Chapter

Observations on Somatic Embryogenesis in *Coffea arabica* L.

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Abstract

Somatic embryogenesis contributes to coffee breeding programs. This is a process of asexual reproduction which is based on the concept of cellular totipotency. Each haploid or somatic cell of the plant tissue has the genetic information necessary to generate a complete and functional plant. The somatic embryogenesis can occur either indirectly or directly. *Coffea arabica* genotypes may respond to direct, indirect, or both. In this species, the indirect somatic embryogenesis is composed of two phases, the callogenesis and the embryogenesis, while the direct pathway occurs in a single phase, without the callogenesis. In *Coffea*, in general, the indirect pathway is induced by the auxin and cytokinin, and the direct pathway with cytokinin only. *C. arabica* genotypes usually respond easily to the indirect route with high production of somatic embryos. But these are inefficient by the direct route because they present low production of embryos and the process occurs for a long time. In this review, emphasis will be given to different events that are part of the somatic embryogenesis of *C. arabica* occurring indirectly and in the direct pathway as well as factors that may affect its control.

Keywords: Callus, leaf explant, plant hormone, embryogenic structures, somatic embryos

1. Introduction

The genus *Coffea* belongs to the Rubiaceae family and has over 100 species [1]. However, only *Coffea arabica* and *Coffea canephora* species are commercial, the former accounting for almost 75% of world coffee production and the latter for the remaining 25%. Brazil is the world's largest producer and exporter of coffee accounting for about 70% of world exports [2]. *C. arabica* has this hegemony for producing pleasant and stimulating drink that is consumed worldwide while *C. canephora* coffee is less palatable and is intended primarily for the instant coffee industry.

Coffea breeding aims to combine genotypes of *C. arabica* and *C. canephora* species to release genetically stable varieties with strong traits of both species [3, 4]. In conventional *Coffea* breeding it takes six to eight selection cycles to generate a new cultivar, which is about 40 years. Each cycle corresponds to five years. But it takes four to five harvests to consistently evaluate a generation [5]. In addition, it is important that evaluations are performed on plants over five years of age to obtain reliable yield data [6]. In the breeding program, during the selection phase intermediate populations of progenies are generated and each one of them has different genetic pattern. Thus each progeny corresponds to a single plant.

In the selection phase, the progenies have the characteristic of heterosis that favors the occurrence of differentiated plants. Some of these plants may have special characteristics such as tolerance to biotic and abiotic factors or high productivity or excellent drink quality or all of these. To confirm if a progeny is special it must be multiplied and evaluated in relation to its agronomic performance in the field. Following this phase, the progeny may be released as a clonal cultivar. Cloning selected materials allows to capture all selection and improvement gains without involving genetic segregation [7].

The multiplication of intermediate genotypes to breeding program is not indicated by seeds because plants resulting from the germination may have genetic segregation that leads to loss of the special features [8]. Thus, it is recommended that these genotypes be vegetatively multiplied to maintain their genetic pattern. The vegetative multiplication of coffee plants has been obtained by cutting, and the species *C. canephora* responds very well to this process [9] while arabica plants are less efficient by this way, having low multiplication rate [10].

Usually *C. arabica* genotypes are vegetatively multiplied by in *vitro* cultivation. *In vitro* culture or plant tissue culture belongs to Plant Biotechnology which comprises culturing cells, tissues or organs under aseptic conditions and using artificial culture media containing different components such as water, minerals, vitamins, carbon source and plant growth regulators [11]. Plant tissue culture involves micropropagation processes such as somatic embryogenesis.

2. Somatic embryogenesis

Somatic embryogenesis is defined as a process in which a zygotic embryo-like bipolar structure develops from a nonzygotic cell with no vascular connection to the original tissue [8, 11–14]. This process is based on the concept of cellular totipotency, where all the somatic cells of plant tissue contain the genetic information necessary to produce a complete and functional plant (Haberlandt 1902 *apud* [15–17]). In somatic embryogenesis there is no gamete fusion, only somatic cells of explant tissue that will be responsible for the formation of somatic embryos. These embryos undergo the same developmental stages as the zygotic embryo (**Figure 1**) that will develop into a plant with a genetic pattern identical to the explant donor plant [18].

The occurrence of somatic embryogenesis is associated with the induction of differentiated explant tissue cells that acquire the embryogenic characteristic, followed by the expression of the somatic embryo [19–22]. Thus, this process consists in the termination of the gene expression model present in the explant differentiated tissue cells that will be replaced by the expression of embryogenic genes [23, 24]. But embryogenic program does not occur in all cells at the same time, only in some of them [14, 17, 25, 26]. Several changes may occur to reprogram a somatic cell to the competent embryogenic stage.

Embryogenic cells have different characteristics, they are unique, small, superficially they are similar to meristematic cells, with isodiametric forms, small vacuoles, stained nuclei, with abundance of organelles, thick cell wall and starch accumulation [22, 26–28]. The formation of somatic embryos is strongly associated with the embryogenic competence of the explant cells. Possibly, the acquisition of embryogenic competence is related to the endogenous level of plant hormones, which favor tissue sensitivity to plant growth regulators present in the culture medium, which modulates events leading to the formation of the somatic embryo [21, 28, 29].

Somatic embryogenesis can be applied to most plant species [30], but adequate conditions must be available for this, such as explant type, culture medium and growing environment condition [28].

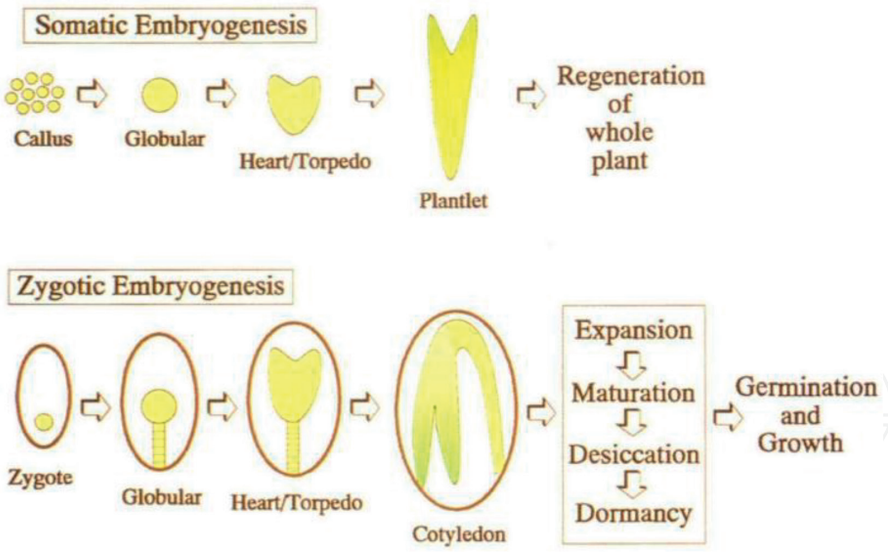

Figure 1.
Schematic representation of the comparison of somatic and zygotic embryogenesis from Ref. [18].

3. Somatic embryogenesis in *Coffea*

Somatic embryogenesis is applied to *C. arabica* and *C. canephora* species for the purpose of vegetative multiplication of elite cultivars, large-scale clonal cultivar, Arabica F1 hybrid [4], to obtain transgenic plants and also as plant differentiation study model [31]. In addition, cloning allows the *in vitro* stock of germplasm cultivars for exchange between research institutions and the preservation of materials since seeds of this species have some degree of recalcitrance [32, 33].

In *C. arabica* the formation of somatic embryos can be obtained either by indirect [34], direct [35] or both somatic embryogenesis (**Figure 2**). Indirect somatic embryogenesis occurs in two phases, callogenesis followed by embryogenesis. On the other hand, direct somatic embryogenesis occurs in one phase without callogenesis. Comparison between these two forms of embryogenesis shows that the direct pathway is more advantageous than the indirect pathway. The direct pathway that occurs in a single phase ends up reducing inputs, labor and cultivation time while the indirect pathway occurs in two phases with higher costs of producing somatic embryos [6, 36, 37]. But although the direct pathway is more advantageous, most studies show that vegetative multiplication of *C. arabica* genotypes is mainly achieved by indirect somatic embryogenesis [6]. In the indirect pathway, explants of this species produce embryos more easily and in high quantity while in the direct pathway the number of formed embryos is smaller and this process occurs in a long time. Studies have indicated that the difficulty of direct pathway occurrence in *C. arabica* genotypes seems to be related to the presence of substances produced by the explant tissue itself [38]. This was demonstrated in explants of *C. canephora* and *Daucos carota* that showed inhibition of direct route when they were cultivated in culture medium with addition of substances secreted by explants of *C. arabica*. This result is possibly related to the difficulty of direct pathway occurrence in *C. arabica* genotypes.

Coffee regeneration via somatic embryogenesis can be obtained from different types of explants (**Figure 3**), such as anthers [39, 40], leaves [34, 41–46] and roots [47, 48]. However, leaf explant is the most used type for the application of direct or indirect somatic embryogenesis in *C. arabica* genotypes, this has been occurring since the pioneering work of Sondhal and Sharp [34]. Normally, these explants are used in rectangular shape with dimensions close to 1.5 × 2 cm^2.

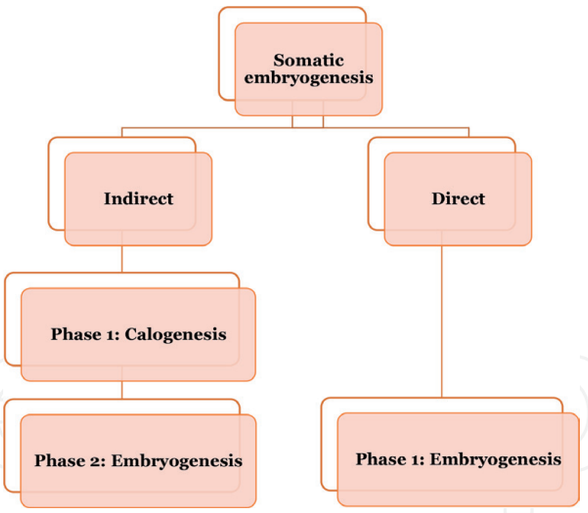

Figure 2.
Details of the occurrence of indirect and direct somatic embryogenesis in Coffea.

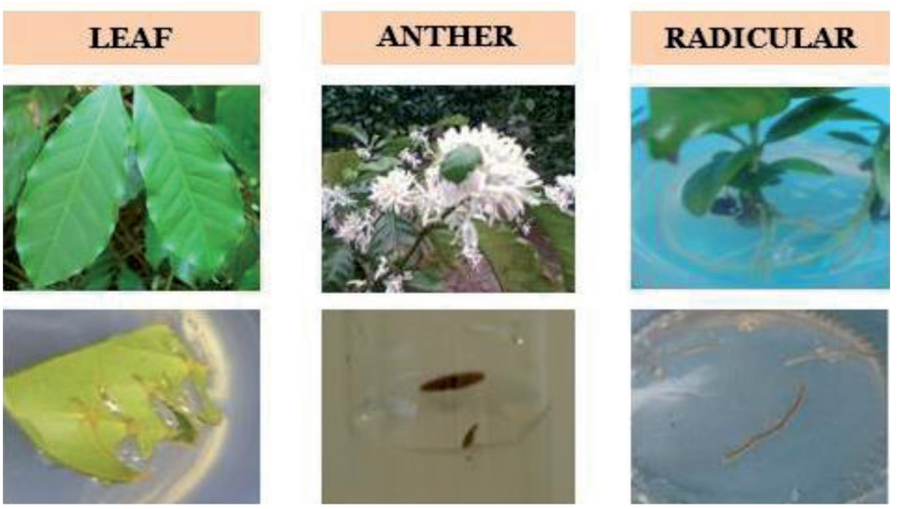

Figure 3.
Types of explants used for the application of indirect and direct somatic embryogenesis in Coffea *genotypes.*

There are indications that *Coffea* somatic embryogenesis is more efficient if applied to leaf explants from *in vitro* seedlings than from leaves collected from plants in the natural environment [49–51], and this response must be related to vegetable hormones endogenous. This aspect may also be related to morphology since *in vitro* seedling leaves have a thicker cuticular layer than those from environmental plants. This characteristic tends to favor greater absorption of culture media components leading to the efficiency of somatic embryogenesis response [51, 52]. In addition, *Coffea* leaf explants from environmental plants can become curled which impairs nutrient absorption from the culture medium.

C. arabica explants remain green until about 60 days after inoculation in the culture medium and after this period they oxidize. This response is verified in explants submitted to direct or indirect route [45, 46]. In general, oxidized explants also have the ability to form calluses or embryos although they appear to be senescent.

In the indirect pathway, somatic embryos of *C. arabica* are formed after about 210 days of explant inoculation in the culture medium [45] while in the direct pathway they can be observed after about 90 days, but in small numbers [46]. On the other hand, there are also genotypes that do not form somatic embryos when submitted to these two embryogenesis pathways and are called recalcitrant [53].

Leaf explants of *C. arabica* cultivar Mundo Novo form somatic embryos in both pathways [45, 46]. But the same genotype may have different capacity for somatic embryogenesis in each of these pathways. Explants of cultivar Mundo Novo and decaffeinated genotypes AC1, AC2 and AC3 formed a greater number of somatic embryos via the indirect route than by the direct one [54].

3.1 Indirect somatic embryogenesis in *Coffea*

In *Coffea*, indirect somatic embryogenesis occurs in two phases, the first is callogenesis followed by embryogenesis that corresponds to the formation of somatic embryos [34, 45, 55–57] (**Figure 4**). Another characteristic of the indirect somatic embryogenesis in this species is the occurrence of somaclonal variation in cloned plants, due to the long time that callus remains *in vitro* [58]. Somaclonal variation is undesirable because it leads to the formation of mutants, which can compromise plant growth and development. On the other hand, its occurrence is desirable to obtain genotypes with genetic variability that can be incorporated into the coffee breeding program [59, 60]. For the induction of explant mutations are cultivated at high concentrations of 2,4 D.

Most studies use the Sondhal and Sharp protocol [34] for the application of indirect somatic embryogenesis in *C. arabica* genotypes and these usually respond with some somatic embryo production even when they have low regenerative capacity.

3.1.1 Callogenesis

For the induction of callogenesis in *Coffea* genotypes in general it is used the protocol with MS medium [61] and the addition of phytoregulators 2,4 D and kinetin and 30 g/L sucrose [34]. At this stage, auxin 2,4 D is used at high concentration [26, 62–65] which causes disturbance of endogenous auxin metabolism of explant tissue leading to cell division [8]. Auxin stress is required to obtain calogenesis in most species [12, 66]. Thus, in the induction of callogenesis, differentiated somatic cells of the explant tissue undergo re-determination, with the occurrence of cell division and proliferation events that form a non-functional cell mass, the callus. But it is also found that the induction of calogenesis in *Coffea* can be obtained in response to the use of other auxin types such as NAA [67, 68] and Picloram [69, 70].

Sucrose is used in high concentration to provide energy for the induction of calogenesis [34]. Biochemical analyzes in *C. arabica* explants indicated the occurrence

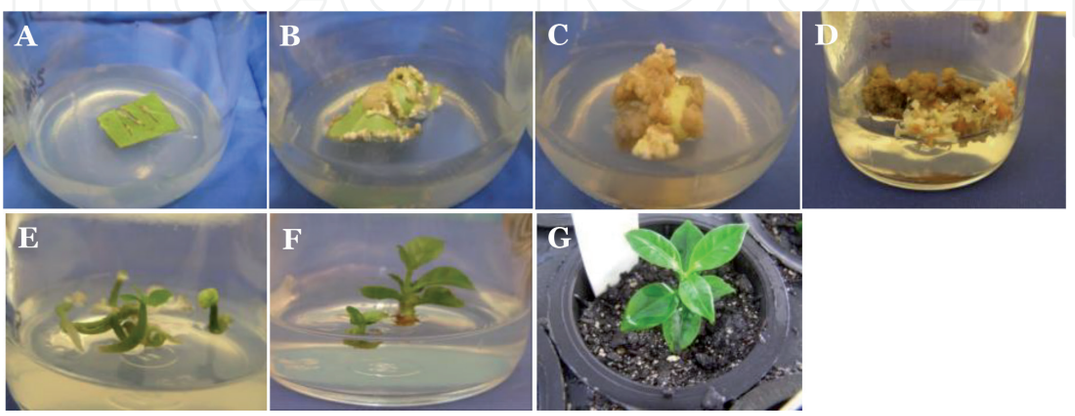

Figure 4.
Indirect somatic embryogenesis in the cultivar Mundo Novo of Coffea Arabica. *(A) Leaf explant with callus presence 15 days after the beginning of cultivation. (B) Calogenesis at 40 days of cultivation. (C) Calogenesis at 90 days of cultivation. (D) Callus with somatic embryos. (E) Embryonic axis. (F) Seedlings. (G) Plant transferred to* ex vitro *environment.*

of high concentration of soluble sugars in the calogenesis phase while it was lower in the formation and maturation of somatic embryos [71].

In the indirect pathway, *C. arabica* leaf explants initiate callus formation from procambium cells around the seventh day after the beginning of cultivation [72]. In general, from the 14th day of cultivation, it is possible to see the occurrence of small callus on the edges of explants that reach up to 3 mm in size [45]. These calluses are usually formed on only one or two sides of the explant and later reach sizes that can occupy the four edges of the explant. In early development, calluses are hyaline, but gradually they tend to oxidize. In a set of explants of the same origin subjected to indirect pathways, it is possible to find calluses that do not develop. They remain small, up to 5 mm in size. However, some of these calluses have the capacity to form somatic embryos, which indicates that they appear to have been "latent" during this time. Furthermore, it is further noted that not all *C. arabica* explants form calli indicating that the somatic embryogenic capacity may vary between explants from the same plant or from the same leaf.

Callus of *C. arabica* can reach sizes up to 30 mm around 90–120 days from the beginning of cultivation. During this period, these calluses may also become oxidized. Oxidation of explants and callus is associated with the high content of phenols present in tissues of this species. It is also observed that normally oxidized calli have the capacity to form somatic embryos although they have an appearance of senescence.

3.1.2 Embryogenesis, embryo induction

For the second phase of the indirect route, MS/2 medium is used, with NAA and kinetin added and 20 g/L sucrose, according to the protocol of Sondhal and Sharp [34]. For this purpose, calli are transferred from the calogenesis induction medium to the embryogenesis medium. In this phase, there is the initiation and development of somatic embryos from certain cells, located in some sectors of the callus, which correspond to embryogenic centers [45, 55, 57]. Somatic embryos are usually formed on the bottom of the callus, which is in contact with the culture medium and also on the top surface of the callus. On the other hand, not all callus form somatic embryos.

The induction of embryogenic cells occurs during a precise moment of callus life [19]. This induction window may vary for identical explants of the same genotype depending on the cultivation conditions used and particularly the hormonal balance [20, 73]. In the same explant, it is possible to find competent or non-competent callus sections, which indicates that genetically identical cells may respond differently to and from a particular stimulus. Embryogenic cells rapidly lose their ability to divide due to the occurrence of the differentiation event, so the moment of callus transfer to embryogenesis is crucial. As the callus gets older, embryogenic cells lose their specificity in forming embryos. Somatic embryo formation is a continuous process and several embryonic stages can occur at the same time, in the same explant, in the same culture [74].

3.2 Direct somatic embryogenesis in *Coffea*

The process of direct somatic embryogenesis in *C. arabica* occurs in a single phase, which is the main feature of this pathway [75] (**Figure 5**). In this case, explant tissue cells are already determined and competent [76] for embryogenic development, before being extracted from the explant donor leaf [77] and may differentiate into somatic embryos soon after the start of cultivation. In this pathway, embryo formation occurs from leaf explant mesophyll cells [27]. The phenomenon

of direct formation of embryogenic tissue in the explant is described as cloning of certain cells [78]. This cloning is a kind of large-scale reproduction of pro-embryos. Thus, pre-embryogenic cells present in explant tissues are cloned and require less epigenetic reprogramming compared to determined embryogenic cells in the indirect process. In the direct route, the need for cellular reprogramming is different from the indirect route.

To respond to the direct pathway, *Coffea* explant cells only need contact with the cytokine-like plant growth regulator and the auxin normally inhibits its occurrence [79]. Explant edge cells differentiate into somatic embryos in response to cytokine in the culture medium [62, 80–83]. The efficiency of the direct pathway also seems to be related to explants from young leaves. On the other hand, in direct somatic embryogenesis, the occurrence of somaclonal variation tends to be minor or absent, since somatic embryos are formed in a single phase.

In the direct pathway, after inoculation of the explants in the culture medium, the formation of embryogenic structures occurs, which can be visualized around the 15th day of culture [75] (**Figure 5A**). Embryogenic structures are formed,

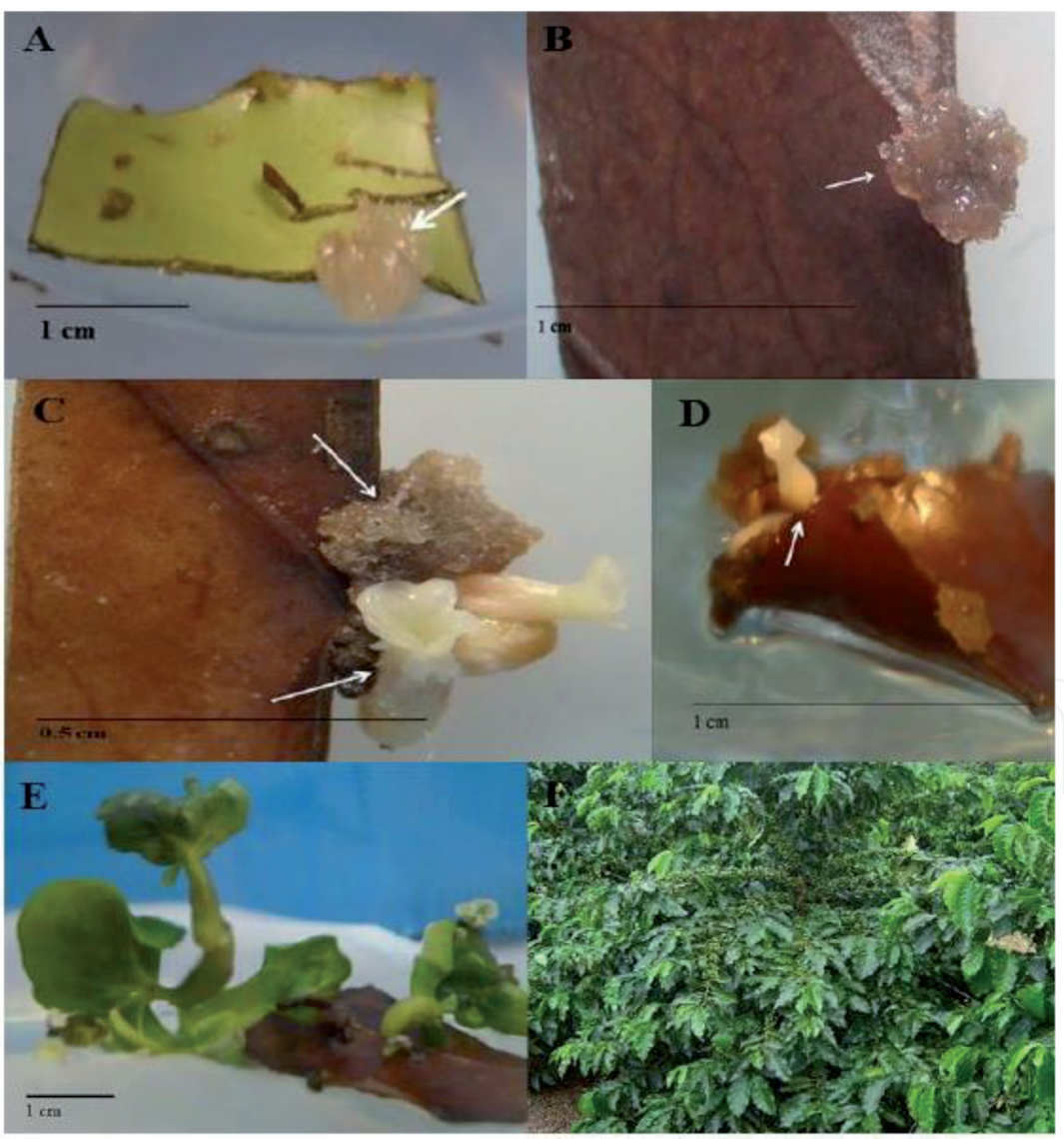

Figure 5.
Direct somatic embryogenesis on explants of C. arabica *Catuaí cultivar maintained in the dark, at 30°C, for 320 days from the beginning of the experiment. (A) Embryogenic structure (arrow), up to 15 days; (B) oxidized embryogenic structure (arrow), up to 70 days; (C) embryos (lower arrow) formed from the embryogenic structure (top arrow), up to 100 days; (D) embryos formed from the border of the explant (arrow), up to 100 days; (E) Plantlets at 320 days; (F) adult plants [75].*

on average, on one or two sides of the rectangular leaf explant. Normally, these structures range in size from 2 to 4 mm (**Figure 5B**) and remain this way until the end of cultivation. About 50 days after the start of cultivation the structures start to oxidize (**Figure 5C**) and by 150 they are completely oxidized [75]. The formation of somatic embryos usually starts from 90 days of cultivation but in low quantity and around 120 days this number tends to increase. Somatic embryos in addition to being formed at the edges of explants (**Figure 5D**) also develop over the surface of embryogenic structures (**Figure 5C**).

3.3 Somatic embryo of *Coffea*

The main product of somatic embryogenesis is the somatic embryo. Somatic embryos are structures that go through different stages of development until they reach that of a plant [18, 28]. These stages of development are perfectly organized, with all the morphological characteristics corresponding to the same stages of development of zygotic embryos [18, 84, 85] being globular, heart, and seedling. Somatic embryos are bipolar morphological structures, presenting radicle, hypocotyl and cotyledons. The somatic embryo does not exhibit endosperm differentiation and is independent of explant tissue after initiation and development. Somatic embryos do not go through the maturation or desiccation phase as in zygotic embryos. This system is not connected to the vascular tissue of the mother or explant during its initiation and development [86]. Moreover, in the direct pathway, it is possible to find somatic embryos at different developmental stages in the same explant [16]. This response pattern can also be found in the indirect callus.

Somatic embryos formed by the direct and indirect pathways are transferred to the germination stage and generally use MS/2 culture medium added 20 g/L sucrose and without plant growth regulators. This same medium can also be used for embryo growth and development to the seedling stage. These observations suggest that *Coffea* somatic embryos may have a hormonal balance that favors differentiation of developmental stages, requiring only nutrients from the culture medium without phytoregulators for germination and growth and development.

4. Factors that influence the occurrence of somatic embryogenesis

The control of the occurrence of somatic embryogenesis in *Coffea* is not yet completely identified. Some authors relate the genetic pattern of the species with the absence or low responsiveness [87]. Knowing the factors that control the occurrence of somatic embryogenesis in *C. arabica* will allow to optimize its application and especially the direct pathway. The high or low capacity of somatic embryogenesis of a species is related to the presence of competent cells or not in the explant, inherent to their totipotency [88]. The maintenance of somatic embryogenesis capacity requires the use of conditions that maintain the proliferation of determined and competent cells [86].

Somatic embryogenesis regeneration capacity is also associated with other factors such as explant donor plant developmental stage, explant donor plant physiological conditions, explant position relative to the plant [89], *in vitro* culture conditions and mainly of plant growth regulators. The seasons influenced the indirect somatic embryogenesis response of plant explants to eight *C. arabica* genotypes in the field [56]. Explants formed more somatic embryos in the fall-winter season than in the spring-summer season.

4.1 In vitro culture condition factors

4.1.1 Lighting

In indirect somatic embryogenesis of *C. arabica*, calli are induced and initiated in the absence or presence of light, but they reach a larger size only if maintained in the absence of light [45, 86]. The size of these callus increases gradually each month, and can reach sizes up to 30 mm.

On the direct pathway, *C. arabica* explants also have difficulty responding in the presence of light. In this way, at the edge of the explants, small structures are formed, which are called embryogenic structures, which remain without change in size and shape in the presence or absence of light.

4.1.2 Cultivation temperature

Cultivation temperature is another factor that may influence the somatic embryogenesis response. Leaf explants of cultivar Catuaí and two hybrids showed higher formation of somatic embryos at 30°C compared to 25°C [90].

4.2 Plant growth regulation

Several studies indicate that phytoregulators play a decisive role in controlling the formation of somatic embryos in *Coffea* leaf explants, which is the most explored aspect on this subject. For the induction of the indirect pathway in *C. arabica*, the auxin/cytokine combination, which is already well established for this species, is generally used. In this pathway, auxin 2,4 D has been the most used to induce callogenesis in *C. arabica* leaf explants. This auxin is considered strong and is also used for the induction of anthers [39, 40] and roots [47, 48].

For induction of the direct pathway, most studies use cytokine without auxin because it tends to inhibit its occurrence. However, the efficiency of the direct pathway response may vary depending on the type and concentration of cytokine employed. A pioneer study showed that 6-BA at 5 μM dose matched direct pathway induction in *C. arabica* explants [91].

The formation of somatic embryos was also obtained from *C. canephora* explants inoculated in MS medium only with addition of different cytokines being 2-iP, ZE, Ki and 6-BA, all at a concentration of 5 μM [92]. Explants formed somatic embryos in the presence of all cytokines, but responses varied according to cytokine type. The 2-iP was more efficient than the ZE, Kin and 6-BA. In this study, it was also found that auxins used at different concentrations inhibited the direct somatic embryogenesis of these genotypes. In another study, Zeatin caused the direct pathway response in *C. canephora* explants [87]. Cytokine 2-iP also caused direct pathway induction in *Coffea* [93, 94]. In another study, it was found that 2-iP concentrations of 7.5 and 10 μM favored a greater number of somatic embryos than the 2.5 and 5 μM doses [95].

Synthetic cytokine 6-benzylaminopurine also has the ability to induce the direct pathway in *C. arabica* explants [69, 91, 96–98]. The 6-BA used at 30 μM concentration caused higher production of somatic embryos than the 10 and 20 μM doses in leaf explants of the cultivar Mundo Novo de *C. arabica* [46]. But although the 6-BA concentration was high, embryo production was reduced and the process also took place over a long time. However, this result is interesting because it favors the cost reduction of clonal seedling formation since 6-BA is a cheaper and available synthetic cytokine than zeatin and 2-iP. Cytokine TDZ

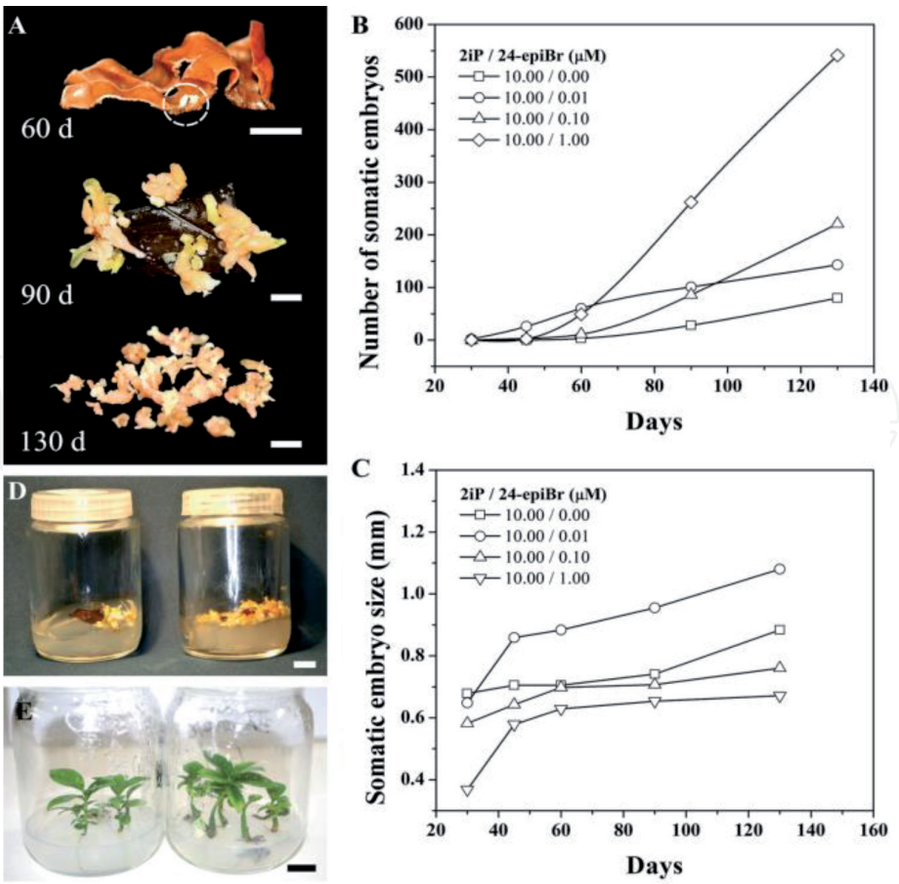

Figure 6.
Direct somatic embryogenesis from leaf explants of C. arabica. *(A) Macroscopic view of coffee leaf explants after 60, 90 and 130 days of culture on induction medium supplemented with 10 μM 2-iP + 1.0 μM 24-epiBR. (B) Number and (C) size of somatic embryos obtained by treatment during the somatic embryogenesis process. (D) Somatic embryo germination. (E) Elongating regenerated plantlets after 90 days on ½MS medium. Bars = 0.5 and 2 cm [99].*

has also been used to induce regeneration of *C. arabica* somatic embryos via the direct pathway [47, 48]. Leaf explants of cultivar IAPAR 59 and *C. arabica* hybrid Sachimor showed direct somatic embryogenesis response in the presence of TDZ at concentrations of 2.27; 4.54; 6.81; 9.08; 13.62 μM, but with low embryo production [96].

The literature shows that it is well established that the *Coffea* direct pathway only occurs in the presence of cytokine, but it is also possible to find studies in which explants of this species formed somatic embryos in the presence of auxin. Explants of cultivar Acaia Cerrado formed somatic embryos when grown in a single culture medium with addition of kinetin, GA_3 and NAA [67]. In another study, it was found that leaf explants of cultivar Mundo Novo submitted to direct pathway showed high production of somatic embryos in response to 2-iP treatment associated with brassinosteroid compared to 2-iP alone control [99]. On the other hand, explants treated with brassinosteroid alone without cytokine formed only embryogenic structures without any occurrence of somatic embryos (**Figure 6**).

4.3 Stress factor

The stress factors have been related to promoting the acquisition of embryogenic competence in different species [20, 66]. Stressful conditions can also influence the acquisition of embryogenic competence in different species [19, 20, 30, 66, 100, 101].

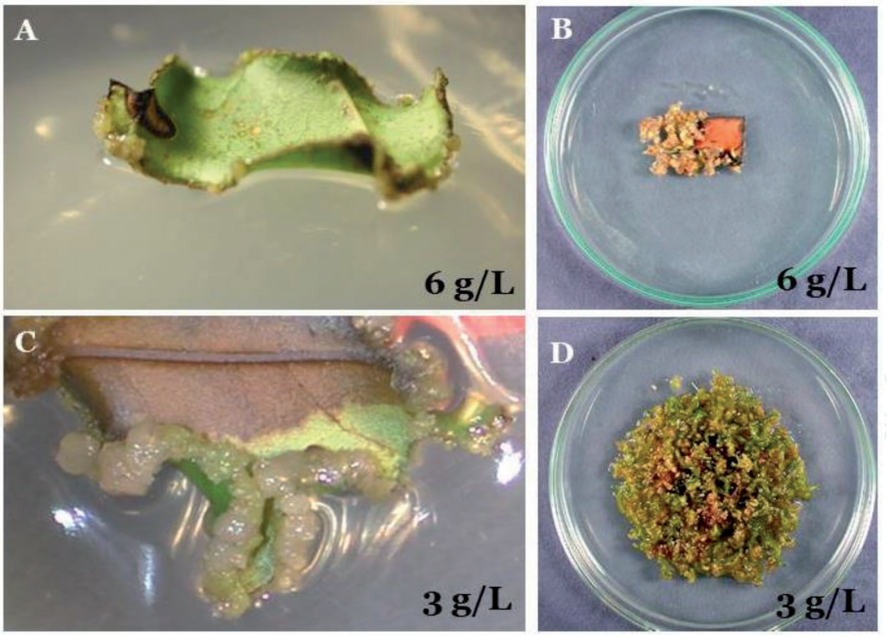

Figure 7.
Effect of 3 and 6 g/L agar on the direct somatic embryogenesis in explants of C. canephora *cultivar "Robusta 2264 Mar" maintained at 25°C in the dark [106]. (A) 6 g/L of agar: explants with curvature without contact with the medium. (B) Explant with few somatic embryos in the presence of 6 g/L agar. (C) 3 g/L of agar: the medium is in contact with the border of the explant. (D) Explant with many somatic embryos in the presence of 3 g/L agar.*

Studies indicate that the occurrence of somatic embryogenesis is strongly related to the exposure of explants to some high intensity stress factor and or the high concentration of plant growth regulator [22].

Osmotic stress treatments alter the explant's environment. This change in tissue/organ growth conditions may represent the estrum that enables cells to undergo changes in developmental processes and make them competent for inductive signals for somatic embryogenesis. Thus, the stress-induction system is composed of two phases: the acquisition of embryogenic competence and the formation of the somatic embryo [102].

Of the responses found in different species, it was shown that the stress-induction system could cause a greater formation of somatic embryos [101–107] although its forms of control and action are unknown and this aspect has been little studied in the culture of coffee plants. It was verified that *C. canephora* explants submitted to direct somatic embryogenesis formed a greater number of embryos in a medium to which 3 g of agar had been added, than in one containing 6 g [106] (**Figure 7**). This result was indirect evidence that altering the osmotic potential of the culture tends to favor the ability of somatic embryogenesis. In another study, it was found that the alteration of the osmotic concentration of the culture medium influenced the embryogenesis response [108]. The use of 7% PEG 6000 caused a greater formation of somatic embryos in foliar explants of the *C. arabica* genotypes AC1 and cultivar Mundo Novo than the use of 5% PEG 6000. This reagent has a high molecular weight and is inert, non-ionic, non-toxic, water soluble [109], not absorbed by vegetable cells and alters the osmotic potential when added to a culture medium.

5. Conclusions

Somatic embryogenesis contributes to coffee crop both in relation to breeding programs and its production chain. Little is known about the factors controlling

somatic embryogenesis in *Coffea* genotypes. But it is known that plant hormones act in controlling the occurrence of this process. In addition, studies have shown that environmental and mainly stress factors applied during the cultivation condition are involved in the control of somatic embryogenesis in *Coffea*.

Nomenclature

MS	Murashige and Skoog
2,4 D	2,4-dichlorophenoxyacetic acid
2-iP	N6-(2-isopentenyl)adenine
NAA	naphthylacetic acid
6-BA	6-benzyladenine
Ki	kinetin
ZE	zeatin
TDZ	thidiazuron
PEG 6000	polyethyleneglycol 6000

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