In Vitro Cellular & Developmental Biology - Plant (2019) 55:695–701
https://doi.org/10.1007/s11627-019-10007-5

PLANT TISSUE CULTURE

Alkaloid synthesis is coupled to shoot morphogenesis in *Argemone mexicana* L. (Papaveraceae) *in vitro* cultures

Miriam Monforte-González[1] · J. Germán Serrano-Gamboa[1] · Cecilia Guízar-González[2] · Ma. Lourdes Miranda-Ham[1] · Felipe A. Vázquez-Flota[1]

Received: 28 March 2018 / Accepted: 8 July 2019 / Published online: 5 August 2019 / Editor: Jorge Canhoto

Abstract

During the induction process of an *in vitro* callus culture of *Argemone mexicana* L. (Papaveraceae), the levels of two benzylisoquinoline alkaloids known as berberine and sanguinarine displayed opposing trends. While the berberine levels steadily decreased from the initial explant stage up to the early proliferation of unorganized parenchymatous cell masses, the sanguinarine content increased. Once the callus culture was established, sanguinarine was the primary alkaloid present and berberine could no longer be detected. However, upon shoot regeneration, the berberine accumulation recovered, but sanguinarine was found in the newly formed leafy tissue. After root formation, sanguinarine was relocated to this organ, whereas berberine was evenly distributed between both tissues. Explants from stem internodes did not form callus, and berberine—plus sanguinarine—containing axillary shoots emerged from lateral buds in the induction medium. In contrast to callus-derived shoots, no root formation was observed. Therefore, alkaloid synthesis in *A. mexicana in vitro* cultures is related to the level of tissue organization in different ways, and while berberine accumulation seems to require the presence of differentiated organs, this is not the case for sanguinarine. Moreover, leafy parts of rootless shoots acquired the capacity to accumulate sanguinarine, which is usually absent in aerial tissues of mature plants. However, when these shoots were rooted, sanguinarine was mainly located in the newly formed roots, while berberine was detected in the shoots at similar levels found in the roots.

Keywords *Argemone mexicana* · Berberine · Sanguinarine · Shoot regeneration

Introduction

Accumulation of the benzylisoquinoline alkaloids (BIA) berberine and sanguinarine follows a tissue and developmental pattern in prickly poppy (*Argemone mexicana* L., Papaveraceae; Xool-Tamayo *et al.* 2017a, b). Because both of these alkaloids have diverse industrial and medicinal applications (Rubio-Piña and Vázquez-Flota 2013), there is a renewed interest to produce them through cell culture technology. In a recent report, rootless shoot cultures of this plant were obtained. The presence of berberine, which is evenly distributed in aerial and underground tissues of *A. mexicana*, was observed in these shoot cultures. Interestingly, accumulation of sanguinarine, which is normally absent in the aerial tissues but abundant in roots, was also recorded (Xool-Tamayo *et al.* 2017a; Vázquez-Flota *et al.* 2018). Likewise, the onset of sanguinarine accumulation has been described in *A. mexicana* leaf-derived cell cultures, concomitantly with loss of berberine (Trujillo-Villanueva *et al.* 2010; Xool-Tamayo *et al.* 2017a). Modifications in the tissue-associated metabolite profiles are not uncommon upon introduction of *in vitro* cultures, as the morphological alterations that tissues undergo during the process also have an impact on their biosynthetic capacity (Murthy *et al.* 2014). However, loss of berberine accumulation in callus cultures derived from *A. mexicana* leaves is noteworthy for two reasons. First, berberine-producing *in vitro* cultures have been obtained from other species, including *Berberis buxifolia* Lam. (Alvarez *et al.* 2009), *Coptis japonica* (Thunb.) Makino (Sato *et al.* 2001), and *Thalictrum minus* L. (Hara *et al.* 1994). This

✉ Felipe A. Vázquez-Flota
felipe@cicy.mx

[1] Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán, Calle 42 No. 130 Chuburná, 97205 Mérida, Yucatán, Mexico

[2] Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, Unidad Zapopán, Camino el Arenero No. 1227 El Bajío del Arenal, 45019 Zapopan, Jalisco, Mexico

suggests that tissue organization is not required for the synthesis and accumulation of this alkaloid. Secondly, in contrast to *A. mexicana*, the presence of sanguinarine has neither been reported in the above-mentioned cultures, nor in the plant species from which they were obtained. Accumulation of sanguinarine in rootless shoot cultures is also remarkable because significant amounts of transcripts that correspond to key biosynthetic genes accumulate in aerial tissues in which the alkaloid is absent, such as stems of mature plants (Xool-Tamayo *et al.* 2017a, b; Vázquez-Flota *et al.* 2018). Therefore, although *A. mexicana* aerial tissues seem to have the capacity to produce and accumulate sanguinarine, in whole plants, it is primarily restricted to their underground parts.

Although berberine and sanguinarine belong to different BIA groups (protoberberine and benzophenanthridine, respectively), they share the early biosynthetic reactions, up to the formation of s-scoulerine, and then diverge into specific branches (see figures; Hagel and Facchini 2012). In this context, it is interesting to find possible interactions among the biosynthetic processes of berberine and sanguinarine in *A. mexicana*. In the present study, the modification in distribution patterns of each alkaloid in association with well-defined morphogenetic events was analyzed. Alkaloid distribution and expression of biosynthetic genes during callus formation from leaf explants to shoot regeneration and rooting were followed. Alkaloid and transcript distributions were also followed along axillary shoot formation from stem internodes, which did not form callus and did not respond to rooting treatments.

Materials and Methods

Plant materials and induction of *in vitro* cultures All chemical used for *in vitro* cultures were tissue culture grade from Sigma-Aldrich® (St. Louis, MO), unless specified. Seeds were germinated *in vitro* in Magenta™ GA-7 vessels boxes (Sigma-Aldrich®) on half-strength Murashige and Skoog (MS, Murashige and Skoog 1962) basal medium, without sucrose or growth regulators. Seeds were disinfected by successive immersions in 80% (*v*/*v*) ethanol, diluted NaClO solution [3% (*v*/*v*) active sodium hypochlorite], and sterile water for 1, 3, and 10 min, respectively, prior to being imbibed overnight in 5 μM gibberellic acid to promote germination. Once the seedlings were about 8 cm high and displayed between four and six pairs of leaves, both leaves and stem internodes were exscinded as explants. To induce callus or shoot formation, explants were placed on semisolid MS media contained in Magenta™ vessels supplemented with 20 g L^{-1} sucrose; 0.045, 0.225, 2.225, 4.45, or 22.25 μM thidiazuron (TDZ); and 0.2% (*w*/*v*) Gelrite™. All media were sterilized by autoclaving 30 min at 121°C (1.4 kg cm^{-2}), after adjusting the pH to 5.8 with 0.1 M HCl.

Six explants per vessel were used, and a total of six boxes per treatment were evaluated. Culture vessels were kept under continuous illumination with a photon density of 50 μmol m^{-2} s^{-1}, provided by a combination of 39-W fluorescent and 60-W incandescent lamps (Phillips, Alto Collection and Studio Collections, respectively; Philips de México, Cd de México, México) at a constant 25°C and 70% relative humidity. Shoots arose either from previously formed callus or directly from explants, depending on the initial tissue used. Newly formed shoots were placed on MS medium supplemented with 5 μM indole-3-butyric acid (IBA) for rooting. In parallel, callus that did not produce leafy structures was exscinded from leaf explants and maintained on MS media with 2 or 8 μM 6-benzylaminopurine (BAP), and 1 μM 1-naphthaleneacetic acid (NAA). Samples of callus, shoots, and roots were collected for alkaloid and transcript analysis in triplicate. The experiment was performed in two independent repetitions. Each portion was analyzed separately in those cases in which cultured tissues displayed sections with different organization levels in the same unit (callus and shoots portions in the same cluster).

Alkaloid extraction and analysis Solvents were from JT Baker (Phillipsburg, NJ). Alkaloids were extracted from 20 mg of freeze-dried powdered tissues (8 to 10 pieces of the different tissues) in 15 mL of methanol with continuous shaking at 50 rpm for 2 h at room temperature (27°C). After incubation, the debris was separated by centrifugation at 1400×*g*, and 1 mL of the supernatant was taken, centrifuged again at 1400×*g* to eliminate any remaining tissue, and the supernatant was reduced to dryness in vacuo. The residue was dissolved in 100 μL of methanol, and 1 μL was loaded on 10 × 20 cm (length × width) thin layer chromatography (TLC) silica gel 60 plate F_{254} (Merck KGaA; Darmstadt, Germany) for separation. Alkaloids were separated with mixtures of *n*-butanol:water:NH_4OH (8:1:1 *v*:*v*:*v*) and benzene:ethanol (9.4:0.6 *v*:*v*), as mobile phases to resolve berberine (R_f 0.32) and sanguinarine (R_f 0.36), respectively (Monforte-González *et al.* 2012). Samples were loaded 1 cm above the lower edge of the plate and chromatographed to up to 1 cm below the upper edge. After separation, alkaloids present in the extracts were identified by matching *Rf* values to commercial standards (Sigma-Aldrich®), and autofluorescence under exposure to λ_{365} radiation provided by a hand-held UV lamp (Spectroline ENF-240C; Westbury, NY). Berberine and sanguinarine displayed bright blue-green and orange-reddish tones, respectively. The identity of the alkaloids and chromatographic coincidences on the plates after separation had been previously confirmed by mass spectroscopy-liquid chromatography (*m*/*z* 370.16 for berberine and *m*/*z* 332.00 for sanguinarine; Monforte-González *et al.* 2012; Vázquez-Flota *et al.* 2018). After separation, alkaloids were quantified by in situ fluorescence in a Camag TLC Scanner 4 (Camag,

Muttenz, Switzerland), controlled by the WinCATS 1.4.10 planar chromatography manager (Camag). The excitation wavelength was set at 330 nm, with a 400-nm K400 Camag cut off filter (Monforte-González *et al.* 2012). Three independent analyses were performed for each tissue, and differences were assessed by Tukey's test at $p < 0.05$.

Nucleic acid analysis Extraction of total RNA from the collected tissues and additional analysis by Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) was performed as described by (Xool-Tamayo *et al.* (2017a, b). Selected transcripts were analyzed to monitor common reactions (*norcoclaurine synthase*; *NCS*), and the berberine- (*s-scoulerine O-methyltransferase*; *SOMT*) and sanguinarine- (*s-cheilanthifoline synthase*; *CheSyn*) committed biosynthetic branches, respectively (Xool-Tamayo *et al.* 2017b). The corresponding primer sequences were (5′ to 3′; forward and reverse) CATCGCTA ATTACGTTCTCAAGAATCA and ATAGTAGTACATGG AATTACCTGGATGGGA for *NCS*; ATCCTATCCATGTC TACGAGGGCTATT and CCAGTACCACCACCAACATC TAACA for *SOMT*; and AGGTCTTCAAGGTGTTGCCC and TCTTTTCCCGCCCGTAACAT for *CheSyn*. Primer design was based on accessions EU 88189, KT984756, and EF451152 for *A. mexicana NCS*, *SOMT*, and *CheSyn* (CYP719A14), respectively (Xool-Tamayo *et al.* 2017a, b). For RT-qPCR, first strand cDNA was synthetized from 1 μg of total RNA that was mixed with 500 ng of oligo (dT) 18 primer, 1 mM of dNTPs, and 200 U of M-MLV Reverse Transcriptase (Invitrogen; Carlsbad, CA), in a total volume of 20 μL, following the manufacturer's instructions. RNA was previously treated with 1 U of DNAse I (Invitrogen) for 30 min at 25°C. The cDNA produced was adjusted to 5 ng and subjected to PCR in a final volume of 25 μL, containing 10 μL of SYBR™ Master Mix (Applied Biosystems™, Warrington, Cheshire, UK), with 10 μM of each primer and 2 U of Taq Pol (Invitrogen). Polymerase chain reactions were performed using Eco™ Real-Time PCR System (Illumina, San Diego, CA), following a program of 35 cycles of 60, 30, and 30 s at 95, 62, and 72°C for DNA denaturation, primer alignment, and amplification, respectively. Previously, the reaction mix was heated for 3 min at 95°C for enzyme activation. Gene transcripts were quantified using the ΔΔCq method (Simon 2003), which compared amounts of the targeted transcript with those of actin, as the reference (Simon 2003). Actin primer sequences were (forward and reverse 5′ to 3′) CACIACTACTGCTAAACGGGAAA and ACATCTGC TGGAAGGTGCTG, respectively). The cycle threshold value (Ct) for each PCR was calculated using the EcoStudy software (Illumina). Samples were analyzed in triplicate and the differences were assessed by Tukey's test at $p < 0.05$.

Results

Morphogenetic response of *A. mexicana* tissues Most treatments resulted in callus formation on the leaf explants. Callus proliferation was observed over the edges of the tissues after 2 wk in culture (Fig. 1*a*) and leafy structures formed on callus, turned into well-formed shoots after two additional weeks (Fig. 1*b*). The organogenetic response differed among TDZ treatments, ranging from 0.3 to 1.8 shoots per explant ($p < 0.05$; one-way ANOVA, Fig. 1*b*). The highest value was registered with 22.25 μM TDZ, and shoots formed on this treatment were used for additional characterization. After remaining one extra week in the induction media (a total of 5 wk), shoots were transplanted to the rooting media, and root development was detected after 1 wk (Fig. 1*c*). These roots continued to extend for the rest of the experimental period for two additional wk.

Internode explants developed axillary shoots from lateral buds in less than 10 d in culture, without any callus formation (Fig. 1*d*). Using the assay conditions, one single shoot formed from each bud, with average responses ranging between 0.5 and 2 shoots per explant in the different treatments (each internode presented two opposite buds). The maximum response was detected with 22.25 μM TDZ. Individual axillary shoots were exscinded from explants and cultured on rooting media, and there was no root formation recorded after 2 wk.

Two sets of shoot cultures, which differed both in origin and in their ability to form roots, were obtained, which were regenerated shoots, which displayed rooting capacity (Fig. 1*c*), and axillary shoots that were non-responsive to rooting treatments (Fig. 1*d*).

Alkaloid content in *in vitro A. mexicana* regenerated and axillary shoots Berberine and sanguinarine were quantified on both sets of *A. mexicana* shoots at different phases of the *in vitro* culture process (Fig. 2). Berberine was the only alkaloid detected in the initial explants of both leaves and stem internodes. However, disorganization of leaf explants into callus tissue resulted in the onset of sanguinarine accumulation, which was simultaneous with the loss of berberine (Fig. 2*a*). It should be noted that the leaf portion in explants kept its original alkaloid profile, which consisted in the presence of berberine, but not sanguinarine. Shoot development from callus tissue restored berberine accumulation and maintained levels of sanguinarine. However, upon rooting the regenerated shoots, the sanguinarine accumulation was switched from the leafy parts to the roots (see the *Shoot*/*Roots* bar cluster in Fig. 2*a*). In contrast, berberine levels in leafy tissues of the regenerated shoots remained similar to those of the roots, once this organ was formed (Fig. 2*a*).

The capacity of sanguinarine accumulation, along with berberine, was also observed in the axillary shoots, which developed from the lateral internode buds. Interestingly, the stem

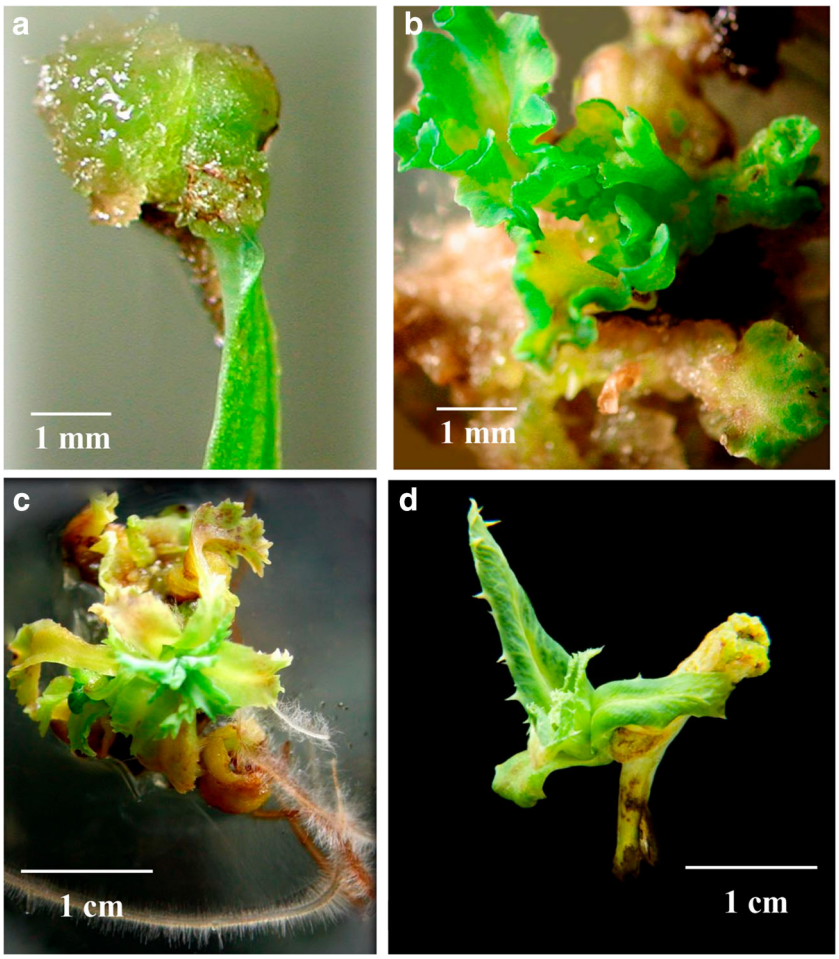

Figure 1. *In vitro* cultures of *Argemone mexicana* tissues. Callus induced from leaves (*a*). Shoot regeneration from leaf-callus (*b*). Rooting of regenerated shoots (*c*). Shoot development from axial stem internode (*d*).

portion of these explants that did not suffer any morphological alteration showed a decreased berberine level but did not acquire the capacity to accumulate sanguinarine (Fig. 2*b*). This suggests that these effects were not related to the culture time but rather to the morphogenetic events, as was also recorded for the leaf explants.

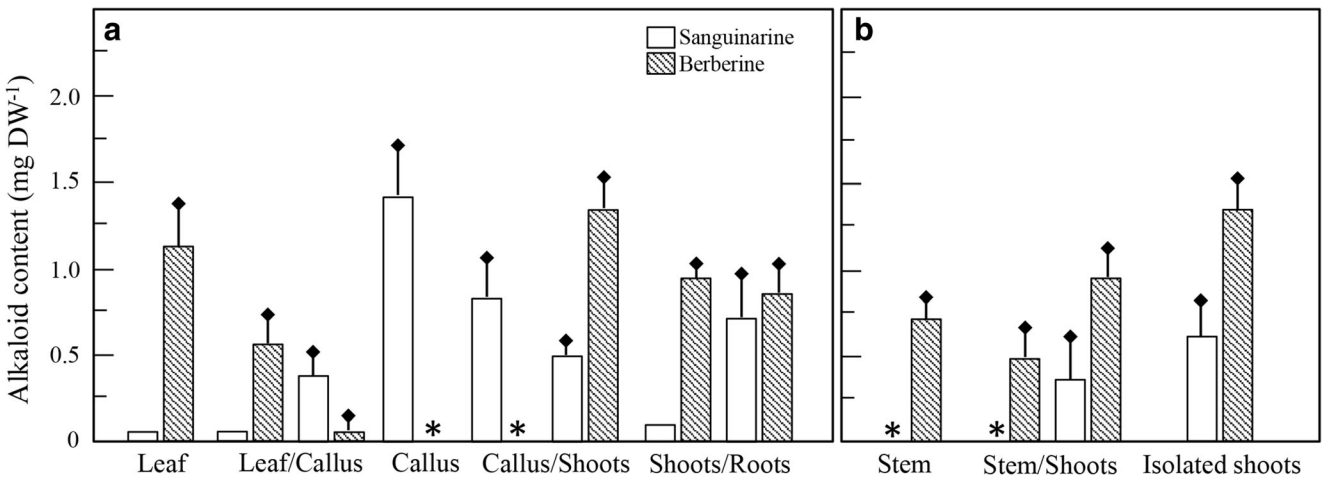

Figure 2. Alkaloid distribution in *Argemone mexicana* tissues submitted to *in vitro* culture. Cultures induced from leaves (*a*) and stem internode explants (*b*). Sets of *bars* above labels interrupted by a *backslash* (/) symbol correspond to masses composed of two different tissues (either leaf or stem explants vs callus and shoots newly formed). Each type of tissue was independently analyzed. *Bars* represent the mean values of three repetitions with standard deviation, whereas *asterisks* (*) represent not detected. *Bars* with the same literal in boldface (berberine) or italics (sanguinarine) presented non-significant differences (Tukey's test at $p < 0.05$).

Tissue distribution of transcripts involved in alkaloid biosynthesis in the *A. mexicana* regenerated and axillary shoots

The distribution of selected transcripts involved in alkaloid biosynthesis was followed through the different morphogenetic stages for both sets of regenerated *A. mexicana* shoots to analyze their biosynthetic capacities. Markers for the early common biosynthetic reactions (*NCS*), and for those specifically committed to either sanguinarine (*CheSyn*), or berberine (*SOMT*), were chosen (Fig. 3). In concordance with the alkaloid profiles of the initial leaf (Fig. 3*a*) and stem internode (Fig. 3*b*) explants, which only presented berberine levels, RT-qPCR revealed high transcript levels for *NCS* and *SOMT*, which differed from those corresponding to *CheSyn*. However, upon leaf differentiation into callus, the *CheSyn* transcripts markedly increased, whereas those for *SOMT* dropped significantly ($p < 0.05$ Tukey's test; Fig. 3*a*). This trend in *CheSyn* and *SOMT* transcript levels was always observed in calluses that were either still attached to the original leaf explants, isolated as an independent culture, or during the development of new shoots (Fig. 3*a*). Transcripts for *SOMT* were recovered in the regenerated rootless shoots, whereas there were similar transcript levels for *CheSyn* (see the *Callus*/*Shoot* bar cluster in Fig. 3*a*). However, when roots were formed, *CheSyn* transcripts were mainly detected in the tissue with shoots that displayed levels just above the detection limit, unlike those observed for *SOMT* transcript distribution (Fig. 3*a*). Therefore, the distribution of both *CheSyn* and *SOMT* transcripts matched those of their corresponding sanguinarine and berberine alkaloids. On the other hand, although some variations were observed, *NCS* transcripts remained in fair amounts, regardless of the tissue organization observed (Fig. 3*a*).

Similar to the regenerated shoots, rootless axillary shoots showed a high *CheSyn* expression level, while the *SOMT* level was constant (Fig. 3*b*), coincidental to the alkaloid distribution (Fig. 2*b*). In addition, stem sections that did not present morphological alterations retained both transcript and alkaloid profiles as the original explants (Fig. 3*b*).

Discussion

This research was conducted to establish a better understanding of morphogenesis-related alkaloid biosynthesis in *A. mexicana* tissues. This was accomplished by developing an organogenesis regenerating protocol, which started with leaf explants on medium containing TDZ. Shoots that emerged from leaf-derived callus were then rooted on IBA (Fig. 1*a*–*c*). Although axillary shoots were also obtained from stem internode explants, these could not be rooted (Fig. 1*d*). The lack of rooting has also been noticed in multiple adventitious shoots derived from *A. mexicana* hypocotyls, which did not develop roots when exposed to different treatments (Xool-Tamayo *et al.* 2017a). Moreover, isolated roots displayed restricted growth in *in vitro* cultures (Xool-Tamayo *et al.* 2017a). This suggests a limited *in vitro* rooting capacity for this plant. In fact, although shoot regeneration from *Argemone* callus was lower than in other Papaveraceae species, such as *Papaver somniferum* L. (Park and Facchini 2000), and *Papaver bracteatum* Lindl. (Rostampour *et al.* 2010), all of the shoots were successfully rooted.

In vitro morphogenetic events affected the accumulation of sanguinarine and berberine differently. Leaf explants presented abundant berberine amounts but lack of sanguinarine accumulation. During callus formation, these explants lost the former, while acquiring the latter (Fig. 2*a*). This effect was directly related to tissue disorganization because explant portions not forming callus retained their original alkaloid profile (Fig. 2*a*). This was also observed in stem explants (Fig. 2*b*).

Interestingly, those callus tissues that were collected from the explants and kept on MS media with 2 μM or 8 μM BAP and 1 μM NAA were able to proliferate and maintain the same

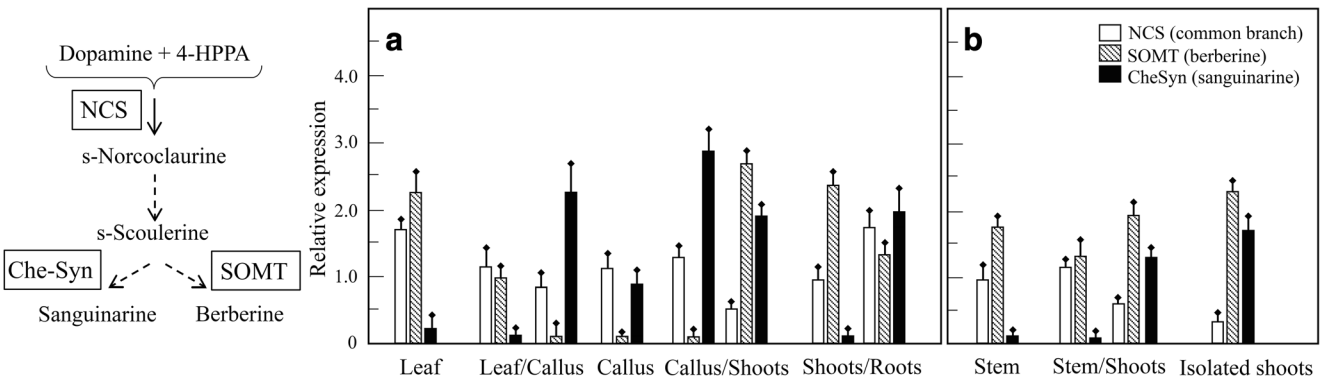

Figure 3. Alkaloid biosynthetic transcript distribution in *Argemone mexicana* tissues submitted to *in vitro* culture. Cultures induced from leaves (*a*) and stem internode explants (*b*). Sets of *bars* above labels interrupted by a *backslash* (/) symbol correspond to masses composed of two different tissues (either leaf or stem explants vs callus and shoots newly formed). Each type of tissue was independently analyzed. *Bars* represent the mean values of triplicates with standard deviation. *Diagram at the left* depicts the condensed biosynthetic sanguinarine and berberine pathways.

alkaloid pattern as those initially collected from the leaf explants (loss of berberine, but presence of sanguinarine) (Guízar-González *et al.* 2012). Moreover, different induction treatments, such as the exposure to methyl jasmonate, salicylic acid, or yeast extract, did not modify this pattern, although sanguinarine accumulation increased (Trujillo-Villanueva *et al.* 2010; Guízar-González *et al.* 2012, 2016).

Although alkaloid biosynthetic capacity is frequently lost during tissue disorganization (Murthy *et al.* 2014), berberine-producing *in vitro* cultures have been reported for other species of the Papaveraceae family (Day *et al.* 1986; Facchini and Bird 1998; Zakaria *et al.* 2011). This suggests that berberine biosynthesis does not require tissue integrity (Hara *et al.* 1994; Sato *et al.* 2001; Alvarez *et al.* 2009). In contrast, the development of a novel sanguinarine accumulating capacity has been observed in hypocotyl-derived *P. somniferum* cell cultures, after fungal elicitation (Facchini *et al.* 1996). Interestingly, sanguinarine was also found in well-differentiated leafy parts of callus-regenerated shoots, but it only occurred in the absence of roots (Fig. 2*a*). Upon root formation, it was switched to this new location (Fig. 2*a*). In contrast, berberine accumulation in these leafy tissues remained unaffected by rhizogenesis. It should be noted that the presence of both sanguinarine and berberine always coincided with *CheSyn* and *SOMT* expression, which are required in the corresponding biosynthetic pathways (Fig. 3*a*), which points to a direct involvement of the tissue in this process.

The acquisition of sanguinarine accumulating capacity, while keeping that of berberine, which was observed in the newly formed leafy tissues of the regenerated shoots, was also noted in axillary shoots, formed from internode stem explants (Fig. 2*b*). Moreover, this trend has also been recorded in hypocotyl-derived *A. mexicana* rootless shoots (Xool-Tamayo *et al.* 2017a). Unfortunately, rooting has only been achieved in callus-regenerated shoots (Fig. 1. X). Rooting has not been reached in neither the axillary shoots obtained in this work nor in those hypocotyl-derived (Xool-Tamayo *et al.* 2017a) in order to confirm the sanguinarine distribution pattern observed in regenerated shoots. However, during seedling development, sanguinarine was detected in newly unfolded leaflets up to the formation of secondary roots, when it markedly decreased in these tissues to be relocated to the roots. In contrast, berberine presence in aerial parts was unaffected by root development (Xool-Tamayo *et al.* 2017b), as it was in the regenerated rooted shoots in the present study (Figs. 2*a* and 3*a*). Taken together, these data suggest that in *A. mexicana*, roots might play a role to define the final alkaloid distribution, rather than to confer biosynthetic capacities. Because synthesis of sanguinarine and berberine utilizes *s*-scoulerine as a common late intermediary (see Fig. 3), underlying molecular mechanisms seem to be related to the preferential expression of the committed biosynthetic pathway for each alkaloid (Fig. 3*a* and *b*).

Funding Information This research was financially supported by CONACYT, Mexico (grant CB-2016-01-0285887).

References

Alvarez MA, Eraso NF, Pitta-Alvarez SI, Marconi PL (2009) Two-stage culture for producing berberine by cell suspension and shoot cultures of *Berberis buxifolia* Lam. Biotechnol Lett 31:457–463. https://doi.org/10.1007/s10529-008-9875-2

Day KB, Draper J, Smith H (1986) Plant regeneration and thebaine content of plants derived from callus culture of *Papaver bracteatum*. Plant Cell Rep 5:471–474. https://doi.org/10.1007/BF00269645

Facchini PJ, Bird DA (1998) Developmental regulation of benzylisoquinoline alkaloid biosynthesis in opium poppy plants and tissue cultures. In Vitro Cell Dev Biol-Plant 34:69–79. https://doi.org/10.1007/BF02823126

Facchini PJ, Johnson AG, Poupart J, De Luca V (1996) Uncoupled defense gene expression and antimicrobial alkaloid accumulation in elicited opium poppy cell cultures. Plant Physiol 111:687–697. https://doi.org/10.1104/pp.111.3.687

Guízar-González C, Monforte-González M, Vázquez-Flota F (2016) Yeast extract induction of sanguinarine biosynthesis is partially dependent of the octadecanoic pathway in cell cultures of *Argemone mexicana* L., the Mexican poppy. Biotechnol Lett 38:1237–1242. https://doi.org/10.1007/s10529-016-2095-2

Guízar-González C, Trujillo-Villanueva KA, Monforte-González M, Vázquez-Flota F (2012) Sanguinarine and dihydrosanguinarine accumulation in *Argemone mexicana* (L) cell suspension cultures exposed to yeast extract. J Mex Chem Soc 56:19–22 https://cicy.repositorioinstitucional.mx/jspui/handle/1003/748

Hagel JM, Facchini PJ (2012) Subcellular localization of sanguinarine biosynthetic enzymes in cultured opium poppy cells. In Vitro Cell Dev Biol-Plant 48:233–240. https://doi.org/10.1007/s11627-012-9426-3

Hara M, Tanaka S, Tabata M (1994) Induction of a specific methyltransferase activity regulating berberine biosynthesis by cytokinin in *Thalictrum minus* cell cultures. Phytochemistry 36:327–332. https://doi.org/10.1016/S0031-9422(00)97070-5

Monforte-González M, Guízar-González C, Rubio-Piña J, Carrillo-Pech M, Vázquez-Flota F (2012) Berberine and sanguinarine quantitation in *Argemone mexicana* L. (Papaveraceae) tissues by TLC-in situ fluorography. J Planar Chromatogr 25:358–360. https://doi.org/10.1556/JPC25.2012.4.14

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Murthy HN, Lee EJ, Paek KY (2014) Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. Plant Cell Tissue Organ Cult 118:1–16. https://doi.org/10.1007/s11240-014-0467-7

Park SU, Facchini PJ (2000) *Agrobacterium*-mediated transformation of opium poppy, *Papaver somniferum*, *via* shoot organogenesis. J Plant Physiol 157:207–214. https://doi.org/10.1016/S0176-1617(00)80192-3

Rostampour S, Sohi H, Dehestani A (2010) *In vitro* regeneration of Persian poppy (*Papaver bracteatum*). Biologia 65:647–652. https://doi.org/10.2478/s11756-010-0079-6

Rubio-Piña J, Vázquez-Flota F (2013) Pharmaceutical applications of the benzylisoquinoline alkaloids from *Argemone mexicana* L. Curr Top Med Chem 13:2200–2207. https://doi.org/10.2174/15680266113139990152

Sato F, Hashimoto T, Hachiya A, Tamura K, Choi KB, Morishige T, Fujimoto H, Yamada Y (2001) Metabolic engineering of plant alkaloid biosynthesis. Proc Natl Acad Sci U S A 98:367–372. https://doi.org/10.1073/pnas.98.1.367

Simon P (2003) Q-Gene: processing quantitative real-time RT–PCR data. Bioinformatics 19:1439–1440. https://doi.org/10.1093/bioinformatics/btg157

Trujillo-Villanueva K, Rubio-Piña J, Monforte-González M, Vázquez-Flota F (2010) *Fusarium oxysporum* homogenates and jasmonate induce a limited sanguinarine accumulation in *Argemone mexicana* cell cultures. Biotechnol Lett 32:1005–1009. https://doi.org/10.1007/s10529-010-0252-6

Vázquez-Flota F, Rubio-Piña J, Xool-Tamayo J, Vergara-Olivares M, Tamayo-Ordoñez Y, Monforte-González M, Guízar-González C, Mirón-López G (2018) Tissue distribution of transcripts involved in the biosynthesis of benzylisoquinoline alkaloids in *Argemone mexicana* L (Papaveraceae). Rev Fitotec Mex 41:13-21 https://www.revistafitotecniamexicana.org/documentos/41-1/2r.pdf

Xool-Tamayo J, Serrano-Gamboa G, Monforte-González M, Mirón-López G, Vázquez-Flota F (2017a) Development of newly sanguinarine biosynthetic capacity in *in vitro* rootless shoots of *Argemone mexicana* L. Mexican prickly poppy. Biotechnol Lett 39:323–330. https://doi.org/10.1007/s10529-016-2250-9

Xool-Tamayo JF, Monforte-González M, Rubio-Piña J, Mirón-López G, Vázquez-Flota F (2017b) Early developmental onset of alkaloid biosynthesis in Mexican poppy (*Argemone mexicana* L) Papaveraceae. Phytochem Lett 20:300–305. https://doi.org/10.1016/j.phytol.2016.12.020

Zakaria RA, Hour MH, Zare Z (2011) Callus production and regeneration of the medicinal plant *Papaver orientale*. African J Biotechnol 10:11152–11156. https://doi.org/10.5897/AJB11.204