Chapter 25
Application of Somatic Embryogenesis to Secondary Metabolite-Producing Plants

Felipe A. Vázquez-Flota, Miriam Monforte-González and María de Lourdes Miranda-Ham

Abstract Plants display an amazing biosynthetic capacity. To date, around 200,000 different chemical compounds have been isolated from them. Only a relatively few of these compounds are common to all plant species, since they are involved in basic or primary cell processes, such as energy metabolism. However, the broader plant chemical diversity corresponds to those compounds showing a restricted distribution among only a few taxonomically related species and which are not involved in primary metabolic pathways. These compounds are called secondary or specialized metabolites and they have important roles in numerous plant-environment interactions. Aside from these functions, secondary metabolites, and the plants bearing them, represent highly regarded commercial products given their pharmaceutical, flavoring, aromatic, coloring, and poisonous properties. In here, we present some selected examples of secondary metabolite-producing plants for which efficient protocols of somatic embryogenesis have been developed. The review covers mainly plants producing fine chemicals, used either in pharmaceutical or food industries. As shown, the development of somatic embryogenesis procedures could respond to two main goals: the genetic transformation of a given plant species, or the massive propagation of selected materials. Furthermore, the use of such protocols for the generation of diversity through indirect embryo formation is also presented.

Abbreviations

2,4-D 2,4-diclorophenoxyacetic acid
ABA Abscisic acid
BA 6-benzyladenine

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F.A. Vázquez-Flota (✉) · M. Monforte-González · M. de Lourdes Miranda-Ham
Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Calle 43 No. 130 Chuburná de Hidalgo, 97205 Merida, Yucatan, Mexico
e-mail: felipe@cicy.mx

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GA Gibberellic acid
Kin Kinetin
NAA Naphthalenacetic acid
TDZ Thidiazuron

25.1 Introduction

Plants are frequently regarded as chemical factories due to their sophisticated biosynthetic capacity. It is estimated that over 200,000 chemicals have been isolated from plants and that up to 20 % of the plant genome could be devoted to the production, packing, and mobilization of these compounds, collectively known as *secondary* or *specialized* metabolites (Scheible et al. 2004; Hartmann 2007). This estimation also includes genes involved in the perception of the environmental cues that trigger the process of synthesis as a response leading to adaptation to new conditions. Plant chemical diversity is derived from the gene plasticity associated to non-fundamental cell processes. Genes involved in secondary metabolism do not show the tight conservation existing in those involved in primary biochemical routes, such as energy metabolism or the synthesis of cellular components. Genes ascribed to secondary metabolism often originate from divergence of those involved in reactions of primary metabolism (Pichersky and Lewinsohn 2011). As a consequence, every plant species presents a unique chemical blend, different from the others.

Nonetheless, even when plant secondary metabolism can display such wide diversity, products often show a restricted distribution. Commonly, only a few plants that are taxonomically related display similar products. Moreover, secondary metabolites accumulate in low amounts in plant tissues, which seem to be directly related to their physiological activities. Secondary metabolites play critical roles in plants' interactions with other organisms, mainly herbivores and microbes, often exerting toxic effects on them. In fact, some plants can be toxic to other plants, and even to themselves; hence, different mechanisms operate to strictly control the synthesis, storage, and distribution of secondary metabolites. Interestingly, only low doses of secondary metabolites are needed to exert their effect, given their affinity to specific cell receptors and thus, prompting a physiological response. This is the result of their structures mimicking those of the actual ligands (Hartmann 2008).

Besides their important role in nature, secondary metabolites, and the plants bearing them, represent highly regarded commercial products. Secondary metabolites have pharmaceutical, flavoring, aromatic, coloring, and poisonous properties. Consumers are willing to pay premium prices for secondary metabolites obtained from natural sources that do not display the health hazards that some chemically obtained products display. One can satisfy hunger with different types of staples, but only caffeine produces such a stimulant effect on us.

25.2 Plant Cell Cultures and Secondary Metabolites

Plant secondary metabolites are found in a number of products that are daily consumed around the world. Some of them include stimulant beverages such as coffee, cocoa, tea, and other brewed infusions; spices, such as pepper, clover, nutmeg, peppermint, and cinnamon; flavorings, such as garlic, saffron, and ginger; and pharmaceuticals, such as opium and belladonna, among others. Other industrial products containing plant secondary metabolites or their derivatives are pesticides (pyrethrins from *Chrysanthemum* or thiophenes from *Tagetes*) and textile dyes (as the natural indigoes from the *Indigofera* genus), to name two of them. Based on their continuous demand and economic value, cell culture technology was applied to the production of secondary metabolites since its early development (Vázquez-Flota and Loyola-Vargas 2003). Earlier approaches focused on the use of cell suspensions in a similar fashion as yeast strains producing antibiotics and other fine chemicals. However, plant cells' biosynthetic capacity was frequently lost or reduced in undifferentiated cultures that lack the required specialization for the synthesis and storage of these metabolites. However, despite the lack of commercial success, in vitro cell cultures have been fundamental to the understanding of mechanisms controlling secondary metabolism (Hartmann 2007, 2008).

Somatic embryogenesis applied to secondary metabolite-producing plants has been directed, either to massive propagation of elite materials, or as one of the strategies involved in genetic improvement. As happens for other economically important plants, interest in the genetic improvement of those producing secondary metabolites mainly focuses on increasing yields per area. This can be achieved by either promoting a higher total accumulation of the product of interest or by reducing the accumulation of less valuable or undesirable byproducts. In both cases, the biosynthetic pathways leading to the synthesis of these products should be known well enough to identify critical steps or regulatory mechanisms controlling the processes. Identification of genes involved in the control of production of secondary metabolites allows their isolation and introduction by genetic transformation techniques. The value of embryogenic cultures of these species is therefore evident. However, recalcitrance is a common problem associated to such species (both for transformation and embryogenesis). This may be explained in terms that most of them are non-model plants (Facchini and De Luca 2008). Although primary embryogenesis is frequently a success, embryo maturation and conversion to entire plants are critical and limiting steps to obtain transformed mature plants (Facchini et al. 2008).

In here, we present some selected examples of secondary metabolite-producing plants for which efficient protocols for somatic embryogenesis have been developed. The review covers mainly plants producing fine chemicals, used either in pharmaceutical or food industries. It should be mentioned that the hormonal treatments applied at the different stages of the in vitro process were kept in the same concentration units (either as mg/L or μM) as in the original reports.

25.3 Somatic Embryogenesis in Plants with Medicinal Applications

25.3.1 Alkaloid-Producing Plants

Alkaloids are basic compounds that include a tertiary nitrogen atom, usually as part of a heterocyclic structure. Most alkaloids are synthesized from amino acids and display powerful toxic effects at low doses given their high affinity to cell receptors, displacing the actual ligands (Hartmann 2007). Due to their complex chemical structures, which often present one or more chiral centers, alkaloids are commonly obtained from various natural sources, rather than from chemical synthesis. Moreover, alkaloid-producing plants are frequently collected in wild areas, which can result in discontinuous supplies to pharmaceutical manufacturers. For these reasons, the development of biotechnological tools for the improvement of alkaloid-producing plants is highly desirable. Three of the most commonly pharmaceutically employed alkaloids are those produced by *Catharanthus roseus*, *Papaver somniferum*, as well as, the tropane alkaloids produced by certain members of the Solanaceae family.

25.3.2 Catharanthus roseus *(Apocynaceae)*

This plant, also known as Madagascar periwinkle, remains as the only commercial source of the antineoplastic dimeric alkaloids vinblastine (VLB) and vincristine (VCR) widely used to treat different types of tumors. In the past, numerous attempts to produce these therapeutically valuable agents using cell culture technology were done without clear results. These unsuccessful attempts have been related to the high tissue and cell specialization involved in their synthesis, which is lost during the induction of in vitro cultures (Facchini and De Luca 2008). Moreover, one of the subunits required for dimer formation (catharanthine) is not accumulated inside the tissues, but rather excreted and kept in the leaf covering wax, excluding it from vindoline, the other moiety needed for obtaining these alkaloids (Yu and De Luca 2013).

The morphogenic response of *C. roseus* cultured in vitro has been found to be genotype-related (Lee et al. 2003). Moreover, differential tissue responses to in vitro culture have been reported (Dhandapani et al. 2008). Although a good embryogenic response was obtained with anthers (Kim et al. 1994) and immature zygotic embryos (Kim et al. 2004), low efficiencies in embryo germination were observed in both cases. Dhandapani and coworkers (Dhandapani et al. 2008) found that the use of TDZ (7.5 μM) on explants from mature zygotic embryos of *C. roseus* "Little Bright Eye" cultivar produced a noticeable embryogenic response (up to 49 %). Embryo maturation and plantlet germination were also accomplished using TDZ (2.5 μM) and IBA (2.4 μM), respectively. In this work, though other explants were employed, including cotyledons, hypocotyls, and petioles, the best embryogenic

response was recorded with zygotic embryos (Dhandapani et al. 2008). Interestingly, hypocotyl explants showed a high embryogenic response when exposed to NAA (Junaid et al. 2006, 2007; Aslam et al. 2011). After formation, embryo maturation and germination to entire plantlets can be achieved in a liquid phase by replacing culture media supplemented with GA and BAP plus IBA, respectively (Junaid et al. 2007). Other complete liquid-phase protocols using hypocotyl derived explants, from the induction of primary embryogenic calli to the formation entire plantlets, have been described (Junaid et al. 2007).

As mentioned above, a high degree of cell organization is required for the formation of the *Catharanthus* alkaloids. Both VLB and VCR contents increased as embryos progressed from calli to their mature stage and during early plantlet development (Aslam et al. 2009, 2011). It is noteworthy to mention that treating embryos with low temperatures at some point through the process markedly promoted alkaloid accumulation, perhaps as a response to the desiccation that the treatment induced (Aslam et al. 2011). Contents of other *Catharanthus* alkaloids, such as ajmalicine and serpentine, also increased as embryo formation proceeds in cell suspensions (Favretto et al. 2001).

Alkaloid accumulation has been studied in mature *Catharanthus* plants obtained through somatic embryogenesis, using hypocotyl explants as starting materials (Filippini et al. 2000). Comparison to the original plant materials revealed no variations in alkaloid contents in leaves and stems, while a noticeable accumulation in flowers of plants derived from somatic embryos was recorded. Similar results were obtained for both pink and white flower cultivars (Favretto et al. 2001).

25.3.3 (Papaveraceae)

Opium has been used for medicinal and recreational purposes since the dawn of civilization. Records of opium usage can be traced as far as 1400 BC and all the major antique Eastern civilizations did know of its properties and made ample use of them (Schivelbusch 1992). Opium is the dried latex from the opium poppy (*Papaver somniferum*) obtained by milking unripened seed capsules through blade-made incisions. Latex, in turn, is the cytoplasm of the laticifer cells, which are part of the highly specialized internal secretory system of this plant. Opium is composed of a number of alkaloids; however, its narcotic effects are mainly due the opiate alkaloids morphine, codeine, and noscapine. The biosynthetic pathway leading to the formation of *Papaver* alkaloids is complex and different cell types are involved (Facchini and De Luca 2008). Therefore, as it frequently occurs in these cases, the biosynthetic capacity is lost in undifferentiated cell cultures (Facchini et al. 2012).

Despite its long relationship with mankind and extensive manipulation, this plant presents a relatively narrow genetic base. As a consequence, efforts for its genetic improvement by traditional means (Levy and Milo 1997) have encountered limited success. Hence, attempts to generate variation using biotechnological tools have

been envisioned for decades (Facchini et al. 2008). Reports of the regeneration of shoots of *P. somniferum* and *P. orientale* plants from embryogenic suspension showed that alkaloid profiles (morphine, codeine and thebaine) were similar to those from seed-germinated plantlets of the same size and morphology (Schuchmann and Wellmann 1983). Similar results were obtained by Day and coworkers when comparing *P. bracteatum* plants derived from somatic embryos and seed-grown plants (Day et al. 1986). These examples indicate that alkaloid synthesis in *Papaver* is relatively stable after passage through in vitro cultures.

Most protocols involve indirect embryogenesis using young developing tissues, such as hypocotyls from approximately two-week-old seedlings, as initial explants. A combination of auxin (NAA or 2,4-D) and cytokinin (BAP or Kin) is employed to induce the embryogenic cultures at doses ranging from 1 to 2.5 mg/L. Transition from embryonic calli or suspensions to somatic embryos and further maturation is achieved by eliminating auxins from culture media (Ovecka et al. 1997; Chitty et al. 2003; Frick et al. 2004). Although shoots readily developed from the mature embryos, root formation has shown to be a limiting step (Ovecka et al. 1997). Roots from developing seedlings can also be used as starting material with good results (Facchini et al. 2008; Pathak et al. 2012). As for hypocotyl-derived calli, hormones should be removed to promote embryo maturation (Facchini et al. 2008). ABA and GA (between 0.1 and 0.2 mg/L) can be added to the culture medium to promote maturation (Pathak et al. 2012).

A highly efficient protocol of indirect embryogenesis was combined with *Agrobacterium tumefaciens*-mediated genetic transformation to confer herbicide resistance to *P. somniferum* by introducing the phosphinothricin acetyltransferase (*pat*) gene (Facchini et al. 2008). Explants (excised root tissues) were transformed prior to the induction of the embryogenic cultures. Rooting of cotyledonary embryos was improved by dissecting individual embryos from clusters and avoiding direct contact with the agar medium using filter paper. Moreover, short exposures to a high IBA concentration (100 mg/l for less than 6 h) and incubation at low temperatures (<20 °C) were also beneficial for the process (Facchini et al. 2008).

Somatic embryogenesis and *Agrobacterium*-mediated transformation has also been used to generate plants over-expressing the first to last step of morphine biosynthesis (codeinone reductase; *PsCor1.1*) (Larkin et al. 2007).

Protocols for direct somatic embryogenesis have been described using hypocotyls and seedling cotyledons (Kassem and Jacquin 2001).

25.3.4 Plants Producing Tropane Alkaloids

Tropane alkaloids are formed either from arginine or ornithine. There are more than 200 of these alkaloids, some of them with pharmacological interest due to their anticholinergic effects caused by binding to the muscarinic acetylcholine receptors at the nervous central system. These alkaloids are common in the Solanaceae and

Erythroxylaceae families (Jirschitzka et al. 2013). Examples of plants producing this type of alkaloids are members of the *Datura* genus, *Atropa belladonna* (the deadly nightshade), and *Hyoscyamus niger* (henbane).

Datura metel (the devil's trumpet) is a shrub, native to India and southern China. It is used in the treatment of a number of ailments, given its anthelmintic, anticancer, antispasmodic, hypotensive, and antiviral properties. Unrestricted gathering of this plant has resulted in a severe reduction of its population (Nithiya and Arockiasamy 2007). As a strategy to preserve this important medicinal plant, a massive propagation procedure using somatic embryogenesis has been developed. Root explants from seedlings were exposed to 4 mg/L BAP resulting in the direct formation of embryos after two weeks. Shoot elongation and root formation were achieved using BAP, GA_3, and IBA (2, 1 and 1 mg/L, respectively) in the culture media. Over 100 entire plantlets per root explant were obtained through this method (Nithiya and Arockiasamy 2007). Another approach for its clonal propagation was the use of hypocotyl sections of androgenic embryos, derived from anthers (Wijesekara and Iqbal 2013).

Hyoscyamus niger. This plant produces hyoscyamine, a powerful anticholinergic agent employed as muscle and gastric spasms relaxant. Scopolamine, which is also found in this plant, has similar medicinal uses, but with stronger side effects (Jirschitzka et al. 2013).

A highly efficient method for plant regeneration through somatic embryogenesis has been described using mature zygotic embryos. One week of exposure to 1 mg/NAA of pre-soaked embryos (16 h in water) resulted in 80 % embryo formation directly on the hypocotyl segment (Tu et al. 2005). Several waves of embryo formation occurred; however, after the first round, an unsynchronized development was observed. Plant formation occurred without transfer of embryos to fresh media; although, auxin elimination promoted their development (Tu et al. 2005). Since high numbers of morphologically normal embryos were obtained, this method has been proposed to be used for the transformation of this valuable medicinal plant (Tu et al. 2005).

25.3.5 Other Select Cases of Medicinal Plants

Terminalia chebula. This is a tree, common to sub-Himalayan forests, that has spread to different parts of India. Seeds are a valuable source of ellagitannic acid, highly demanded in the tannery industry. These also contain the hexapeptide cherbuin, which displays significant antispasmodic activity. Dried fruits are used in different medical preparations as adjuvants and they are prescribed in Ayurvedic medicine against a number of diseases, including leprosy, jaundice, epilepsy, and hiccough. Tree propagation is made by seeds, but low germination rates have been determined. Therefore, biotechnological approaches such as multiple shoot cultures (Shyamkumar et al. 2003) and somatic embryogenesis protocols have been developed (Anjaneyulu et al. 2004).

Seed cotyledons and mature zygotic embryos of *T. chebula* were used as explants on MS medium supplemented with 30 g/L sucrose and 2,4-D and Kin (1 and either 0.01 or 0.1 mg/L, respectively) for callus induction. After 6–8 weeks, somatic embryos developed on the same media composition and their maturation was achieved by increasing sucrose to 50 g/L. A good embryo to plantlet conversion (nearly 50 %) was achieved with 0.5 mg/L BA (Anjaneyulu et al. 2004). Interestingly, an *Agrobacterium*-mediated transformation protocol for this species has been described (Shyamkumar et al. 2007), which combined with embryogenic lines, will allow the genetic modification of this important Indian medicinal tree.

Paris polyphylla. This is a shrub, commonly known as "Chonglou" in Chinese, which is widely used in several Chinese medical preparations to treat conditions that include parotiditis and certain types of tumors. Rhizomes accumulate steroidal saponins with antitumor effects, as well as anthelmintic and antifungal properties (Xiao et al. 2009). Due to effectiveness of using rhizome for medicinal treatments, entire plants are collected from wild areas in Asian forests, endangering populations both in China and India. Using immature zygotic embryos as initial explants, direct somatic embryogenesis has been achieved using agar solidified 0.5× MS media. A good embryogenic response (higher than 30 %) was obtained using hormone-free media (Raomai et al. 2014). Moreover, when primary embryos were isolated and cultured on the same media, secondary, morphologically normal, embryos were formed. This response, however, was reduced after few subcultures, but pretreatment with 1 M mannitol for 12 h re-established the embryogenic potential (Raomai et al. 2014). Cotyledonary *Paris* embryos germinated to form entire seedlings, showing shoots, rhizomes, and radicles when they were exposed to 0.5 mg/L GA_3. Complete transition to healthy and vigorous plantlets required an exposure to 0.05 mg/L BA and 0.1 mg/L NAA (Raomai et al. 2014).

Thymus hyemalis. This is a herb, native to the West Mediterranean region, known as winter thyme. It belongs to the Lamiaceae family and produces essential oils in the leaves with antifungal, antibacterial, and insecticide properties, as well as antioxidant activity. Leaves also produce diterpenes such as thymol, carcavol, and borneol (Tepe et al. 2011), as well as other metabolites such as flavonones, rosmarinic acid, and triterpenes. Due to its wide use as a medicinal plant, as well as food preservative and seasoning, it is collected from wild populations, mainly in the Southeastern Iberic Peninsula (Jordán et al. 2006). In order to avoid plant over-extraction from its environment, a procedure for its massive in vitro propagation through somatic embryogenesis was formulated. Nodes of in vitro maintained shoots were used to generate embryogenic calli by exposure to 1.8 and 0.5 μM 2,4-D and NAA, respectively. Over 85 % of the explants formed embryogenic calli, which were allowed to develop for four weeks. Embryos were formed on calli exposed to 4.44, 0.54, or 4.65 μM of BA, NAA or Kin, respectively (Nordine et al. 2014). Embryo germination was achieved in hormone-free medium. Although over 90 % of the embryos formed shoots, rooting was not as efficient; nevertheless, it was induced ex vitro, by transplanting them to a 2:1 mixture of peat and vermiculite (Nordine et al. 2014). The complete process, from callus induction to nursery-established plantlets, took around three months to complete. Thus, this

scheme has been proposed for genetic improvement through gene transformation-embryogenesis scheme or to be integrated in a conservation program to avoid plant-over extraction from nature (Nordine et al. 2014). It has not been yet established if the patterns of secondary metabolites in plants derived from somatic embryos are comparable to those arising from seed germination.

25.4 Somatic Embryogenesis in Plants Used as Food Seasonings

Colorants, either dyes or pigments, represent a valuable market. In 2010, it was worth 1.8 billion dollars and has been globally increasing by 4.5 % annually. Natural colorants represent about one third of the total market (0.66 billion dollar), but it is increasing at a 6.7 % annual rate. Some projections estimate that by 2020 natural colorants' market will be worth around 2.0 billion dollars (see Caro et al. 2012). The food industry is the main consumer of natural colorants, followed by soft drinks and alcoholic beverages. Public concerns about possible health hazards associated to food colored with synthetic compounds have increased due to reports linking red and yellow additives to cancer and children hyperactivity (Nigg et al. 2012; Potera 2010). Although there is still some controversy, major manufactures of processed food products have turned to natural colorants, charging premium prices to consumers willing to acquire more natural products. Higher demands of natural colorants result from volumes required to replace the synthetic ones, but also from the larger quantities of these compounds used to obtain similar shades due to their sensitivity to storage and manipulation (Delgado-Vargas et al. 2000).

In the following paragraphs, selected examples of somatic embryogenesis of plant used to produce natural dyes are presented. Since, as it was mentioned above, main health concerns refer to red and yellow-orange shades, plants producing these colorants have been included, namely saffron and annatto for reds and curcuma and marigold for yellows. Horticultural plants, such as beet, berries, carrots, and chili peppers, which may also use as colorant sources, were not considered.

25.4.1 Crocus sativus *(Iridaceae)*

Saffron is the spice derived from the dry styles of *Crocus sativus* flowers. It is highly appreciated due to its intense red color and bitter taste and aroma, caused by the accumulation of three major carotenoids: crocetin glycosides, picrocrocin, and safranal (Carmona et al. 2006). Important medicinal properties, as an anticonvulsant, antidepressant, and anticancer agent, have also been claimed for this plant (Akhondzadeh et al. 2005). Saffron can reach high prices based on the difficulties encountered for its cultivation and also by the large amount of flowers (about

120,000) required to obtain 1 kg of the spice (Carmona et al. 2006). Since *Crocus* is male sterile, plants do not set seeds and are propagated using vegetative corms, which are produced in a reduced number (three to four per plant) in each growing season. Moreover, crop improvement is limited to the selection and vegetative propagation of naturally occurring variants (Carmona et al. 2006). The use of mass propagation techniques, such as somatic embryogenesis, could certainly help to overcome these limitations.

An efficient method to produce *Crocus* cormlets that will be used as propagation materials was developed, using leaf bases as initial explants. Direct formation of embryos was observed on MS medium supplemented with 2.5 μM TDZ and 2.0 μM picloram (Devi et al. 2014). Secondary embryo formation occurred on the same medium, but only after reducing picloram to 1.0 μM. These embryogenic cultures retained its capacity for over three years. A high conversion rate was recorded when embryos were cultured on media containing TDZ and picloram. Shoots, formed from these embryos, were propagated in BAP and NAA MS media (27 and 1 μM, respectively). Interestingly, in vitro formed shoots produced cormlets similar in size and behavior to those formed by field plants (Devi et al. 2014).

Retention of embryogenic capacity of *Crocus* (over 10 years) was also reported by Blazquez and coworkers in corm-derived cultures maintained on 2,4-D and BAP (Blazquez et al. 2009). Differentiation of somatic embryos from these cultures was achieved switching auxin to NAA (Blazquez et al. 2009).

25.4.2 Bixa orellana*(Bixaceae)*

The annatto plant is a perennial woody shrub from where the red pigment bixin is obtained. Bixin is an apocarotenoid accumulated in the seed arile and is used in the food industry to color different products, such as cheeses, charcuterie, and sauces (Rivera-Madrid et al. 2006). Problems with the production of bixin relate to genetic variation among populations resulting from the conventional heterozygous seed propagation of the plant (Rivera-Madrid et al. 2006). Propagation through somatic embryogenesis (Paiva Neto et al. 2003a) and other in vitro culture techniques (Paiva Neto et al. 2003a; Da Cruz et al. 2014) represents an interesting alternative to overcome this limitation (see also Monteiro Matos et al.; Chap. 14, this book).

Immature zygotic embryos exposed to a combination of 2,4-D and Kin (2.3 and 4.5 μM, respectively) on MS media supplemented with 1.0 g/L of activated charcoal turned to directly formed embryos after 25 days (Paiva Neto et al. 2003a). Mature embryos did not respond to any of the hormonal treatments assayed. Moreover, embryogenic response was related to the genotype employed (Paiva Neto et al. 2003a, b). Although a good primary embryogenic response was observed (close to 70 % of the explants formed embryos), transition to mature stages was very poor, with less than 5 % of the embryos reaching the cotyledonary

phase (Paiva Neto et al. 2003a). Interestingly, although *Bixa* embryos displayed both root and shoot meristems and a well-formed vascular system, very low number of them developed to form complete plants (Paiva Neto et al. 2003a).

25.4.3 Curcula longa *(Zingiberaceae)*

This plant from the Zingiberaceae family is also known as turmeric and frequently called the golden spice for its characteristic yellow-orange color. It is a common ingredient of sauces, mustards, soups, and butter, just to mention a few (Gang and Ma 2008). Color is the result of phenolic compounds accumulated in the rhizomes, collectively known as curcuminoids [i.e., curcumin (1,7-bis (4-hydoxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione), demethoxy-curcumin, and bismethoxy-curcumin]. Rhizomes are also used for medicinal purposes as an antihelmintic and antiviral, and most recently, for its antioxidant and anticancer activities (Gang and Ma 2008). Turmeric is propagated vegetatively through underground rhizomes, which result in a relative narrow genetic base. In fact, cultivars with highest curcumin contents are susceptible to fungal and bacterial infections, as well as to nematode infestation.

Indirect somatic embryogenesis from young inflorescences of *C. longa* (He and Gang 2014) has been reported and this method resulted in embryogenic suspensions' which were used in genetic transformation procedures. Use of Gamborg's B5 medium supplemented with 5 and 30 mg/L of NAA and BAP resulted in the formation of friable calli that later gave way to embryogenic suspensions. One week-old calli were co-cultivated with an infective *Agrobacterium tumefaciens* strain for 20 min and thereafter maintained on MS medium with 0.5 mg/L Kin and 30 mg/L NAA for embryo maturation. Shoot formation was achieved on a medium with similar composition, but reducing Kin to 0.3 mg/L (He and Gang 2014). Rootless shoots developed when Kin was eliminated and NAA concentration reduced to 10 mg/L (He and Gang 2014). Although this procedure was highly efficient, passage through callus phase might introduce undesirable genetic instability. Using leaf bases as initial explants, a direct embryogenic process was described by Raju and co-workers (Raju et al. 2015). Preincubation of explants in the dark on solid MS medium supplemented with 4.5 μM 2,4-D followed by a change to liquid media with 1.3 μM BAP and light exposure. Over 90 % of the explants formed primary embryos, from which secondary embryos readily arose by doubling BA concentration (Raju et al. 2015). Embryo maturation and further germination was achieved on half-strength MS media with 1.4 μM GA_3, although it was not indispensable. Over 80 % of the embryos produced entire plants (Raju et al. 2015).

Procedures for the somatic embryogenesis of other *Curcuma* species, such as *C. amada* and *C. caesia* (Raju et al. 2013), have also been reported.

25.5 Concluding Remarks

Plants that produce secondary metabolites represent a valuable natural resource. They can be considered either for commercial purposes, in order to obtain active principles for pharmaceutical formulations and other products, or as part of the heritage of ancient cultures around the world. Biotechnological approaches based on these plants are, therefore, focused on three main goals: increasing products yields, efficient propagation, and preservation of rare varieties. Somatic embryogenesis could be applied to all those objectives. In this way, the availability of embryogenic cell lines might allow the development of genetic transformation protocols designed to introduce regulatory or limiting step genes. Moreover, indirect embryo formation (after callus tissue was formed) may generate genetic variation in those cases where vegetative traditional propagation may have reduced the genetic base. However, once elite materials have been selected, clonal propagation is required in order to keep the genetic stability of those traits. Somatic embryogenic propagation offers the means to achieve such end.

On the other hand, secondary metabolite-producing plants are frequently collected in wild forests, which, in some cases are endangering wild populations. Propagation through somatic embryogenesis may help to restore such populations.

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