Protoplasts

Isolation and culture of coffee protoplasts (wall-less cells) has been done in callus (Sondhal *et al.* 1980), in leaves (Orozco 1984) and from embryogenic cell suspensions (Acuña 1987). Schopke *et al.* (1987) also reported somatic embryogenesis and plantlet regeneration using protoplasts isolated from cell suspension-derived somatic embryos of *C. canephora*. These authors attained plantlet regeneration by subculturing globular embryos on media free of growth regulators. Acuña (1991) reported plant regeneration from protoplasts from an embryogenic cell suspension of *C. ara-*

Table 4 Current *coffea* sp. transfomation studies.

Coffea species	Explant used	Transformation method	Results	Reference
C. arabica	Somatic embryos	*A. tumefaciens*	CryiAc. Csr1-1 integration, plant regeneration	Leroy *et al*. 2000
	Leaves, embryogenic tissues	Biolistic delivery	GUS expression	Rosillo *et al*. 2003
	Embryogenic tissue, somatic tissue	Electroporation	GUS expression, secondary embryo formation	da Silva and Yuffá 2003
	Embryogenic tissue, somatic embryos	*A. tumefaciens*	GFP expression, CaMxMT1 integration, transformed embryogenic tissue plant	Ogita *et al*. 2004
	Embryogenic tissue	Biolistic delivery	GUS and BAR gene integration, plant regeneration	Cunha *et al*. 2004
	Embryogenic tissue	*A. tumefaciens*	Antisense ACC and BAR integration, plant regeneration	Ribas *et al*. 2006
	Transformed root	*A. rhizhogenes*	Growth and branching stimulation in hairy roots	Alpizar *et al*. 2008
C. canephora	Embryogenic tissue	*A. tumefaciens*	GUS and BAR gene integration, plant regeneration	Ribas *et al*. 2006
	Leaf explants, embryogenic tissue	*A. tumefaciens*	DsRFP and NPTII gene integration, plant regeneration	Canche-Moo *et al*. 2006

bica Caturra.

The several reported procedures for coffee protoplast regeneration differ widely, especially in terms of the growth regulators and culture media utilized. Different growth regulators can be used in protoplast cultures to produce plantlet regeneration. For instance, the embryogenic tissue used for protoplast isolation can be induced with an auxin/cytokinin mixture (Schopke *et al.* 1987; Schopke 1989; Spiral 1991) or by cytokinin alone (Yasuda *et al.* 1986; Acuña 1991; Tahara *et al.* 1994). Tahara *et al.* (1994) also reported formation of somatic embryos from protoplasts obtained with an improved and simplified method using embryogenic callus from young leaves of mature *C. arabica* trees.

Genetic transformation

Advances in *in vitro* coffee techniques have facilitated manipulation of the coffee plant at the cellular and molecular levels. This makes coffee suitable for biotechnological breeding programs, including genetic transformation. Genetic transformation has been revolutionizing plant science and agriculture because it provides the opportunity to introduce traits from different species. In addition to its direct application in commercial breeding programs, plant transformation is a powerful instrument in plant science research. Conventional breeding techniques have helped solve many agronomic problems in coffee production, with remarkable success in selecting and producing cultivars for rust resistance, changes in ripening period and plant architecture. Compared to conventional techniques, genetic transformation technology provides the advantage of producing new cultivars with desirable traits in less time. Traits such as insect resistance, herbicide resistance, cup quality and tolerance to abiotic stress (e.g. drought or frost) would be very difficult to develop using traditional breeding techniques. Most coffee transformation research has focused on embryogenic tissues and somatic embryos (**Table 4**). Torpedo-shaped embryos show a high potential for secondary somatic embryogenesis, favouring regeneration of transgenic plants (Spiral *et al*. 1993; Leroy *et al*. 2000).

Hatanaka *et al*. (1999) used two tissue culture systems for transgenic plant regeneration: indirect somatic embryogenesis in *C. arabica* using a modified half-strength MS medium (Murashige and Skoog 1962) containing a combination of 1 µM N-(2-chloro-4 pyridyl)-N'-phenylurea (4-CPPU) and 5 µM 2,4-dichloro-phenoxyacetic acid (2, 4-D); and direct embryogenesis in *C. arabica* and *C. canephora* using half-strength MS plus 20 µM 2iP with fresh medium subcultures made at 3-week intervals. In an alternative method, the hypocotyl segments were co-cultivated with *Agrobacterium* before embryogenic tissue production, still using half-strength MS medium plus Indol acetic acid, kinetin and the appropriate selective agent. The first transformed coffee plants used NPTII genes and kanamicyn as the selective agent (Spiral *et al*. 1993). A double selection approach was applied by Hatanaka *et al*. (1999) in which putative transgenic callus was initially selected under an increasing hygromycin concentration and small plantlets were selected in a kanamycin-containing medium.

Use of antibiotic-resistant markers in transformed plants has raised some consumer concern, and in response the use of herbicide-resistant genes or positive selection marker genes is recommended. Glufosinate has been very effective as a selective agent in production of transformed *C. canephora* after use of particle bombardment or *Agrobacterium tumefaciens* as the transformation system (Ribas *et al*. 2005). Positive selection systems are an alternative to antibiotics. In a study using somatic embryos of four *Coffea* species, Penna *et al*. (2002) demonstrated that explants could grow in the presence of mannose as the carbohydrate source, but not in different xylose concentrations (Samson *et al*. 2004). Very few research groups have successfully transformed *Coffea* using electroporation, biolistic delivery or *Agrobacterium* methods.

Electroporation

Ever since the first report of the transformation of *C. arabica* somatic embryos by electroporation (Barton *et al*. 1991), establishing optimum conditions for plantlet survival has proved a challenge. da Silva and Menéndez-Yuffá (2003) determined the optimum conditions for transformation of coffee somatic embryos by electroporation, for use prior to electroporation of the torpedo-shaped somatic embryos with pCambia3201 plasmid. They obtained eight regenerated plantlets showing GUS expression and BAR genes after the embryos were pre-treated for one hour in an enzymatic solution (2% cellulose and 1% macerozyme in 5 mM MES, 0.5 M mannitol and 25 mM $CaCl_2$ at pH 5.8) incubated in a potassium aspartate buffer pH 5.8 at 28°C for 3 hours and then electroporated at 900 uF, 375 V/cm.

Biolistic delivery

Direct gene transfer by particle bombardment in transformation using *Agrobacterium* prevents and eliminates the complex plant/bacterium interrelationship (Gray and Finer 1993). Van Boxtel *et al*. (1995) provided the first description of transient expression of the GUS marker gene following biolistic delivery of foreign DNA into coffee tissues (callus, suspension, somatic embryos and leaves). Using this method, Ribas *et al*. (2005) reported successful regeneration of transgenic coffee plants. They used pCambia3301 plasmid containing the GUS report and BAR genes (both confer resistance to ammonium glufosinate) as a selectable markers, selected transformed *in vitro* tissue and then applied herbicide screening for *in vivo* identification of *C. canephora* plants. Cunha *et al*. (2004) used PCR to detect and describe transformation of *C. arabica* using biolistic delivery in callus with *npt*II and GUS genes.

Agrobacterium

Transformation of coffee tissues by different strains (LBA4404, C58, EHA101, EHA105) of *Agrobacterium tumefaciens* is the most frequently reported method in *Coffea* sp. Using wild *A. tumefaciens* strains, Ocampo and Manzanera (1991) were the first to report transformation of *C. arabica*, although they were unable to produce regenerated plants. More recently, Leroy (1997, 2000) has reported on somatic embryo transformation in *C. canephor*a and *C. arabica* with *A. rhizogenes* A4 and/or *A. tumefaciesn* LBA4404. In this study, insect resistance was provided by using the TDNA-region containing the CRY1Ac gene from *Bacillus thurigiensis*, resistance to the herbicide chlorsulfuron was conferred by the CSR1-1 gene and the GUS gene reporter was used. Up to 80% of the resulting callus manifested the deep blue colour characteristic of GUS-transformed tissue. Leroy and Dufour (2004) were able to transform 20 different genotypes of *C. arabica* or *C. canephora* by co-cultivation of embryogenic callus with *A. tumefaciens*. Ribas *et al*. (2005) transformed *C. canephora* plants using *A. tumefaciens* EHA105, including the BAR gene to produce plants tolerant to ammonium glufosinate. The most frequently used *Agrobacterium* concentrations (OD_{600nm}) in these experiments range from 0.5 (Leroy and Dufour 2004) to 0.2 (Ribas *et al*. 2005). Other kinds of strategies have also been used to improve *Agrobacterium* infection, such as application of acetosyringone during co-cultivation (Ribas *et al*. 2005) and vacuum infiltration (Canche-Moo *et al*. 2006). However, use of this technology requires adequate weed management, since constant application of a single herbicide will eventually generate tolerant weed populations, a predicament that will require careful consideration in the future (Bates 2005).

The principal insect pests of coffee are coffee berry borer, (*Hypothenemus hampei*) and leaf miner (*Perileucoptera coffeella*). Guerreiro-Filho *et al*. (1998) were the first to report the potential use of genes from *Bacillus thurigiensis* to protect against leaf miner through selection of proteins (CryIA, CryIB and CryIE) with toxicity towards *Perileucoptera* sp. In another approach, Grossi de Sá *et al*. (2004) reported an α-amylase inhibitor from *Phaseolus vulgaris* as a potential target gene for introduction in coffee plants to control against coffee berry borer. *In vitro* assays demonstrated that this protein can inhibit coffee berry borer growth and development.

The first report of RNA interference (RNAi) technology in *Coffea* was by Ogita *et al*. (2003, 2004), who controlled it with the CaMV35S promoter and nopaline synthase (NOS) terminator. They observed transcript down-regulation in the CaMXMT1 (theobromine synthase gene) and CaMXT1 genes (the latter is involved in the methylation steps of caffeine biosynthesis), and produced *C. canephora* plantlets exhibiting a 70% reduction in theobromine and caffeine in leaves compared to a control.

Coffee plant transformation can also be used to validate gene functions through over-expression or gene knock-out, providing insights into several facets of coffee plant research. Identification and characterization of specific and/or inducible coffee promoters (Marraccini *et al*. 2003; Satyanarayana *et al*. 2005) allow more refined regulation of the gene. Genetic transformation of coffee still requires analysis of its potential risks and benefits, and its possible impacts in the market and on consumer acceptance of genetically-modified coffee products.

Metabolite biosynthesis

The ability to produce coffee cell cultures from callus cultures which maintain the ability to produce caffeine and theobromine, and release these purine alkaloids into the medium, has existed for over 20 years (Keller *et al.* 1972; Waller *et al.* 1983). In coffee plants, caffeine is synthesised from xanthosine via 7-methyl xanthosine, 7-methyl xanthine and theobromine. S-adenosyl methionine (SAM) is the actual source of the methyl groups (Ashihara and Susuki 2004). N-methyltransferase activity, indicative of caffeine biosynthesis in coffee plants, has been detected in cell-free extracts prepared from cultured cells (Baumann *et al.* 1983). Simulation of caffeine biosynthesis in suspension coffee cells and the *in situ* existence of 7-methylxanthosine has been reported (Schulthess and Baumann 1995), and N-methyltransferase proteins, the enzyme responsible for methylation of theobromine leading to caffeine formation in coffee, have been purified (Mazzafera *et al.* 1994; Mosli *et al.* 1997; Moisyadi *et al.* 1998).

Ashihara (2001) proposed that caffeine is synthesized through multiple methylation of xanthine derivatives. Subsequently, cDNA for 7-methylxanthine methyltransferase (MXMT or theobromine synthase) was successfully cloned from coffee plants (Ogawa *et al.* 2001; Mizuno *et al.* 2003). Mizuno *et al*. (2003) attained isolation of a bifunctional coffee caffeine synthase (CCS1) clone from coffee endosperm by reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends using previously reported sequence data for the theobromine synthases (CTSs). They reported more than 80% identity in the CCS1's predicted amino acid sequences.

Aluminium toxicity

The signal transduction cascade involving lipids has received recent attention, and it is now well known that external signals such as hormones, growth regulators and environmental stress may play important roles in their regulation. Signalling cascades involving lipid-derived compounds seem to operate the same way in plant cells as they do in animal cells (Gawer 1999).

Plant biologists have become more aware of the importance of environmental issues in agriculture, and a significant portion of research in this area has focused on the mechanisms of aluminium phytotoxicity at the cellular level. Aluminium (Al) is the most abundant metallic constituent in the earth's crust; only the non-metals oxygen and silicon are more abundant. Aluminium is never found naturally as a free metal, rather it is always present as aluminium silicate or as a silicate of Al mixed with other metals such as sodium, potassium, iron, calcium or magnesium (Foy 1978).

Aluminium toxicity is considered to be the most important growth-limiting factor for plants in highly acid soils (Conner *et al.* 1985; Matsumoto 2000; Kochian 2004). A characteristic of Al phytotoxicity is rapid inhibition of root elongation. This is now recognized as the principal symptom of Al toxicity, and has become widely accepted as a measure of Al-stress in plants. This inhibition may be related to different biochemical processes involving membrane phospholipids and several enzymes such as phospholipase C (PLC) and phospholipase D (PLD). These are important elements in the phosphoinositide signal cascade, which is related to Ca^{2+} homeostasis. Martinez-Estevez and Hernandez-Sotomayor (2003) found that PLC activity decreased and lipid kinase activities were modified when *C. arabica* cell suspension was incubated with Al^{3+}.

Phosphatidic acid (PA) is emerging as a very important secondary messenger lipid in plants (Testerink 2005); for instance, it is important in stress signalling (Munnik 2001). It is generated via the PLC/GDK pathway and by PLD activity (Munnik *et al.* 1995). PA is considered an essential cell component because of its function in signal cascades and novo phospholipids biosynthesis. Ramos-Diaz *et al.* (2007) recently reported evidence showing reductions in PA levels to be an effect of PLC inhibition induced by Al-toxicity in *C. arabica* suspension cells.

The cumulative data on Al interactions has shown the importance of its effect on different signal transduction pathways such as phosphoinositide, protein phosphorylation and polyamines, in addition to the possible participation of anion channels and their role in the excretion of organic acids as an Al tolerance mechanism in plants. Coffee breeding has clearly focused on improving important agronomic

characteristics such as flowering, yield, bean size, cup quality, caffeine content, disease resistance, drought resistance and now Al toxicity tolerance. Determining the dynamic interaction of Al, or other metals in soil, with cell structures such as the cell wall, plasma membrane, several proteins and the nucleus is a very promising area of research because understanding the tolerance mechanism of plants against metals like Al may help in resolving questions about other plant toxicities.

BIODIVERSITY AND BIOTECHNOLOGY

Germplasm evaluation and characterization of genetic variability are research strategies in the worldwide search for solutions to present and futures challenges in crop productivity, adaptation to environmental change and development of improved crop varieties. Major crops included in this research include coffee, cacao, chilli and tomato. The data generated from this research will promote crop genetic improvement and provide substantial agricultural benefits.

Many questions about the genetic diversity of internationally important crops such as coffee and cacao have been answered using molecular biology techniques like RAPD (Random Amplified DNA Polymorphism), AFLP (Amplified Fragment Length Polymorphism) and Microsatellite SSR (Simple Sequence Repeats).

Development of specific molecular markers for coffee has led to identification of wild genotypes with traits that are potentially useful in genetic development of domestic genotypes. The worldwide coffee market has been facing chronic overproduction of low quality varieties (Robusta and un-washed Arabicas) which has been depressing prices. Genetic molecular biology research and biotechnology have been employed intensively in recent years to isolate the gene(s) codifying for caffeine, with the purpose of creating a plant without this alkaloid.

During the second half of the 20th century, most breeding programmes (e.g. Brazil, Colombia, Kenya, India, Costa Rica, Honduras) produced positive results in the crusade against several organisms affecting coffee production. These programmes were successful in developing cultivars resistant to leaf rust (*Hemileia vastatrix* Berk. and Br), root-knot nematodes (*Meloidogyne* sp.) and coffee berry disease (*Colletotrichum kahawae* sensu Hindorf) by crossing the Timor Hybrid with *C. arabica* cultivars.

Founded in 2002, the Brazilian Coffee Genome Project is aimed at making modern genomic resources available to researchers working with coffee in different aspects of its production chain. The partial sequencing of anonymous cDNA clones (expressed sequence tags-ESTs) is a rapid and cost-effective method for generating data on the coding capacity of genomes (Wolfsberg 1997). In plants, the EST approach was initially used for the model species *A. thaliana* (Hofte *et al.* 1993) and rice (Yamamoto 1997). Recently, an EST database was released based on sequences from approximately 47,000 cDNA clones and with a special focus on developing seeds of *C. canephora* (Chenwei *et al.* 2005). The coffee EST database from *C. arabica*, *C. canephora* and *C. racemosa* includes identifications of more than 30,000 different unigenes, is a vital tool in functional genetic analysis and an invaluable contribution to knowledge of the structure and evolution of the coffee genome (Vieira *et al.* 2006).

After more than two years, Brazil is now using the coffee DNA map to create a database containing 200,000 DNA sequences and 35,000 genes linked to coffee plant growth and development, and influence different aromas and caffeine levels in the tropical bean. This data will also help to create varieties resistant to diseases and environmental change with the goal of increasing production of gourmet, organic and caffeine-free beans.

With the exception of *C. arabica*, all *Coffea* species, including *C. canephora* (2n=2x=22), are diploid and most are self-sterile. *C. arabica* is the only tetraploid (2n=4x=44), self-sterile species. In addition, it is allotetraploid and manifests diploid-like meiotic behaviour (Grassias 1975; Lashermes *et al*. 2000b). The limited genetic diversity detected among Arabica cultivars is largely the consequence of limited introductions and its self-pollinating nature. This lack of genetic diversity limits the potential for germplasm improvement, and the very few spontaneous interspecific coffee hybrids have been used exhaustively for improving disease and pest resistance in Arabica cultivars. Perhaps the most extensively used hybrid is the Timor Hybrid, a spontaneous interspecific cross between *C. arabica* and *C. canephora*, according to recent molecular evidence (Lashermes *et al.* 1993; Orozco-Castillo *et al.* 1994; Lashermes *et al*. 2000a). Another spontaneous interspecific hybrid between *C. arabica* and *C. liberica* increased genetic diversity among *C. liberica* introgressed lines and was used as the main genetic resource for developing rust resistance in coffee breeding programs in India (Prakash *et al.* 2002).

Research effort has focused on development of co-dominant and multi-allelic molecular markers across the entire coffee genome, and increased availability of these markers will allow analysis of population structure and development of genetic maps. Several genetic maps of *C. canephora* are already available (Lashermes *et al.* 2001; Paillard *et al.* 1996; Crouzillat *et al.* 2004). Development of *C. arabica* genetic maps is less advanced due to its polyploid status and reduced diversity. However, there is one recent report of use of AFLP analysis to build a genetic linkage map of a pseudo-F2 Arabica population derived from a cross between the Mokka hybrid and Catimor cultivar (Pearl *et al.* 2004).

Identification of quality-related genes is the prime objective of several research groups. This is an indispensable prerequisite for development of efficient and rapid quality breeding strategies based on marker-assisted selection (MAS) or genetic modification (GMO) approaches. Using GMO construction, a DNA fragment can be identified as a structural or regulatory gene in a biosynthetic pathway leading to a quality precursor, but data on nucleotide variation levels in natural populations and links between detected polymorphisms and variability in the quality precursors need to be carefully verified (Leroy *et al.* 2006).

CONCLUDING REMARKS

Coffee's worldwide importance cannot be overstated. It is one of the most valuable primary products in world trade, and its cultivation, processing, trade, transport and marketing generate employment for millions of people. Coffee is crucial to the economy and politics of many developing countries, and in many of the least developed countries coffee exports account for a substantial part (up to 80%) of foreign exchange earnings. Coffee is also traded on major futures and commodity exchanges.

Advances in biotechnological techniques, particularly during the past 20 years, promise substantial potential benefits for coffee breeders and offer the possibility of developing new varieties. Development of molecular markers in coffee trees has opened new perspectives in breeding for resistance to parasites, resistance to abiotic stresses (frost, long dry season), biochemical and physical bean traits, and plant features adapted to mechanical harvesting.

Combining use of biotechnology tools (embryogenic cultures, plant transformation) with the inherent advantages of genetic engineering, and applying this combination with a consciousness about its possible positive and negative implications will greatly expand the ability to characterize agronomically important genes for genetic improvement of *Coffea* species.

The excellent results achieved in *in vitro* propagation by somatic embryogenesis offers the possibility of mass hybrid propagation of both *C. arabica* and *C. canephora*. The commercial success of somatic embryogenesis will directly depend on production costs, added agronomical and technological value of the selected hybrids, and rigorous control of somaclonal variation risk.

Production of transgenic coffee plants is an important

contribution and improved utilization of genetic resources has the potential to restore coffee production to long-term economic sustainability. New findings from genome research indicate that there is tremendous genetic potential locked up in wild and cultivated germplasm resources.

Biotechnology is able to offer new ideas and techniques for application in agriculture because it uses the conceptual framework and technical approaches of molecular biology to develop commercial processes and products. Plant breeding has benefited from introduction of genetic engineering techniques, and the knowledge they generate on gene structure and function. Advances in cellular developmental and molecular genetics combined with traditional breeding can target and achieve improvements in specific agronomic, processing and quality traits. The promising results of *in vitro* coffee plant regeneration systems constitute an important breakthrough in various *in vitro* manipulations of regeneration processes. Genetic transformation methods, such as direct DNA uptake, gene bombardment and mediation by *Agrobacterium* sp., and vacuum infiltration are now known to be reliable techniques for coffee. This progress has allowed coffee breeders to implement projects aimed at developing plants resistant to pests and disease, regulate chlorogenic acid synthesis an/or overproduce the amino acids (cysteine and methionine) involved in coffee flavour (a commercially vital trait). Improvements in gene silencing techniques have produced revolutionary advances in molecular breeding programmes. The best example of this is down-regulation of the caffeine pathway, a model for future research towards molecular breeding to improve processing characteristics.

In summary, the enormous body of basic data now available on coffee encompasses cellular level research on signalling proteins, studies on the molecules which interact with the plasma membrane from the outside (abiotic and biotic factors) or inside, and reports on the genes involved in coffee cell development and describing the coffee genome. This data foundation is supporting further biological and physiological research on coffee and will be critical in working towards the larger goal of genetic improvement of *Coffea* species.

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