

## CAPÍTULO 1

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### Secondary somatic embryogenesis and its potential biotechnological applications

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#### Abstract

Secondary somatic embryogenesis is a powerful biotechnological tool in the genetic improvement of plants. However, up to date, it has only been described in 148 species (7 Gymnosperms (G), 141 Angiosperms between 35 monocots (M) and 106 dicots (D)) in different explants, in particular zygotic embryos (G: 100%, M: 54%, D: 48%), mainly indirectly (G: 100%, M: 67%, D: 66%), and asynchronous (G: 100%, M: 100%, D: 96%), of unicellular origin (G: 63%, M: 63%, D: 67%); directly differentiating the secondary embryos in particular from the apical zone of higher stage primary embryos, plants regenerating in more than 90% of the species. The combination of growth regulators (single auxin, only cytokinin, or combination auxin + cytokinin) for the primary and repetitive embryogenesis stage varies with the species and explant type. However, in the second stage, the media is used without any hormones, with low auxin concentration, or increasing the cytokinin. The potential and avant-garde system used is poorly described; this review supports future research in this regard, given insights into the genetic improvements of many species with agro-ecological interest.

**Keywords:** *Agrobacterium*, biolistic, cyclic embryogenesis, repetitive embryogenesis, temporary immersion, unicellular origin.

#### 1. Introduction

The system with the higher regenerative rate *in vitro* is somatic embryogenesis, and it is defined as the process in which develops a bipolar structure called embryos from a single somatic cell (without any cell fusion), being structurally and functionally identical to the derived zygotic embryo from the union of gametic cells in sexual embryogenesis. The first report was described using

the dicotyledonous *Daucus carota* in the late 50s of the 20th century. More than 100 species have been reported (NEUMANN; KUMAR; IMANI, 2020). Frequently, it occurs in a larger proportion by the indirect way (presence of a transition phase called callus); it is given that exogenous hormonal stimuli must induce the competent cells. Consequently, these generate a morphogenic reprogramming for the plant's differentiation from the developed somatic embryos, and it can originate from one or more somatic cells, with subsequent implications in the genetic homogeneity of the developed plants (FEHÉR; BERNULA; GÉMES, 2016; NEUMANN; KUMAR; IMANI, 2020).

A relevant aspect in the micropropagation efficiency is the synchrony of the process, which in most species is asynchronous, as somatic embryos with the same stage are not seen at the same time (WÓJCIKOWSKA; GAJ, 2016). In some species, this regenerative process has a high cyclic capacity. It is determined by establishing two types of somatic embryogenesis, where the first one called "primary" is distinguished in the formation of somatic embryos directly from the explant or indirectly through the callus. At the same time, the "secondary" is characterized in the differentiation of new somatic embryos, generally directly on the surface of the primary embryos, and this process can be repeated in more than one cycle (PÉREZ; CHAN; SÁENZ, 2006). Thus, repetitive somatic embryogenesis reports began at the '80s ending and the beginning of the '90s of the last century. In the "solo" bibliographic revision of this topic, up to date was indicating 82 species following 10 Gymnosperms; 72 Angiosperms divided into eight monocotyledons and 64 dicotyledons (RAEMAKERS; JACOBSEN; VISSER, 1995).

The regenerative system through somatic embryogenesis is an efficient biotechnological tool that can be applied in the genetic improvement of different species with agro-ecological interest since it can be coupled in the processes of micropropagation and genetic engineering (LOYOLA, 2016). However, up to date, the use of the system is limited to a few species, particularly forestal ones. In this regard, the objective of this review is to detail update all the important and inherent aspects of the regenerative process and its potential applications. Furthermore, after 25 years of the first review, to support future research not only in the described species but also in others, whose resources for a human could be improved and increased by this way.

## 2. Conceptualization of secondary somatic embryogenesis

The somatic embryogenesis (also known as asexual or adventitious embryogenesis) consists of the development of bipolar structures (with two vegetative apices, the apical of the stem and the subapical of the root) called "embryos", from somatic cells that form the tissues. So, these cells are not the product of sexual cells fusion of gametes during fertilization, similar in sexual or zygotic embryogenesis, where the zygotic embryo is differentiated, being structurally and functionally identical in both types of embryos (LOYOLA, 2016). Comparing organogenesis and somatic embryogenesis as regenerative systems *in vitro*, the last one had the highest replicative rate in most plant species; therefore, it is frequently used in mass micropropagation and conventional or genetic engineering breeding programs (LOYOLA, 2016).

Regarding the pathway can be two types, direct and indirect. The direct pathway is characterized by the direct differentiation of somatic embryos from the explant. While the indirect is the second where the somatic embryos develop from callus (a dedifferentiated cell mass), considered a transitional phase of the regenerative process, being able to imply the appearance of genetic variations in the regenerated plants to the high rates of cell division in the callus. Thus, this can lead to mutations or permanent genetic aberrations without molecular repair mechanisms. The genetic uniformity of the variety of elite cultivars in propagation is not ensured; however, this does not occur in most cases (FEHÉR; BERNULA; GÉMES, 2016).

Furthermore, another important aspect from the point of view of plant breeding is the origin of somatic embryos, which can be unicellular or multicellular, in epidermic or sub epidermic layers of the explant or callus of embryogenic nature (QUIROZ *et al.*, 2006). In the first case, the embryos are formed from a single cell, which divides anticlinally, distinguishing perfectly at the end the embryo isolated from the maternal tissue, united only by the suspensor (WILLIAMS; MAHESWARAN, 1986). Although, in the second case, the embryos are observed without suspensor and fused to the tissue that gave them origin because these were initially the product of the anticlinal cell division of more than one cell, being visualized sometimes bodies of fused embryos (HACCIUS; BHANDARI, 1975). Thus, the probability of genetic mosaicism or chimeras is

higher in the multicellular origin, not assuring the genetic homogeneity of the regenerated plants, an aspect that is feasible with the unicellular origin.

Concerning the synchronization during the differentiation of the somatic embryo, it can be synchronous or asynchronous. The synchronous is characterized for having only one type of stage observed simultaneously due to the cells that gave rise to them and divided at the same time. In contrast, the asynchronous refers to the differentiation of somatic embryos at different times, being observed simultaneously in different proportions of embryos according to the stage (WÓJCIKOWSKA; GAJ, 2016).

Regarding the cyclic capacity of somatic embryogenesis, two types can be defined as primary. Firstly, where only a series of somatic embryos are differentiated, and the second as secondary, repetitive, or cyclic, where somatic embryos are differentiated in more than one cycle, being denominated as primary to the somatic embryos that developed first from the explant or callus, and secondary to those that generally differentiate directly from the surface of the primary embryos, being able to repeat cyclically in the newest embryos (PÉREZ; CHAN; SÁENZ, 2006).

### **3. Cyclic somatic embryogenesis in Gymnosperms**

Only seven forestal species of gymnosperms of the family Pinaceae are reported in this work. Although Raemakers *et al.* (1995) indicate ten species, after complete revision of different reports, it was determined in a conclusive and expressly way that currently, only this number of species described secondary somatic embryogenesis. Moreover, in this group of plants, only zygotic embryos were used as explant, with an indirect and asynchrony regenerative pathway at 100%, originating somatic embryos in a unicellular way in 63% of the species. In comparison, in two (25%), it was multicellular, and only in one (12%), both origins were reported (Table 1).

In the primary somatic embryo stage, where the cyclic embryogenesis occurs, 86% is torpedo and 14% in the cotyledon. While the zone of differentiation of secondary embryos was in greater degree (57%) in the hypocotyl, followed by the cotyledon (29%) and the meristem (14%), the regenerating plants (81-98%) only in 5 of the 7 species (Table 1).

**Table 1.** Gymnosperms with Cyclic embryogenesis somatic

| SPECIES   | EXPLANT |   | REGENERATIVE VIA | ORIGIN OF THE SOMATIC EMBRYOS       |          |     |     |     |           |     |   |       |                      | DIFFERENTIATION ZONE AND TYPE FROM PSE TO SSE |   | SECONDARY SOMATIC EMBRYOGENESIS (SSE) |     |     |   |           |   |    |  | PERCENTAGE OF PLANT DIFFERENTIATION FROM SSE (%) | REFERENCES |
|---|---------|---|------------------|-------------------------------------|----------|-----|-----|-----|-----------|-----|---|-------|----------------------|---|---|---------------------------------------|-----|-----|---|-----------|---|----|--|--|------------|
|   |         |   |                  | PRIMARY SOMATIC EMBRYOGENESIS (PSE) |          |     |     |     | CYTOKININ |     |   |       |                      |   |   | AUXIN                                 |     |     |   | CYTOKININ |   |    |  |  |            |
|   |         |   |                  | (mg/l)                              |          |     |     |     | (mg/l)    |     |   |       |                      |   |   | (mg/l)                                |     |     |   | (mg/l)    |   |    |  |  |            |
|   |         |   |                  | 2,4-D                               | Picloram | NAA | IAA | BAP | K         | TDZ | Z | 2,4-D | Picloram             |   |   | NAA                                   | IAA | BAP | K | TDZ       | Z |    |  |  |            |
| <i>Abies numidica</i> De Lannoy & Carrieré(P <sup>1</sup> )         | ZE      | I | M                | -                                   | -        | -   | -   | 1   | -         | -   | - | -     | A/<br>C <sup>b</sup> | -   | - | -                                     | -   | -   | - | 1         | - | 90 | Vooková <i>et al.</i> (2003); Vooková y Kortumák (2006)  |  |            |
| <i>Larix decidua</i> x <i>leptoeuropaea</i> Mill. (P <sup>1</sup> ) | ZE      | I | M                | 2                                   | -        | -   | -   | 0.5 | -         | -   | - | -     | A/<br>T <sup>b</sup> | 2   | - | -                                     | -   | 1   | - | -         | - | 98 | Saly <i>et al.</i> (2002)  |  |            |
| <i>Picea abies</i> (L.) H.Karst.(P <sup>1</sup> )                   | ZE      | I | U<br>M           | 2.2                                 | -        | -   | -   | 1.1 | -         | -   | - | -     | A/<br>T <sup>b</sup> | -   | - | -                                     | -   | 1.8 | - | -         | - | 81 | Nagmani <i>et al.</i> (1987); Von Arnold (1987); Mo <i>et al.</i> (1989); Mo & Von Arnold (1991) |  |            |
| <i>P. glauca</i> (Moench) Voss (P <sup>1</sup> )                    | ZE      | I | U                | 2.2                                 | -        | -   | -   | 1.1 | -         | -   | - | -     | A/<br>T <sup>m</sup> | -   | - | -                                     | -   | 0.5 | - | -         | - | 0  | Nagmani <i>et al.</i> (1987); Kong & Yeung (1992)  |  |            |
| <i>Picea glauca</i> x <i>engelmanni</i> (P <sup>1</sup> )           | ZE      | I | U                | 2.2                                 | -        | -   | -   | 1.1 | -         | -   | - | -     | A/<br>T <sup>b</sup> | 1.1   | - | -                                     | -   | 0.5 | - | -         | - | 0  | Nagmani <i>et al.</i> (1987); Eatsman <i>et al.</i> (1991)                                       |  |            |
| <i>Pinus pinaster</i> Ait. (P <sup>1</sup> )                        | ZE      | I | U                | 0,5                                 | -        | -   | -   | 0,5 | -         | -   | - | -     | A/<br>T <sup>c</sup> | 2   | - | -                                     | -   | 1   | - | -         | - | 90 | Klimaszewska <i>et al.</i> (2009)  |  |            |
| <i>Pseudotsuga menziesii</i> (Mill.) (P <sup>1</sup> )              | ZE      | I | U                | 0,5                                 | -        | -   | -   | 0,5 | -         | -   | - | -     | A/<br>T <sup>c</sup> | 2   | - | -                                     | -   | 1   | - | -         | - | 98 | Lelu <i>et al.</i> (2018)  |  |            |

**FAMILY.** (P): Pinaceae. **HABIT.** (t): tree. **EXPLANT.** ZE: zygotic embryo. **REGENERATIVE VIA.** D: direct; I: Indirect. **ORIGIN OF THE SOMATIC EMBRYOS.** (U): unicellular; (M): multicellular. **SOMATIC EMBRYO.** PSE: primary; ESS: secondary. **DIFFERENTIATION TYPE PSE TO SSE.** G: globular; T: torpedo; C: cotyledonal. **SYNCHRONIC OF THE SSE.** (SIN). S: Synchronic; A: asynchrony. **DIFFERENTIATION ZONE PSE TO SSE.** (t): root; (b): hypocotyl; (e): epicotyl; (m): meristem. **AUXINS.** 2,4-D: 2,4-dichlorophenoxyacetic; **PICLORAM:** 4-amino-3,5,6-trichloropicolinic acid; **NAA:** 1-naphthaleneacetic acid; **IAA:** indole-acetic acid. **CYTOKININS:** BAP: 6-benzyl-amino purine; K: 6-furfurylamino purine or Kinetin; **TDZ:** thidiazuron; Z: zeatin.

Accordingly, the relationship of growth regulators in primary and secondary somatic embryogenesis (Table 1), it was found that only in *Abies numidica* was used cytokinins, in the primary (BAP) and the secondary (TDZ),

while in the rest of the species (86%) used in the primary, auxin plus cytokinin (2,4-D+ BAP), but in the secondary in two species (29%; *Picea abies* and *Picea glauca*) was induced only with cytokinin (BAP). On the contrary, the remaining five species (71%) were induced by combining auxin and cytokinin (2,4-D+BAP). Secondary embryo maturation is another important aspect prior to the regeneration, which is achieved mostly with the growth substances used in the cyclic embryogenesis phase. However, it is used at that stage 10 mg/L of ABA, 3% maltose, and 10% polyethylene glycol for *Abies numidica* (VOOKOVÁ; MATUSOVÁ; KORMUTÁK, 2003). In this sense, in *Pseudotsuga menziesii*, ABA is used as the only hormonal inducer that increases the secondary embryogenic frequency by 62% using 10.5 mg/l of it (WALTHER; WAGNER; RASCHKE, 2022).

#### **4. Cyclic somatic embryogenesis in Angiosperms: monocotyledons group**

In the bibliographic review carried out by Raemakers *et al.* (1995), eight species of monocotyledons with herbaceous habit were indicated, 25 years later in this work, 35 species with repetitive somatic embryogenesis were reported, 86% herbaceous and 14% tree (palms), being 37% grasses, 14% palms (tree-like), 14% muses, 9% orchids, and the remaining 9 (26%) species of different families (Table 2).

The most used explant was the zygotic embryo (48%), followed by the inflorescence (15%) and then leaf (13%), with the indirect regenerative way in 68% of the species. The only way found in anther, inflorescence, ovary, corm, stem, and cotyledon, while the direct way was found exclusively in the meristem, unlike both ways was described in zygotic embryos and leaves. Likewise, the differentiation of somatic embryos was asynchronous, originating unicellular in 58% of the species, multicellular in 8 (20%) and four (10%) with both origins (Table 2).

On the other hand, the type of primary somatic embryo where cyclic embryogenesis occurs, globular is 94% and 3% for cotyledonary and 3% torpedo, differentiating 100% of the secondary embryos in the apex zone, regenerating plants in all species, in more than 70% in 24 of them (Table 2).

**Table 2.** Cyclic Somatic Embryogenesis in Angiosperms species (monocotyledons)

Table 2. Cyclic Somatic Embryogenesis in Angiosperms species (monocotyledons)

| SPECIES  | EXPLANT |   |   | ORIGIN OF THE SOMATIC EMBRYOS |     |     |   |      |     |   |     | DIFFERENTIATION ZONE AND TYPE FROM PSE TO SSE | SECONDARY SOMATIC EMBRYOGENESIS (SSE) |          |     |     |           |     |     |       | PERCENTAGE OF PLANT DIFFERENTIATION FROM SSE (%)                                     | REFERENCES   |        |          |     |     |           |   |     |                    |
|--|---------|---|---|-------------------------------|-----|-----|---|------|-----|---|-----|---|---------------------------------------|----------|-----|-----|-----------|-----|-----|-------|--|--|--------|----------|-----|-----|-----------|---|-----|--------------------|
|  |         |   |   |                               |     |     |   |      |     |   |     |   | AUXIN                                 |          |     |     | CYTOKININ |     |     |       |  |  | AUXIN  |          |     |     | CYTOKININ |   |     |                    |
|  |         |   |   |                               |     |     |   |      |     |   |     |   | (mg/l)                                |          |     |     | (mg/l)    |     |     |       |  |  | (mg/l) |          |     |     | (mg/l)    |   |     |                    |
|  |         |   |   |                               |     |     |   |      |     |   |     |   | 2,4-D                                 | PICLORAM | NAA | IAA | BAP       | K   | TDZ | 2-IP  |  |  | 2,4-D  | PICLORAM | NAA | IAA | BAP       | K | TDZ | Z/2IP <sup>2</sup> |
| <i>Allium cepa</i> L. (A <sup>b</sup> )  | ZE      | I | M | 2                             | -   | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | 2                                     | -        | -   | -   | 0.5       | -   | -   | 70    | Saker (1997); Fereol <i>et al.</i> (2002)  |  |        |          |     |     |           |   |     |                    |
| <i>Avena sativa</i> L. (P <sup>b</sup> )   | ZE      | D | U | 1.8                           | -   | -   | - | 0.5  | -   | - | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | 25        | -   | -   | 20    | Eudes <i>et al.</i> (2003); Borji <i>et al.</i> (2018)                               |  |        |          |     |     |           |   |     |                    |
| <i>Asparagus officinalis</i> L. (As <sup>b</sup> )                                     | A       | I | U | 1                             | -   | 0.1 | - | -    | 0.2 | - | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | 0.1 | -   | 0.5       | -   | -   | 100   | Feng & Wolyn (1993); Li & Wolyn (1996)   |  |        |          |     |     |           |   |     |                    |
| <i>Alstroemeria</i> L. (Al <sup>b</sup> )  | ZE      | I | U | 4                             | -   | -   | - | 1    | -   | - | -   | A/<br>G <sup>a</sup>                          | 1                                     | -        | -   | -   | 0.5       | -   | -   | 85    | Schaik <i>et al.</i> (1998)  |  |        |          |     |     |           |   |     |                    |
| <i>Baloskion tetraphyllum</i> (Labill.) B.G. Briggs & L.A.S. Johnson (R <sup>b</sup> ) | C       | I | M | 0.2                           | -   | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | 0.2                                   | -        | -   | -   | -         | -   | -   | 100   | Panaia <i>et al.</i> (2004; 2011);   |  |        |          |     |     |           |   |     |                    |
| <i>Bactris gasipaes</i> Kunth (Ar <sup>b</sup> )                                       | ZE      | I | M | -                             | 2.4 | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | -         | -   | -   | 88    | Steinmacher <i>et al.</i> (2007); Steinmacher <i>et al.</i> (2011)                   |  |        |          |     |     |           |   |     |                    |
| <i>Cocos nucifera</i> (L.) (Ar <sup>b</sup> )  | ZE      | I | M | 13                            | -   | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | 1.3                                   | -        | -   | -   | 8.8       | -   | -   | 82    | Pérez-Núñez <i>et al.</i> (2006)   |  |        |          |     |     |           |   |     |                    |
| <i>Cynodon dactylon</i> x <i>Cynodon transvaalensis</i> (P <sup>b</sup> )              | I       | I |   | 1                             | -   | -   | - | 0.01 | -   | - | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | 1         | -   | -   | 100   | Li & Qu (2002b)  |  |        |          |     |     |           |   |     |                    |
| <i>Dactylis glomerata</i> L. (P <sup>b</sup> )   | L       | D | U | -                             | 8.7 | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | -                                     | 8.7      | -   | -   | -         | -   | -   | 100   | Conger <i>et al.</i> (1983); Sonstad y Conger (1986); Trigiano <i>et al.</i> (1989); |  |        |          |     |     |           |   |     |                    |
| <i>Dendrobium</i> sp. Sw. (O <sup>b</sup> )  | L       | D | M | -                             | -   | -   | - | -    | -   | 4 | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | -         | 0.2 | -   | 25    | Chung <i>et al.</i> (2005; 2007)   |  |        |          |     |     |           |   |     |                    |
| <i>Elaeis quineensis</i> Jacq. (Ar <sup>b</sup> )                                      | ZE      |   |   | -                             | 25  | -   | - | -    | -   | - | 3   | A/<br>G <sup>a</sup>                          | 0.01                                  | -        | -   | -   | -         | -   | -   |       | Rajesh <i>et al.</i> (2003)  |  |        |          |     |     |           |   |     |                    |
|  | L       | I | U |                               | 1   |     |   |      |     |   |     |   |                                       |          |     |     |           |     |     | 59-75 | Te-chato & Hilae (2007); Inpuay & Te-chato (2012)                                    |  |        |          |     |     |           |   |     |                    |
| <i>Euterpe precatoria</i> (Ar <sup>b</sup> )   | ZE      | I | U | -                             | 4   | -   | - | -    | -   | - | 0.8 | A/<br>T <sup>a</sup>                          | -                                     | -        | 0.1 | -   | -         | -   | -   | 46    | 93   | Barbosa <i>et al.</i> , 2022                                 |        |          |     |     |           |   |     |                    |
| <i>Gladiolus x grandiflorus</i> (P <sup>b</sup> )                                      | CO      | I | U | 2                             | -   | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | 1.1       | -   | -   | 0.1   | 100  | Remotti (1995)   |        |          |     |     |           |   |     |                    |
| <i>Hordeum vulgare</i> L. (P <sup>b</sup> )  | ZE      | D | U | 1.8                           |     |     |   | 0.5  |     |   |     | A/<br>G <sup>a</sup>                          | -                                     | -        | -   | -   | 25        |     |     |       | 100  | Eudes <i>et al.</i> (2003)                                   |        |          |     |     |           |   |     |                    |
|  |         | I | M |                               | 4   |     |   |      |     |   |     |   |                                       |          |     |     |           |     |     |       |  | Ryschka <i>et al.</i> (1991); Kachhwaha <i>et al.</i> (1997) |        |          |     |     |           |   |     |                    |
| <i>Lepidosperma drummondii</i> Benth. (C <sup>b</sup> )                                | ZE      | I | M | 0.2                           | -   | -   | - | -    | -   | - | -   | A/<br>G <sup>a</sup>                          | 0.2                                   | -        | -   | -   | -         | -   | -   | 100   | Panaia <i>et al.</i> (2011)  |  |        |          |     |     |           |   |     |                    |





promoters in 5% of the cases, being used mostly only auxin (11% with Picloram; 32% with 2,4-D) or auxin plus cytokinin (47% with 2,4-D+ BAP; 11% with Picloram + 2-IP; 5% with ANA+ TDZ) similar trend in anther (50% with 2,4-D+ ANA; 50% with 2,4-D+ ANA+K), inflorescence (17% with 2,4-D; 17% with 2,4-D+ANA+AIA; 50% with Picloram+ANA+AIA; 17% with 2,4-D+ BAP) and ovule (50% with Picloram 50% with 2,4-D+ BAP), using only auxin (2,4-D) in corm, stem and cotyledon (100%), auxin plus cytokinin (2,4-D+ BAP) in meristem (100%) and in leaf segments with auxin (33% with 2,4-D; 67% with Picloram) or cytokinin (100%: TDZ). On the other hand, in the secondary embryogenesis induction medium, the absence of growth regulators was observed from 21% in zygotic embryos, 17% in inflorescences, while the cases with only cytokinin were 36% in zygotic embryos (BAP), 20% in leaves (TDZ), 33% in inflorescences (BAP), 50% in meristem (BAP) and 100% in corm (BAP) and stem (BAP), additionally the use of only auxin was reported in coleoptile (100% with 2,4-D), and leaf (40% with 2,4-D; 20% with Picloram), decreasing the use of only auxin and auxin plus cytokinin in zygotic embryos (5% with 2,4-D+ K; 11% with ANA+TDZ; 16% with 2,4-D+ BAP) and ovules (50% with Picloram; 50% with 2,4-D+ ANA+ BAP).

Furthermore, in the secondary somatic embryogenesis phase of *Asparagus officinalis*, other growth regulators, such as ABA and acymidol, increase the frequency of repetitive embryos. In this order, ABA (0.5 mg/L) increases from 77 to 95%, while acymidol (0.2 mg/L) increases from 99 to 101%, being better this last substance for this process (LI; WOLYN, 1996). Similarly, the concentration and type of carbon source affect the degree of differentiation of secondary embryos, where 6% sucrose increases up to 61% and 0.2 M Mannitol 78% in *Elaeis quineensis* (TE-CHATO; HILAE, 2007).

The maturation of secondary embryos, before their regeneration, is achieved in the medium of differentiation of the same. However, in some cases, other growth substances are additionally used, such as 0.5 mg/L GA3 in *Paris polyphylla* (RAOMANI; KUMARIA; TANDON, 2014), NAA 0.1 mg/L and 0.75 mg/L ancimidol in *Asparagus officinalis* (LI; WOLYN, 1996) or without growth regulators in MS medium in *E. quineensis* (TE-CHATO; HILAE, 2007).

## 5. Cyclic somatic embryogenesis in angiosperms: dicotyledonous group

The only preceding bibliographic compilation of 64 dicotyledonous species with secondary somatic embryogenesis was done by Raemakers *et al.* (1995), while this work reports 106 species, 39 herbaceous, and 67 trees, distributed into 44 families (Table 3).

Independently of the species habits, 16 different explants were used. The intact zygotic embryo represents 42%, followed by leaves with 19%, cotyledon with 9%, anther with 6%, inflorescences with 5%, nucellus with 2%, a zygotic embryo without meristem with 2%, and the remaining ones (corm, petiole, petale, pistils, meristem, microspore, stem and root) with 1% each; these explants regenerated exclusively by the direct way with meristems and microspores, and by the indirect way only with segments of corm, inflorescences, petiole and stem, and by both ways in anthers (D: 33%; I: 67%), cotyledon (D:40%; I:50%; both: 10%), intact zygotic embryos (D: 39%, I:54%; both: 7%), zygotic embryos without meristems (D: 50%; I: 50%), root (D: 67%; I: 33%) and nucellus (D: 33.3%; I: 33.3%; both: 33.3%). Correspondingly, the differentiation of somatic embryos was asynchronous in 96% of the species, originating unicellular in 62% of the species, multicellular in 26 (25%) and 14 (13%) with both origins.

The differentiation of secondary somatic embryos was presented in primary somatic embryos torpedo type in 88% of the species, followed by the cotyledonary (10%) and globular (2%), mostly (80%) in the hypocotyl zone and to a lesser extent in the root (18%), apex (2%) and epicotyl (1%), regenerating plants in 96 out of the 106 species (Table 3).

Regarding the combination of plant growth regulators in the primary and secondary somatic embryo induction media (Table 3), it was found only with intact zygotic embryos, the use of media lacking growth substances for primary embryogenesis in 13% of herbaceous species and 4% of forestal species, using mostly only auxin (29% with 2,4-D; 11% with ANA; 3% with IBA), cytokinin alone (11%: BAP), or auxin plus cytokinin (29% with 2,4-D+BAP or K; 18% with ANA+BAP, 8% AIB+BAP, 5% AIB+ K, 3% AIA+BAP, 3% AIB+2-IP, 3% AIB+BAP+K, 3% 2,4-D +2-IP; 3% with AIA+DPU), as well as in stem sections (11% with 2,4-D; 11% with BAP, K, 2-IP or Z; 11% with 2,4-D +BAP; 22% with ANA+BAP; 11% with ANA+BAP+K), using only auxin (100%: 2,4-D) or cytokinin (100%: K) in zygotic embryos without meristem, also using only cytokinin or auxin

plus cytokinin in corm (50%: BAP+Z; 50%: 2,4-D+Z), cotyledon (9% with TDZ; 9% with 2,4-D; 45% with 2,4-D+BAP; 9% with AIB+BAP; 19% with AIB+K; 9% with AIB+BAP+K) and meristem (50% with BAP; 50%: 2,4-D+BAP), while using auxin plus cytokinin in microspore, petale (100%: 2,4-D+K), petiole, pistiles (100%: 2,4-D+BAP) and finally with media with only auxin or auxin plus cytokinin in anther (11,1% with ANA; 55.6% with 2,4-D+BAP ; 22.2% with ANA+BAP; 11,1% with 2,4-D+BAP+K), inflorescence (28.6% with 2,4-D; 28.6% with 2,4-D+BAP; 14.3% with 2,4-D+K; 14.3% with 2,4-D+DPU; 14.3% with AIB+BAP+K), nucellus (33% with 2,4-D; 67% with 2,4-D+BAP), leaf (15.4% with 2,4-D; 3.8% with IBA or Picloram, 30.8% with 2,4-D+BAP, 23.2% with 2,4-D+K, 3.8% with 2,4-D+TDZ or DPU, 3.8% with 2,4-D+NAA+K, 15.4% with ANA+BAP, 3.8% with AIB+BAP) and root (33.3%: AIB; ANA+BAP; AIB+K).

The induction in the cyclic embryogenesis, it is detailed that increasing in the use of medium devoid of growth promoters in 23% of herbaceous species and from 19% in the tree or forestal species, using intact zygotic embryos, as well as when using zygotic embryos without meristem (100%), petal (100%), cotyledon (25%), leaf (19%) and anther (12,5%). In media with hormones, in anther (13% with ANA; 38% with ANA+BAP; 13% with 2,4-D+BAP+K; 13% with ANA+AIB+Z), only cytokinin and auxin plus cytokinin in corm (50%: BAP+Z ; 50%: ANA+BAP), inflorescence (28% with BAP; 14% with K; 14% with Z; 14% with 2,4-D+BAP; 14% with 2,4-D+BAP +TDZ) and nucellus (67% with BAP; 33% with ANA+BAP+K), auxin alone, cytokinin alone, and auxin plus cytokinin in intact zygotic embryos (10% with 2,4-D or ANA; 4% with AIA; 2% with ANA+AIA+AIB; 15% with BAP; 4% with K; 8% with ANA+BAP; 4% with AIB+BAP; 2% with AIB+Z; 2% with AIA+BAP or K; 8% with 2,4-D+BAP; 2% with 2,4-D+Z; 2% with Picloram+BAP), leaf (4% with 2,4-D, ANA or AIB; 12% with Picloram; 4% with K, 12% with BAP, 4% with ANA+BAP, 4% with AIB+BAP or Z, 4% with 2,4-D+ANA+K; 4% with 2,4-D+DPU, SA, TDZ or SA, 8% with 2,4-D+K; 12% with 2,4-D+BAP), cotyledon (8.3% with AIA or 2,4-D; 8.3% with BAP; 8.3% with AIB+BAP; 17% with AIB+K; 8.3% with AIB+BAP+K; 8.3% with 2,4-D+BAP; 8.3% with 2,4-D+BAP+K), stem (12.5% with ANA or 2,4-D; 25% with 2-IP; 25% with ANA+BAP ).

On the other hand, some species in the differentiation stage of secondary somatic embryos used another growth regulator such as ABA, which increases

the rate of cyclic embryogenesis. Thus from primary cotyledonary embryos, secondary somatic embryos increase by 73% in *Aralia cordata* with 0.2 mg/L (LEE; LEE; SOH, 1998) and by 87.5% with 3 mg/L in *Calliandra tweedii* (HEIKRUJAMA *et al.*, 2014), 100% with 0.03 to 0.3 mg/L in *Daucus carota* (OGATA *et al.*, 2005), or improving their morphology with 0.01 mg/L in *Aesculus hippocastanum* (CÁLIĆ; DEVRNJA; MILOJEVIĆ, 2012).

Similarly, another substance is AgNO<sub>3</sub> at 6.7 mg/L that influences the cyclic embryogenesis rate by increasing 90% in *Coffea canephora* (KUMAR; RAMAKRISHNA; RAVISHANKAR, 2007), as well as the action of 0.1% activated carbon in *Sorbus pohuashanensis*, where it increases by 60.5% (YANG *et al.*, 2012a). Furthermore, the concentration and type of carbon source affect the degree of secondary embryo differentiation, where 6% sucrose increases by 80 and 100% respectively in *Chrysanthemum indicum* (NAING *et al.*, 2013) and *Morus alba* (AGARWAL *et al.*, 2004), as well as in *Q. suber*, which increases by 100% when using 4% of this disaccharide or 3% glucose (BENALI; LAMARTI, 2019). Similarly, it increases by 100% in *Ocotea catharinensis* when using 0.5 M Mannitol (SANTA CATARINA *et al.*, 2004). Finally, in *Q. suber*, the use of amino acids increases the secondary embryogenic rate by 80% with 438.3 mg/L of L-Glutamine and 73% with 396.3 mg/L of L-Asparagine (RAHMOUNI *et al.*, 2020); however, when using a lower concentration (30 mg/L) of L-Glutamine the increase was 35%, while when using 30 mg/L of Casein hydrolysate the increase was 36% (ALI; AHMAD, 2021).

Other factors of *in vitro* culture that influence the development of repetitive embryos in some species are temperature and pH. Thus, in *Hovenia dulcis*, a maximum of 66.7% is obtained at 30°C; however, 20°C is optimum for the development of cotyledonary embryos and their conversion into plants (YANG *et al.*, 2013). It increases from 70 to 84% at pH 3.5-5 in *Albizia lebbeck* (SAEED; SHAHZAD, 2015) or to 100% at pH 7 in *Glycine max* (SANTAREM *et al.*, 1997). While in the case of light, 16 h of photoperiod improved the plant regeneration by 35% in *Olea europaea* (MAZRI; NACIRI; BELKOURA, 2020).

Furthermore, related to the secondary embryo maturation prior to their regeneration, is achieved simultaneously at the differentiation stage. Regardless, in some species, other growth substances are used, such as GA<sub>3</sub>, in *Santalum album* at 0.5 mg/L (RAI; MCCOMB, 2002), in *Carthamus tinctorius* at 0.3 mg/L

(KUMAR; KUMARI; CASTAÑO, 2008) and 1 mg/L (KUMAR; KUMARI, 2010) and 0.1 mg/L in *Olea europaea* (MAZRI; NACIRI; BELKOURA, 2020). Similarly, ABA is used at 1 mg/L in *Rosa hybrida* (LI *et al.*, 2002) and 0.5 mg/L in *Cinnamomum camphora* (SHIN *et al.*, 2010) and *Cyclamen persicum* (YOU *et al.*, 2011). In addition, MS salts were at 1/2 in *Dianthus caryophyllus* (KARAMI; DELJOU; KARIMI, 2008) *Rosa hybrida* (BAO *et al.*, 2012), or to 1/3 *Panax ginseng* (KIM *et al.*, 2012).

On the other hand, the increase in the number of cycles of secondary somatic embryos increases the obtaining of genetically stable plants, as was found in *Hevea brasiliensis*, evaluating the number of chromosomes and the variation rate of EST-SSRs loci, showed the chromosome number of the regenerated plants was similar to the mother tree and the rate of EST-SSRs was considered low (2.61%), fundamentally due to the unicellular origin of somatic embryos, according to the study of Wang *et al.* (2017).

Finally, the culture time in the medium significantly influences the greater number of secondary somatic embryos, according to Montoya *et al.* (2022) in *Theobroma cacao*, obtaining an average of 15.2 cyclic embryos per primary somatic embryo (globular and cotyledonary stage), in a medium without hormones after 30 days of culture, decreasing at longer incubation times (40 to 80 days).



[illegible]

|  |                        |        |        |     |   |          |                       |          |   |                  |                      |     |     |          |                       |     |   |                  |           |   |
|--|------------------------|--------|--------|-----|---|----------|-----------------------|----------|---|------------------|----------------------|-----|-----|----------|-----------------------|-----|---|------------------|-----------|---|
| <i>Coriandrum sativum</i> L.<br>( <b>Ap<sup>b</sup></b> )                          | C S                    | I      | U      | 1   | - | -        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | 1   | -   | -        | -                     | -   | - | -                | 90        | Murthy <i>et al.</i> (2008)                                     |
| <i>Crocus vernus</i> (L.) ( <b>I<sup>h</sup></b> )                                 | CO                     | I      | M      | 1   | - | -        | -                     | -        | - | 0.5 <sup>a</sup> | A/<br>T <sup>h</sup> | -   | -   | 0.5      | -                     | 2   | - | -                | 100       | Sivanesan <i>et al.</i> (2012)<br>Blazquez <i>et al.</i> (2009) |
| <i>Cucumis sativus</i> L. ( <b>Cu<sup>b</sup></b> )                                | T                      | I      | M      | -   | - | -        | -                     | -        | - | 0.2 <sup>a</sup> | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | -   | - | 0.2 <sup>a</sup> | 82        | Ziv & Gadasi (1986)<br>Cade <i>et al.</i> (1990)                |
| <i>Cyclamen persicum</i> Mill<br>( <b>Pr<sup>h</sup></b> )                         | L                      | I      | M      | 2   | - | -        | -                     | 0.2      | - | -                | A/<br>G <sup>a</sup> | 2   | -   | -        | -                     | 0.2 | - | -                | 100       | You <i>et al.</i> (2011)  |
| <i>Datura metel</i> L. ( <b>So<sup>h</sup></b> )                                   | ZE <sup>cm</sup>       | D      | M      | -   | - | -        | -                     | -        | 2 | -                | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | -   | - | -                | 50        | Wijesekara & Iqbal (2013)                                       |
| <i>Daucus carota</i> L. ( <b>Ap<sup>h</sup></b> )                                  | ZE <sup>cm</sup><br>ZE | I<br>D | U      | 1   | - | -        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | -   | - | -                | 100<br>93 | Tokuyi <i>et al.</i> (1995)<br>Ogata <i>et al.</i> (2005)       |
| <i>Dianthus caryophyllus</i> L.<br>( <b>C<sup>h</sup></b> )                        | I                      | I      | U      | 0.2 | - | -        | -                     | 0.2      | - | -                | A/<br>T <sup>h</sup> | 0.2 | -   | -        | -                     | 0.2 | - | -                | 95        | Karami <i>et al.</i> (2008)                                     |
| <i>Eleutherococcus<br/>senticosus</i> Maxim ( <b>Ar<sup>l</sup></b> )              | ZE                     | D      | U<br>M | 1   | - | -        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | -   | - | -                | 65        | Yang <i>et al.</i> (2012b); Zhou <i>et al.</i> (2014)           |
| <i>Eriobotrya japonica</i> (Thunb.)<br>Lindl. ( <b>Ro<sup>b</sup></b> )            | A                      | I      | M      | 0.5 | - | -        | -                     | 2        | - | -                | A/<br>T <sup>h</sup> | -   | -   | 0.2      | 0.2 <sup>a</sup>      | -   | - | 0.0 <sup>a</sup> | 73        | Junqiang <i>et al.</i> (2008)<br>Yang <i>et al.</i> (2012)      |
| <i>Eucalyptus citriodora</i> Hook.<br>( <b>M<sup>l</sup></b> )                     | ZE                     | I      | U<br>M | -   | - | 3        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | -   | -   | -        | 3 <sup>a</sup>        | -   | - | -                | 50        | Muraidharan <i>et al.</i> (1989)                                |
| <i>Eucalyptus globulus</i> Labill.<br>( <b>M<sup>l</sup></b> )                     | ZE                     | I      | U<br>M | -   | - | 3        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | -   | -   | -        | 3 <sup>a</sup>        | -   | - | -                | 50        | Pinto <i>et al.</i> (2008)                                      |
| <i>Fagus sylvatica</i> L. ( <b>Fa<sup>l</sup></b> )                                | ZE                     | I      | M      | 5   | - | -        | -                     | -        | - | -                | A/<br>T <sup>h</sup> | 1   | -   | -        | -                     | -   | - | -                | 10        | Vreitez <i>et al.</i> (1992)                                    |
| <i>Fraxinus americana</i> L. ( <b>O<sup>l</sup></b> )                              | ZE                     | I      | M      | 1   | - | -        | -                     | 1        | - | -                | A/<br>T <sup>h</sup> | -   | 0.2 | -        | -                     | 0.2 | - | -                | 20        | Bates <i>et al.</i> (12993)                                     |
| <i>Garcinia indica</i> Choisy. ( <b>Ci<sup>l</sup></b> )                           | ZE                     | D      | M      | -   | - | -        | -                     | 5        | - | -                | A/<br>C <sup>h</sup> | -   | -   | -        | -                     | 5   | - | -                | 70        | Thengane <i>et al.</i> (2006)                                   |
| <i>Gladiolus x Gladiolus</i> L. ( <b>I<sup>h</sup></b> )                           | CO                     | I      | M      | -   | - | -        | -                     | 0.2      | - | 0.2 <sup>a</sup> | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | 0.2 | - | 0.2 <sup>a</sup> | 100       | Remotti (1995)  |
| <i>Glycine max</i> L. ( <b>F<sup>h</sup></b> )                                     | ZE<br>D                | I<br>D | U      | -   | - | 10<br>40 | -                     | -        | - | -                | A/<br>T <sup>h</sup> | -   | -   | 10<br>40 | -                     | -   | - | -                | 100<br>77 | Liu <i>et al.</i> (1992)  |
| <i>Ipomea batatas</i> L. (Lam)<br>( <b>Co<sup>h</sup></b> )                        | L<br>P                 | I      | U      | 2.5 | - | -        | -                     | 0.3      | - | -                | S/<br>T <sup>h</sup> | 0.2 | -   | -        | -                     | -   | - | -                | 100       | Zheng <i>et al.</i> (1996)                                      |
| <i>Helianthus maximiliani</i><br>(Schrader) ( <b>Ar<sup>h</sup></b> )              | L                      | D      | U      | -   | - | 0.5      | -                     | 0.5      | - | -                | A/<br>T <sup>h</sup> | -   | -   | 0.5      | -                     | 0.5 | - | -                | 100       | Vasic <i>et al.</i> (2001)                                      |
| <i>Hepatica nobilis</i> L. ( <b>Ra<sup>h</sup></b> )                               | ZE                     | I      | M      | -   | - | 0.2      | -                     | 0.0<br>2 | - | -                | A/<br>T <sup>h</sup> | -   | -   | 0.0<br>2 | -                     | 0.2 | - | -                | 52        | Szewczyk & Pawłowska (2015)                                     |
| <i>Hevea brasiliensis</i> (Willd & A.<br>Juss) Müll. Arg. ( <b>E<sup>l</sup></b> ) | A                      | I      | U      | -   | - | 0.0<br>2 | -                     | 0.5      | 3 | -                | A/<br>T <sup>h</sup> | -   | -   | 0.0<br>2 | -                     | 0.5 | 3 | -                | 75        | Hu <i>et al.</i> (2010)   |
| <i>Hovenia dulcis</i> Thunb. ( <b>Rh<sup>l</sup></b> )                             | ZE                     | I      | U      | 1   | - | -        | -                     | -        | - | -                | S/<br>T <sup>h</sup> | -   | -   | -        | -                     | 0.1 | - | -                | 43        | Yang <i>et al.</i> (2013)                                       |
| <i>Juglans hindsii</i> (Jeps.) Jeps.<br>ex R.E.Sm. ( <b>J<sup>l</sup></b> )        | C                      | D      | U      | -   | - | -        | 0.0<br>1 <sup>b</sup> | -        | 2 | -                | A/<br>T <sup>r</sup> | -   | -   | -        | 0.0<br>1 <sup>b</sup> | -   | 2 | -                | 100       | Tulecke & Mcgranahan (1985)<br>Polito <i>et al.</i> (1989)      |
| <i>J. nigra</i> L. ( <b>J<sup>l</sup></b> )  | ZE                     | D      | U      | -   | - | -        | 0.0<br>1 <sup>b</sup> | -        | 1 | -                | A/<br>T <sup>h</sup> | -   | -   | -        | -                     | -   | - | -                | -         | Jariteh <i>et al.</i> (2011)                                    |
| <i>J. nigra x regia</i> ( <b>J<sup>h</sup></b> )                                   | C                      | D      | U      | -   | - | -        | 0.0<br>1 <sup>b</sup> | -        | 2 | -                | A/<br>T <sup>r</sup> | -   | -   | -        | 0.0<br>1 <sup>b</sup> | -   | 2 | -                | 100       | Tulecke & Mcgranahan (1985)<br>Polito <i>et al.</i> (1989)      |
| <i>J. regia</i> L. ( <b>J<sup>l</sup></b> )  | C                      | D      | U      | -   | - | -        | 0.0<br>1 <sup>b</sup> | 1        | 2 | -                | A/<br>T <sup>h</sup> | -   | -   | -        | 0.0<br>1 <sup>b</sup> | 1   | 2 | -                | 48        | Fang <i>et al.</i> (2022)                                       |



|  |              |             |   |                         |   |   |            |          |     |                       |  |                     |   |     |                               |          |                       |   |
|--|--------------|-------------|---|-------------------------|---|---|------------|----------|-----|-----------------------|--|---------------------|---|-----|-------------------------------|----------|-----------------------|---|
| <i>Limnanthes alba</i> Benth. ( <b>L<sup>h</sup></b> )             | ZE           | D           | U | 0,0<br>2                | - | -   | -          | -        | -   | 0,0<br>2 <sup>a</sup> | A/<br>T <sup>h</sup>                   | 0,0<br>2            | - | -   | -                             | -        | 0,0<br>2 <sup>a</sup> | Southworth & Kwiatkowski (1991)   |
| <i>Linum usitatissimum</i> L. ( <b>Li<sup>h</sup></b> )            | S            | I           | U | -                       | - | 0,5   | -          | 0,5      | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | 0,5 | -                             | 0,5      | -                     | 3 Tejavathi et al. (2000)   |
| <i>Liquidambar styraciflua</i> L. ( <b>Al<sup>i</sup></b> )        | I            | I           | U | 3                       | - | -   | -          | -        | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | -        | 0,0<br>1 <sup>a</sup> | 100 Merkle et al. (1997)  |
| <i>Magnolia delbata</i> Zucc. ( <b>Ma<sup>i</sup></b> )            | ZE           | I           | U | 1                       | - | -   | -          | -        | -   | -                     | A/<br>T <sup>h</sup>                   | 1                   | - | -   | -                             | -        | -                     | 37 Chávez et al. (2020)   |
| <i>Magnolia</i> spp. L. ( <b>Ma<sup>i</sup></b> )                  | ZE           | D           | U | 2                       | - | -   | -          | 0,3      | -   | -                     | A/<br>T <sup>h</sup>                   | 2                   | - | -   | -                             | 0,3      | -                     | 25 Merkle & Wiecko (1990)   |
| <i>Malus domestica</i> Borkh. ( <b>Ro<sup>i</sup></b> )            | N            | D           | U | 0,4                     | - | -   | -          | 0,0<br>4 | -   | -                     | A/<br>C <sup>h</sup>                   | -                   | - | 0,2 | -                             | 0,2      | 0,2                   | 70 Daigny et al. (1996)   |
| <i>M. pumila</i> Mill. ( <b>Ro<sup>i</sup></b> )                   | N            | D           | U | 0,4                     | - | -   | -          | 0,0<br>4 | -   | -                     | A/<br>C <sup>h</sup>                   | 1                   | - | -   | -                             | 0,5      | -                     | 50 James et al. (1984)  |
| <i>Mangifera indica</i> L. ( <b>An<sup>i</sup></b> )               | N            | I           | U | 2                       | - | -   | -          | -        | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | 0,5      | -                     | 55 Litz et al. (1984)   |
| <i>Manihot esculenta</i> Crantz ( <b>E<sup>h</sup></b> )           | M L<br>L     | D<br>I      | U | 0,0<br>1<br>1<br>4<br>4 | - | -   | -          | 0,1      | -   | -                     | A/<br>T <sup>h</sup><br>C <sup>h</sup> | 0,0<br>1<br>12<br>4 | - | -   | -                             | 0,1      | -                     | 35 Szabados et al. (1987)<br>Groll et al. (2001)<br>43 Wongtiem et al. (2011)<br>13 Anuradha et al. (2015)<br>10 <sup>a</sup><br>80 |
| <i>Medicago sativa</i> L. ( <b>F<sup>h</sup></b> )                 | C<br>L       | I<br>I      | U | 2<br>1                  | - | -   | -          | 0,3      | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | -        | -                     | 50 Lupotto (1986)<br>Sangra et al. (2019)   |
| <i>Medicago truncatula</i> Gaertn. ( <b>F<sup>h</sup></b> )        | L            | I           | U | 1                       | - | -   | -          | -        | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | -        | -                     | 34 Das Neves et al. (1999)  |
| <i>Morus alba</i> L. ( <b>Mo<sup>i</sup></b> )                     | ZE           | I           | U | 2                       | - | -   | -          | 0,5      | -   | -                     | A/<br>T <sup>h</sup>                   | 0,0<br>5            | - | -   | -                             | 0,0<br>1 | -                     | 52 Agarwal et al. (2004)  |
| <i>Myrtus communis</i> L. ( <b>M<sup>i</sup></b> )                 | C<br>S       | I           | U | 1                       | - | -   | -          | 1        | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | -        | -                     | 10 Parra & Amo (1998)   |
| <i>Ocotea catharinensis</i> Mez. ( <b>Lu<sup>i</sup></b> )         | ZE           | I           | U | 4                       | - | -   | -          | -        | -   | -                     | A/<br>C <sup>h</sup>                   | 4                   | - | -   | -                             | -        | -                     | 2,5 Moura et al. (1993)   |
| <i>Olea europaea</i> L. ( <b>O<sup>i</sup></b> )                   | L<br>C<br>ZE | I<br>M<br>D | U | -                       | - | 0,0<br>5 <sup>a</sup><br>5,8<br>2,8<br>6,2<br>5 | 0,1<br>2,8 | -        | -   | -                     | A/<br>T <sup>i</sup>                   | -                   | - | -   | 0,0<br>5b<br>0,0<br>5b<br>0,1 | 0,1      | -                     | 90 Rugini & Caricato (1995)<br>75 Pires et al. (2020)<br>35 Mazri et al. (2020)<br>Benelli et al. (2001)                            |
| <i>Panax ginseng</i> C.A.Mey. ( <b>Ar<sup>h</sup></b> )            | C            | I           | U | 1                       | - | -   | -          | 0,1      | -   | -                     | A/<br>T <sup>h</sup>                   | -                   | - | -   | -                             | -        | -                     | 88 Kim et al. (2012)<br>Kim et al. (2010)   |
| <i>Panax vietnamensis</i> Ha et. Grushv. ( <b>Ar<sup>h</sup></b> ) | L            | I           | U | 1                       | - | 0,5   | -          | -        | 0,2 | -                     | A/<br>G                                | 1                   | - | 0,5 | -                             | -        | 0,2                   | 66 Diem et al. (2022)   |
| <i>Passiflora cincinnata</i> Mart. ( <b>Pa<sup>h</sup></b> )       | A            | I<br>M      | U | 4                       | - | -   | -          | 1        | -   | -                     | A/<br>T <sup>h</sup>                   | 4                   | - | -   | -                             | 1        | -                     | 100 Lemes et al. (2021a)  |
| <i>Petiveria alliacea</i> L. ( <b>Pe<sup>h</sup></b> )             | L            | D           | U | 5                       | - | -   | -          | -        | 5   | -                     | A/<br>T <sup>h</sup>                   | -                   | 4 | -   | -                             | -        | -                     | 5 Cantelmo et al. (2013)  |

|   |    |   |   |     |   |     |                       |     |          |   |   |   |   |                      |     |   |          |          |          |     |     |   |    |     |  |   |                                  |
|---|----|---|---|-----|---|-----|-----------------------|-----|----------|---|---|---|---|----------------------|-----|---|----------|----------|----------|-----|-----|---|----|-----|--|---|----------------------------------|
| <i>Pharbitis nil</i> L. (M <sup>b</sup> )   | ZE | D | M | -   | - | 3   | -                     | -   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | 0,2<br>+ | 2        | -   | -   | - | -  | 100 | Jia & Chua (1992)  |   |                                  |
| <i>Piper nigrum</i> L. (Pi <sup>b</sup> )   | ZE | D | U | -   | - | -   | -                     | -   | -        | - | - | - | - | A/<br>T <sup>r</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | 75  | Nair & Gupta (2006)  |   |                                  |
| <i>Polyscias filicifolia</i> (C. Moore ex<br>E. Fourn.) L. H. Bailey (Ar <sup>r</sup> ) | L  | I | M | 0,5 | - | -   | -                     | 1   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | 90  | Śliwińska <i>et al.</i> (2008)                                   |   |                                  |
| <i>Populus ciliata</i> Wall. & Royle<br>(Sa <sup>l</sup> )                              | L  | I | U | 0,4 | - | -   | -                     | -   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | 0,2      | -        | 0,4      | -   | -   | - | -  | 100 | Cheema (1989)<br>Huang <i>et al.</i> (2015)                      |   |                                  |
| <i>Prunus avium</i> L. (Ro <sup>b</sup> )   | ZE | I | U | -   | - | -   | 0,0<br>5 <sup>o</sup> | 0,1 | 0,1      | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | 0,1      | 0,1<br>+ | -        | -   | -   | - | -  | 18  | Garin <i>et al.</i> (1997)                                       |   |                                  |
| <i>Prunus cerasus</i> L. (Ro <sup>l</sup> )   | C  | D | U | 2   | - | -   | -                     | 0,1 | -        | - | - | - | - | A/<br>T <sup>r</sup> | 2   | - | -        | -        | 0,1      | -   | -   | - | -  | -   | -  | Tanga <i>et al.</i> (2000)  |                                  |
| <i>Prunus persica</i> (L.) Stokes<br>(Ro <sup>l</sup> )                                 | C  | D | U | 5   | - | -   | -                     | 2   | 2        | - | - | - | - | A/<br>T <sup>b</sup> | 1   | - | -        | -        | 0,2      | 0,2 | -   | - | -  | 100 | Bhansali <i>et al.</i> (1990)                                    |   |                                  |
| <i>P. avium</i> x <i>P. pseudocerasus</i> (Ro <sup>b</sup> )                            | R  | D | U | -   | - | 1   | -                     | 0,1 | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | -   | -  | Pesce & Rugini (2004)   |                                  |
| <i>P. incisa</i> x <i>P. serula</i> (Ro <sup>b</sup> )                                  | ZE | I | U | -   | - | -   | -                     | 1   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | -   | -  | Druart (1990)   |                                  |
| <i>Quercus alba</i> L. (Fa <sup>l</sup> )   | ZE | D | U | 1   | - | -   | -                     | 1   | -        | - | - | - | - | A/<br>T <sup>b</sup> | 0,1 | - | -        | -        | 0,1      | -   | -   | - | -  | 3   | Gingas & Lineberger (1989)                                       |   |                                  |
|   |    | I |   |     |   |     |                       |     |          |   |   |   |   |                      |     |   |          |          |          |     |     |   |    |     |  |   |                                  |
| <i>Q. bicolor</i> Willd. (Fa <sup>l</sup> )   | I  | I | U | 1   | - | -   | -                     | 1   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | 1        | -   | -   | - | -  | 20  | Gingas (1991)  |   |                                  |
| <i>Q. brantii</i> L. (Fa <sup>l</sup> )   | ZE | I | U | 0,8 | - | -   | -                     | -   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | 100 | Faizy <i>et al.</i> (2019)                                       |   |                                  |
| <i>Q. ilex</i> L. (Fa <sup>l</sup> )  | S  | I | U | -   | - | 2   | -                     | 0,5 | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | 0,0<br>6 | -        | -        | -   | -   | - | -  | 67  | Martínez <i>et al.</i> (2017)                                    |   |                                  |
| <i>Q. rubra</i> L. (Fa <sup>l</sup> )   | ZE | D | U | 1   | - | -   | -                     | 1   | -        | - | - | - | - | A/<br>T <sup>b</sup> | 0,1 | - | -        | -        | 0,1      | -   | -   | - | -  | 3   | Gingas & Lineberger (1989)                                       |   |                                  |
|   |    | I |   |     |   |     |                       |     |          |   |   |   |   |                      |     |   |          |          |          |     |     |   |    |     |  |   |                                  |
| <i>Q. robur</i> L. (Fa <sup>l</sup> )   | ZE | I | U | -   | - | 2,4 | -                     | -   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | -   | -  | Zegzouti <i>et al.</i> (2001)                                       |                                  |
|   | L  | M |   |     |   |     | 0,0<br>6              | 0,1 |          |   |   |   |   |                      |     |   |          |          |          |     |     |   |    | 48  | Mallón <i>et al.</i> (2012)<br>Puiderrajols <i>et al.</i> (2001) |   |                                  |
| <i>Q. suber</i> L. (Fa <sup>l</sup> )   | S  | I | U | -   | - | -   | -                     | 2   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | -   | -  | -   | El Maataqui <i>et al.</i> (1990) |
|   |    | D | M |     |   |     |                       |     |          |   |   |   |   | A/<br>T <sup>r</sup> |     |   |          |          |          |     |     |   |    |     |  | Fernandez <i>et al.</i> (1995)<br>Puiderrajols <i>et al.</i> (2001) |                                  |
|   | L  |   |   |     |   |     |                       | 0,2 |          |   |   |   |   |                      |     |   |          |          |          |     |     |   |    | 35  |  |   |                                  |
| <i>Robinia pseudoacacia</i> L.<br>(F <sup>l</sup> )                                     | ZE | D | U | 4   | - | -   | -                     | 0,3 | -        | - | - | - | - | A/<br>T <sup>b</sup> | 4   | - | -        | -        | 0,3      | -   | -   | - | -  | 100 | Merkle & Wiecko (1989)   |   |                                  |
| <i>Rosa chinensis</i> (Ro <sup>b</sup> )  | L  | I | U | 3   | - | -   | -                     | -   | 0,5      | - | - | - | - | A/<br>T <sup>b</sup> | 1   | - | -        | -        | -        | -   | 0,1 | - | -  | 40  | Cai <i>et al.</i> , 2022   |   |                                  |
| <i>Rosa hybrida</i> (Ro <sup>l</sup> )  | L  | I | U | 10  | - | -   | -                     | -   | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | -        | -        | -        | -   | -   | - | -  | 100 | Li <i>et al.</i> (2002a)<br>Bao <i>et al.</i> (2012)             |   |                                  |
|   |    |   |   | 3   |   |     |                       |     |          |   |   |   |   |                      |     |   |          |          |          |     |     |   | 56 |     |  |   |                                  |
| <i>Rosa rugosa</i> Thunb. (Ro <sup>l</sup> )  | L  | I | U | 4   | - | -   | -                     | -   | 0,0<br>5 | - | - | - | - | A/<br>T <sup>b</sup> | 1   | - | -        | -        | 0,0<br>1 | -   | -   | - | -  | 88  | Xing <i>et al.</i> (2014)  |   |                                  |
| <i>Santalum album</i> Linn. (St <sup>l</sup> )  | ZE | D | U | -   | - | -   | -                     | -   | 0,0<br>1 | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | 1        | -        | -        | -   | -   | - | -  | 65  | Rai & McComb (2002)  |   |                                  |
| <i>Sorbus pohuashanensis</i> (Hance) Hedi. (Ro <sup>l</sup> )                           | ZE | I | U | -   | - | 1   | -                     | 0,1 | -        | - | - | - | - | A/<br>T <sup>b</sup> | -   | - | 0,0<br>1 | -        | -        | -   | -   | - | -  | 40  | Yang <i>et al.</i> (2012a)                                       |   |                                  |
| <i>Syreitenia macrophylla</i> Jacq.<br>(Me <sup>l</sup> )                               | L  | I | U | 1   | - | -   | -                     | 2   | -        | - | - | - | - | A/<br>T <sup>r</sup> | -   | - | -        | -        | 1        | -   | -   | - | -  | 19  | Gatica <i>et al.</i> (2019)<br>Quiala <i>et al.</i> (2021)       |   |                                  |

|   |    |   |   |     |   |   |   |      |   |   |    |                |     |   |   |   |     |   |     |                                    |
|---|----|---|---|-----|---|---|---|------|---|---|----|----------------|-----|---|---|---|-----|---|-----|------------------------------------|
| <i>Theobroma cacao</i> L. (MI <sup>h</sup> )    | I  | I | U | 2   | - | - | - | 0.2  | - | - | -  | A/             | -   | - | - | - | 5   | - | 100 | Maximova et al. (2002)             |
|   |    |   | M | 2   | - | - | - | 0.1  | - | - | -  | C <sup>h</sup> | 2   | - | - | - | 0.5 | - | 20  | Ajjaha & Hartatib (2019)           |
|   | A  |   |   |     | - | - | - | -    | - | - | -  | A/             | -   | - | - | - | -   | - | 70  | Montoya et al. (2022)              |
|   | PE |   |   | 1   | - | - | - | 0.3  | - | - | -  | G              | -   | - | - | - | -   | - |     |                                    |
|   |    |   |   |     |   |   |   |      |   |   |    | A/             | -   | - | - | - | -   | - |     |                                    |
|   |    |   |   |     |   |   |   |      |   |   |    | C <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
| <i>Tilia amurensis</i> Rupr. (MI <sup>h</sup> ) | ZE | I | U | 1   | - | - | - | -    | - | - | -  | A/             | 1   | - | - | - | 0.1 | - | 72  | Kim et al. (2006a)                 |
|   |    |   |   |     |   |   |   |      |   |   |    | C <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
| <i>Trifolium repens</i> L. (F <sup>h</sup> )    | ZE | D | U | -   | - | - | - | 0.8  | - | - | -  | A/             | -   | - | - | - | 0.8 | - | 100 | Maneswaran & Williams (1964; 1965) |
|   |    |   |   |     |   |   |   | 5    | - | - | -  | T <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
| <i>Vitis latifolia</i> L. (V <sup>h</sup> )     | A  | I | U | 4   | - | - | - | 2    | - | - | -  | A/             | -   | - | 2 | - | -   | - | 14  | Salunkhe et al. (1999)             |
|   |    |   |   |     |   |   |   |      |   |   |    | T <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
| <i>V. rupestris</i> Scheele (V <sup>h</sup> )   | L  | I | U | 1   | - | - | - | 1    | - | - | -  | A/             | -   | - | 1 | - | -   | - | 13  | Martinelli et al. (1993; 2001)     |
|   |    |   |   |     |   |   |   |      |   |   |    | T <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
| <i>V. vinifera</i> L. (V <sup>h</sup> )         | ZE | D |   | -   | - | - | - | 0.2  | - | - | -  | A/             | -   | - | - | - | 0.2 | - | 21  | Emershad & Ramming (1994)          |
|   | I  |   |   | 0.8 | - | - | - | -    | - | - | 1c | T <sup>h</sup> | -   | - | - | - | 2   | - | 5   | Hao et al., 2022                   |
|   |    | I | U |     | - | - | - | 2    | - | - | -  |                | -   | - | - | - | -   | - |     |                                    |
|   | I  | I |   | 1   | - | - | - | -    | - | - | -  |                | -   | - | - | - | -   | - |     | Capriotti et al. (2022)            |
|   | A  |   |   |     | - | - | - | 1    | - | - | -  | A/             | 0.5 | - | - | - | -   | - |     |                                    |
|   | PI |   |   | 1.1 | - | - | - | -    | - | - | -  | T <sup>m</sup> | -   | - | - | - | -   | - |     |                                    |
|   | A  | I |   |     | - | - | - | 4    | - | - | -  |                | 0.5 | - | - | - | 1.1 | - | 80  | Zhou et al. (2014)                 |
|   |    |   |   | 0.5 | - | - | - | -    | - | - | -  |                |     | - | - | - | 1.1 | - |     |                                    |
| <i>Zizyphus jujuba</i> Mill. (Rh <sup>h</sup> ) | C  | I | U | 1   | - | - | - | 0.1* | - | - | -  | A/             | -   | - | - | - | 0.2 | - | 93  | Kim et al. (2006b)                 |
|   |    |   |   |     | - | - | - | 0.1  | - | - | -  | T <sup>h</sup> | -   | - | - | - | -   | - |     |                                    |
|   |    |   |   |     |   |   |   | h    | - | - | -  |                | -   | - | - | - | -   | - |     |                                    |

**FAMILY.** (A): Amaranthaceae; (Al): Altingiaceae; (An): Anacardiaceae; (Ap): Apiaceae; (Ar): Araliaceae; (As): Asteraceae; (Bi): Bixaceae; (B): Brassicaceae; (BU): Burceraceae; (C): Caricaceae; (Cr): Caryophyllaceae; (Co): Convolvulaceae; (Cl): Clusiaceae; (Cu): Cucurbitaceae; (E): Euphorbiaceae; (F): Fabaceae; (Fa): Fagaceae; (G): Gentianaceae; (I): Iridaceae; (J): Juglandaceae; (L): Limnathaceae; (La): Lardizabalaceae; (Lu): Lauraceae; (Li): Linaceae; (Ma): Magnoliaceae; (MI): Malvaceae; (Me): Meliaceae; (Mo): Moraceae; (M): Myrtaceae; (O): Oleaceae; (Pa): Passifloraceae; (Pe): Petiveriaceae; (Pi): Piperaceae; (P): Polygonaceae; (Pr): Primulaceae; (Ra): Ranunculaceae; (Rh): Rhamnaceae; (Ro): Rosaceae; (Ru): Rubiaceae; (Sa): Salicaceae; (St): Santalaceae; (So): Solanaceae; (T): Theaceae; (V): Vitaceae. **HABIT.** (t): tree; (h): Herbaceous. **EXPLANT.** A: anther; C: Cotyledon; CO: Cormo; ZE: zygotic embryo; ZE<sup>m</sup>: zygotic embryo without meristem; L: leaf; I: Inflorescence or flower; P: petiole; PE: Petal; PI: Pistils; M: meristem; MM: male meristem; MI: microspores; N: nucella; R: root; S: stem. **REGENERATIVE VIA.** D: direct; I: Indirect. **ORIGIN OF THE SOMATIC EMBRYOS.** (U): Unicellular; (M): Multicellular. **SOMATIC EMBRYO.** PSE: primary; SSE: secondary. **SYNCHRONIC OF THE SSE. (SIN).** S: synchronic; A: asynchrony. **DIFFERENTIATION TYPE PSE TO SSE.** G: globular; T: torpedo; C: cotyledonal. **DIFFERENTIATION ZONE PSE TO SSE.** r: root; h: hypocotyl; e: epicotyl; mc: mid-cotyledonary co. **AUXINS.** 2,4-D: 2,4-dichlorophenoxyacetic; **PICLORAM:** 4-amino-3,5,6-trichloropicolinic acid; **NAA:** 1-naphthaleneacetic acid; **IAA:** indole-acetic acid; **IBA:** Indole-3-butyric acid. **CYTOKININS:** AS: Adenine Sulphate; BAP: 6-benzyl-amino purine; K: 6-furfurylaminopurine or Kinetin; TDZ: thidiazuron; 2-IP: 2-isopentenyl adenine; DPU: 1,3-Diphenylurea; Z: zeatin.

## 6. Biotechnological applications of repetitive somatic embryogenesis.

In general, the applications of somatic embryogenesis are ontogenetic studies, gene expression, molecular genetics, and micropropagation of species of commercial interest, genetically modified or not, with promising characteristics (LOYOLA; OCHOA, 2016). Corresponding to secondary somatic embryogenesis is the massive micropropagation of elite varieties according to the agronomically important characteristic, particularly through Temporary Immersion (TI) systems and the efficient regeneration of transgenic plants from transformed primary somatic embryos.

In relation to the massive multiplication of secondary embryos by IT and the subsequent efficient regeneration, from globular primary somatic embryos, they multiply more than 24 times in secondary embryos in cycles of 1 min of immersion and six h in dryness in *Camellia sinensis* (AKULA; BECKER; BATESON, 2000) or with primary torpedo embryos with cycles of 1 min of immersion and 8 h without medium, developing from 86 to 90% of secondary embryos in *Q. robur* (MALLÓN; COVELO; VIEITEZ, 2012). Accordingly to the system, the mass of primary embryos initiated must be considered, as indicated by Steinmacher *et al.* (2011) and Yang *et al.* (2012b) in *Eleutherococcus senticosus* and *Bactris gasipaes*, respectively, which obtain the highest yield with 100 mg.

Another interesting aspect is obtaining plants with improved characteristics, increasing their ploidy, incubating primary somatic embryos in colchicine. Thus, the 29.9% of polyploid plants without abnormalities and with beneficial agronomic characteristics were regenerated from secondary somatic embryos of *Panax vietnamensis*, incubating primary somatic embryos in the globular stage with 0.3 to 0.5% colchicine for 48 h. The tetraploid plants obtained showed better growth than the diploid plants, as they presented a larger size of the stomata and a lower stomatal density (DIEM, PHONG, TUNG, 2022).

Regarding genetic transformation, few works reported using primary somatic embryos with the subsequent regeneration of transgenic plants resulting from the differentiation of secondary embryos from these. Among the systems used, the main one is the indirect by *Agrobacterium tumefaciens*, followed by the direct ones were Biobalistics and electroporation.

In the first case, in *J. regia*, transforming globular somatic embryos with the *nptII* gene (pCGN200), 70% of secondary embryos are obtained from these plants with the insert then verified by Southern blot (MCGRANAHAN *et al.*, 1988). With the *gus* gene (pCGN7001), only 2.5% of *gus* positive plants are obtained (MCGRANAHAN *et al.*, 1993), while with the *badhx* gene (pBI21) that confers tolerance to salt stress, 80% of transformation of globular primary embryos is achieved, regenerating 5.5% transgenic plants, after the *gus* gene test (BOLAGH *et al.*, 2020). In *Medicago sativa*, inserting the *nptII* and *gus* genes, 16% of GUS-positive plants are regenerated from secondary embryos differentiated from globular primary embryos transforming with pGA472 (NINKOVIĆ; MILJUŠ;

NEŠKOVIĆ, 1995), or using the plasmid pCambia2301 in primary cotyledonary embryos 90% of secondary embryos are differentiated, of which 15.2% were positive to GUS and molecularly by PCR (LIU *et al.*, 2013). Similarly, in *Rosa rugosa*, inserting the *nptII* and *gus* genes (pBI121) in globular primary embryos, 74% of secondary embryos positive for *gus* are achieved, but only 11.4% of these regenerate plants (XING *et al.*, 2014), while with *Eriobotrya japonica* with globular primary embryos using pEG121 with the *nptII* gene, 22% of transformed plants are obtained, regenerated from differentiated secondary embryos (LIN; LI; ZONG, 2021). In *Q. ilex*, the plasmid pK7TA4 carrying the genes *nptII*, *rol*, *gus*, and *cstI1* is transferred, the latest encoding the antifungal protein "thaumatin", which confers tolerance to the pathogen *Phytophthora cinnamomi*; in primary globular somatic embryos, obtaining from 2.8 to 66.7% of transgenic plants, after the colorimetric evaluation of *gus* and the molecular evaluation by PCR (CANO *et al.*, 2020). In *Brassica oleracea*, the *hpk1* and *bar* (pSHX004) genes that confer tolerance to salt stress and herbicide, respectively, were inserted in primary somatic embryos, obtaining 7.33 of transgenic plants, after molecular evaluation by PCR of the presence of both genes, as well as the evaluation in the field where the transformed lines had greater resistance to high concentrations of NaCl and herbicide (PAVLOVIĆ *et al.*, 2020). In *Vitis vinifera*, employing primary torpedo embryos in mid-cotyledonary stage, the pCAMBIA2301 plasmid is transferred with the *nptII*, achieving 80% of regenerated plants molecularly positive by PCR (ZHOU; DAI; CHENG, 2014), while in *Hevea brasiliensis* with the same plasmid and gene, achieving 4.06 % of regenerated plants GUS positive using cotyledonary primary somatic embryos (HUANG; LI; LI *et al.* 2015), improving its expression by cocultivation the same type of primary embryos with the bacteria for 84 hours, also verifying molecularly by PCR, according to Udayabhanu *et al.* (2022). In *Q. suber*, in globular and primary torpedo embryos, the pK7TA4 plasmid is transferred with the *nptII*, *gfp*, *rol* and *cstI1* genes, it is the last encoding for an antifungal protein, observing 60% of *gfp*-positive regenerated plants, of which 100 % carried the transgenes and 83.3% over-expressed the *cstI1* gene product, when molecularly evaluated by real-time PCR, showing that 38.9% of the transgenic lines obtained were tolerant to the fungus, in *in vitro* assays with the pathogen fungal (CANO; MARTÍNEZ; COUSELO, 2021). In *Passiflora cincinnata*, the plasmid pCAMBIA1304 carrying the *hptII*, *gus* and *nos* genes is

transferred into segments of primary cotyledonary somatic embryos, increasing the transformation efficiency to 21.4% by applying 30 s of sonication, when evaluating the presence of the gene by PCR *gus* (LEMES; PAIM; BARCELOS, 2021b). In *Theobroma cacao*, the *hptII* and *gfp* genes are inserted through the plasmid pCAMBIA2300 in cotyledonary segments of secondary somatic embryos, the transgenic frequency being higher (11.6%) with the *Agrobacterium* AGL1 strain and with explants from 4 to 10 mm, by performing the colorimetric evaluation of the green fluorescent protein and molecular PCR of the *nptII* and *gfp* genes (JONES; ZHANG; TUCKER, 2021).

On the other hand, by bombardment, De Guglielmo *et al.* (2010) used torpedo type somatic embryos of *C. arabica* to efficiently transfer the *Cry-1ac* gene (pUBC) that confers resistance to Lepidoptera, at a pressure of 70 psi and 14 cm distance, regenerating by secondary somatic embryogenesis 100% of uniformly transformed plants, after the molecular evaluation of the gene by PCR, Southern blot, as well as by Reverse Transcription-PCR. Likewise, in *C. sinensis*, Furukawa *et al.* (2020) inserted the gene *nptII* and *gus* (pRI201) in primary embryos at a pressure of 80 psi and 6 cm of distance, differentiating secondary embryos positive to GUS, which is 39% became transformed plants. On the other hand, the electroporation method was only reported in leaf, embryogenic callus, and primary somatic embryo (torpedo type) of *C. arabica*, at capacitance of 900  $\mu$ F and voltages of 375, 625, and 875 V/cm with pCambia3201 with *gus* and *bar* genes. Although this plasmid gives resistance to herbicides, transformation (100%) was found only in primary embryos previously enzymatically treated (1 h in 2% cellulose, 1% macerosime) and electroporated at 375 V/cm, regenerating by cyclic embryogenesis in the liquid medium, 100% *gus* positive plants, verifying molecularly by PCR that the genes were transferred (FERNÁNDEZ; MENÉNDEZ, 2003).

## 7. Conclusions

So far, secondary somatic embryogenesis has been reported in detail in 148 species (7 Gymnosperms and 141 Angiosperms) by using different explants. The main pathway was found indirectly and asynchronously, of unicellular origin in more than 60% of the cases, regenerating plants in more than 90% of the species. The combination of growth regulators (only auxin, only cytokinin, or

auxin+ cytokinin) for the stage of primary and repetitive embryogenesis varies accordingly to the plant species and explant type. However, the use of growth-regulators free media increases, decreasing the auxins concentration and increasing cytokinins in the phase of secondary embryogenesis. Despite the potential and versatile biotechnological application of cyclic embryogenesis, there are still very few works on it, so the information in this bibliographic review will be of great help for new research in these species and others where this process has not been reported yet.

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