Morphohistological development of the somatic embryo of *Typha domingensis*

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Abstract

Background. The sustainable methods of propagation for *Typha domingensis* through somatic embryogenesis can help to mitigate its current condition of ecological marginalisation and overexploitation. Then, the hypothesis established that the variation of the concentration of auxin and light conditions in sequential stages of culture generate different morphogenetic routes that can be monitoring by morphohistological markers.

Methods. Murashige and Skoog medium at half ionic strength, 3% sucrose and 0.1% ascorbic acid were used in the induction, proliferation and embryogenic maturation. Induction started with aseptic germinates cultured in 0.5 mg L^{-1} of 2,4-dichlorophenoxyacetic. Four concentrations of 0 to 2 mg L^{-1} of 2,4-dichlorophenoxyacetic, that generated four embryogenic lines, were evaluated in darkness. Maturation of the somatic embryo took place, in each embryogenic line, without auxin and under light and dark conditions.

Results. The yellow and brown callus, as well as oblong and scutellar somatic embryos were recorded in the methodological sequence. The embryogenic differentiation was described with histological analysis. The induced cultures produced both somatic embryos in a small proportion. The percentages of the yellow callus on the explant and of suspended cells in the embryogenic proliferation were greater with the three concentrations of 2,4-dichlorophenoxyacetic. While, the brown callus predominated without auxin. The somatic embryo developed under light and dark conditions, and presented globular, oblong, scutellar and sparsely coleoptilar stages.

Discussion. The combined effect of auxin concentrations and light-dark conditions generated conditions that favoured the development of embryogenic calluses and somatic embryos (globular, oblong, scutellar and coleoptilar) in an asynchronous process with respect to the stages of embryogenic induction, proliferation, and maturation. Indeed, differentiation and cellular

organization of this process were compatible with descriptors of the embryogenic stages recorded by other aquatic and terrestrial monocotyledons.

Keywords: emerging aquatic macrophyte, embryogenic maturation, sustainable propagation, somatic embryogenesis, histodifferentiation

Introduction

Anthropogenic impacts on wetlands threaten environmental processes and services related to native aquatic vegetation. The emerging rooted macrophyte *Typha domingensis* Pers. (bulrush) is a frequent component of the herbaceous associations that dominate the wetlands of Central and North America (Reddy *et al.* 2010). This emerging rooted macrophyte sequesters and stores carbon from the atmosphere provides critical habitats that sustain a high biodiversity and purifies eutrophic and polluted water (Thorp *et al*. 2006; Mitsch *et al.* 2013).

Typha populations invade commercial croplands located in flood areas, for which reason they are subject to control measures (Mora-Olivo *et al.* 2013; Harrison *et al.* 2017). One agricultural management strategy used in the case of *Oryza sativa* substitutes cultivars with genetically improved varieties that have an allelopathic effect on weeds (Jarchow & Cook 2009). Paradoxically, the genus *Typha* has been proposed as raw material in the production of biofuel due to its ideal fatty acids composition and lignocellulosic biomass, and it is planned to justify its use through a sustainable production model (Liu *et al.* 2012; He *et al.* 201; Ruiz-Carrera *et al.* 2016). Therefore, its populations are threatened by fragmentation, changes in land use and agricultural practices in wetlands (Thorp *et al*. 2006; Erwin 2009; Palomeque *et al*. 2017).

The technological challenge to solve the uncertain future of *T. domingensis* will be to develop propagation methods that are independent of its extraction from the natural environment in order to sustain both its re-population and the supply of raw material.

The *in vitro* technology of asexual or somatic embryogenesis is applied to conserve and propagate germoplasm, and to sustainably produce genetic varieties (von Arnold *et al*. 2002; Sánchez-Chiang & Jiménez 2010; Reed *et al*. 2011). It has contributed greatly to explain the physiological, biochemical and molecular mechanisms of the sexual embryogenic process (Quiroz-Figueroa *et al*. 2006; Smertenko & Bozhkov 2014; Mahdavi-Darvari *et al*. 2015). However, its application in the case of aquatic monocotyledons has been scant.

Somatic embryos (SE) develop from somatic cells and are similar to zygotic embryos (haploid or diploid), but differ among genotypes, *in vitro* induced embryogenic routes and the sequential development of the somatic embryogenesis method. Histological studies have helped understand these method-related differences (Máthé *et al.* 2000; Burris *et al.* 2009; Vega *et al.* 2009). Somatic embryogenesis is a multi-phases *in vitro* culture process that implies the previous installation of the cellular capacity to respond to external molecular signals (von Arnold *et al*. 2002). During the inductive phase, the activation of signals by auxins causes cellular re-programmation towards embryogenic differentiation (Elhiti *et al.* 2013; Fehér 2015). Embryogenic induction has been possible in aquatic species such as *Phragmites australis* (Máthé *et al.* 2000), *Brasenia schreberi* (Oh *et al.* 2008) and *T. angustifolia* (Rogers 2003), with stimuli from the 2,4-D auxin. However, in advanced stages, the elimination of or a reduced concentration of auxin favours the development of a competent embryo (von Arnold *et al*. 2002; Quiroz-Figueroa *et al.* 2006; Smertenko & Bozhkov 2014). Also, the variation of light condition has influenced the formation and maturation of SE at the anatomical and biochemical levels (von

Aderkas *et al.* 2015; Klubicová *et al.* 2017). The SE passes through the same development stages as the zygotic embryo, and it is possible to follow its morphogenetic route on a map that details the cellular and tissue markers of the development stages of the somatic embryogenesis (Radoeva & Weijers 2014).

The hypothesis was based on the factors that stimulate and regulate the process of somatic embryogenesis of *T. domingensis* in morphogenetic routes that can be monitoring by morphohistological markers. Therefore, the concentration of the embryogenic 2,4-D phytoregulator and the extreme conditions of light in the sequential stages of crop condition determine the expression of embryogenic competence and drive the somatic embryo morphogenesis. Thus, the purpose of the study was to describe the morphohistological process that leads to the maturation of the *T. domingensis* SE by modifying the process of embryogenic proliferation along a 2,4-D gradient and in contrasting light-dark conditions during maturation.

Materials & Methods

Preparation of the germinates

Mature *T. domingensis* seeds were collected in the catchment area of the Grijalva river in the city of Villahermosa (17º59' N and 92°57' W), located in the basin of the Grijalva-Usumacinta rivers. Seeds with no perianth were obtained following the methods of Lorenzen *et al.* (2000) and were pre-sterilized in 30% (v/v) ethanol for 10 min and thereafter sterilized in 10% (v/v) bleach (Cloralex, Mexico) solution for 10 min, rinsed three times in water sterile type 2 pure (México) and cultured under aseptic conditions. The seeds germinated in the sterile culture unit in a ratio of 1:50 g mL^{-1} purified water. The culture container was a 5 cm Ø 7 cm high glass flask with a

Magenta® polycarbonate lid, previously autoclaved with the aqueous medium at 121 °C and 104 kPa every 25 min in Esterilizer SM300 (Yamato scientific, Japan).

Phases of somatic embryogenesis

The production of SE generally involves three main phases: (1) induction, (2) proliferation of embryogenic cultures and (3) development of embryos (von Arnold 2002; Saenz *et al*. 2006). The culture medium in the three embryogenic phases was prepared with the mixture of basic salts of Murashige and Skoog (1962) at half the ionic strength ($MS_{0.5}$), MS vitamins, 3% sucrose and 10 mg L^{-1} ascorbic acid as antioxidant, all components of the medium were products Sigma-Aldrich (St. Louis, MO). The culture medium was sterilised under the conditions described for the germination. The macroscopic embryogenic products described in the section of evaluated responses were transferred to a fresh medium using a 6" straight round-pointed tweezer in a laminar flow hood (VECO, Mexico), in order to satisfy the objectives of each somatic embryogenesis phase. The culture time of each phase was 28 days.

Environmental control

The cultures were incubated under 16 h photoperiod with light intensity of 20 µmol $m^{-2}s^{-1}$ (Quantum light meter, Spectrum Technologies, Inc), provided by cool white fluorescent lamps (Phillips, E.U.A) at 28±2 °C during the germination and throughout the experiments. The culture units with three germinates were stirred at 125 rpm. The cultures in darkness were kept in closed darkness.

Embryogenic evaluation

Two independent experiments were carried out to analyse the culture environment of the different stages of development of the somatic embryogenesis of *T. domingensis.* Embryogenic induction started with aseptic germinates (9 days) cultured in 0.5 mg L^{-1} of 2,4-D in a dark environment

(N=48). The first experiment evaluated the embryogenic proliferation at four concentrations: 0, 0.5, 1 and 2 mg L^{-1} of 2,4-D and in darkness. Embryogenic lines of each treatment of 2,4-D were named to brown callus 0 mg L^{-1} of 2,4-D (BC0), yellow callus 0.5 mg L^{-1} of 2,4-D (YC1), yellow callus 1 mg L^{-1} of 2,4-D (YC2), and yellow callus 2 mg L^{-1} of 2,4-D (YC3). In the second experiment, the maturation of the SE took place starting from the cultures of the four embryogenic lines of the first experiment, but with no phytoregulator in the culture medium and under light (L) and dark (D) conditions, generating eight new embryogenic lines (BC0D, YC1D, YC2D, YC3D and BC0L, YC1L, YC2, YC3L).

Evaluated responses

The scale for absence and presence in the cultures of the products of embryogenic origin (percentage of adhered to the explant and of suspended) was used in the three embryogenic phases. Yellow callus, brown callus, oblong SE (oSE), scutellar SE (scSE) and cellular suspensions were identified according to their morphology and following Dodeman (1997), Fehér *et al.* (2003), von Arnold *et al.* (2002) and Quiroz-Figueroa *et al.* (2006). These observations were made weekly with a Zeiss Stemi DV4 stereomicroscope (Zeiss, Göttingen, Germany).

Description of the histological process

The embryogenic products, adhered to the explant and suspended, were collected from 30% of the culture units at each phase. The embryogenic products representative of each treatment were preserved in a FAA (formaldehyde-acetic acid-ethanol) solution for 24 h, dehydrated in a graded ethanol series of 70 to 100% (30 min per step) and clarified with 1:1 ethanol-xylol and 100% xylol for 1 h (Filonova *et al.* 2000). The embryogenic structures were then embedded in xylol:paraffin (Paraplast®, Sigma-Aldrich, St. Louis, MO) using a Reichert-Jung Mod 8044 automatic tissue embedding center (Cambridge Instruments GmbH, Buffalo, NY) in order to

obtain 6 µm thick serial cross-sections with a Reichert-Jung Mod. Hn 40 sliding microtome (Cambridge Instruments GmbH, West-Germany). Toluidine blue and hematoxylin-eosin, both at 0.2%, were used for dyeing. The histological preparations were analysed using a Zeiss Axiostar Plus photo-microscope (Carl Zeiss, Göttingen, Germany) equipped with a Zeiss Axio Cam model MRc5 digital camera (Carl Zeiss, Göttingen, Germany). The analysis of the differentiation of embryogenic cells and tissues was qualitative and the descriptions were compared with histological markers described for species of the same order (Máthé *et al.* 2000; Meneses *et al.* 2005; Burris *et al.* 2009; Vega *et al.* 2009). In addition to oSE and scSE, were identified as response variables to the embryogenic products globular SE (gSE) and coleoptilar SE (colSE).

Statistical analyses

Normality (Kolmogorov-Smirnov) and homocedasticity (Cochran) tests were applied to the embryogenic products of each experiment in order to decide on the application of univariate and multivariate parametric (ANOVA) and non parametric (Kruskal-Wallis) tests. The *a posteriori* comparison of averages was carried out using Fisher's technique. The statistical probability value was $p<0.05$ Statistica (StatSoft V8, 2007).

Results

Embryogenic induction

Of the induced cultures, 73% formed yellow calli, 30% brown calli and 50% suspended cells. The production of oSE (Figure 1g) and scSE (Figure 1j) occurred earlier in 6.25% of the cultures in this phase.

Embryogenic proliferation

The proliferation of calli on the explant and of suspended cells was significant due to the 2,4-D (Table 1). The percentage of yellow calli adhered to the explant and of suspended cells was greater in the presence of 2,4-D; the number of cultures with oSE and scSE and all the embryos increased less with respect to the first stage (Table 1). In contrast, brown calli on the explant predominated without 2,4-D.

Maturation of the somatic embryo

With respect to the previous phase, light decreased the proliferation of yellow calli in the cultures and increased brown callus in the BC0L to YC2L embryogenic lines. In darkness, the yellow callus remained unchanged, except in embryogenic line YC3D which recorded a notable increase (Table 2). The cultures with suspended yellow calli were relatively similar among the 2,4-D embryogenic lines ($p<0.05$), although both in light and darkness they produced abundant suspended cells due to the friability of this callus. In the absence of 2,4-D, the light and darkness controls presented the greater number of cultures with brown calli adhered to the explant, but no production of SE. In contrast, embryogenic line YC3L presented the greater percentage of cultures with total SE, dominating the scSE on the explant and the suspended oSE, which in turn coincided with the release of the brown callus of the explant. The same occurred in YC1, but to a lesser degree and with no formation of scSE.

Histological descriptions

The calli of *T. domingensis* presented embryogenic cells and early and late embryogenesis (Figure 1). The nodular yellow callus (Figure 1a) presented zones of great mitotic activity formed by small and isodiametric cells, with strongly dyed prominent nuclei (Figure 1b) and zones with acquisition of embryogenic adeptness (Figure 1c). The three culture phases promoted proembryogenesis and early and late embryogenesis (Figure 1). Proembryogenesis was

associated with the presence of nodular yellow calli through the formation of induced proembryogenic masses (PEM). The gSE originated in the PEM presenting radial development and differentiation of three primary meristematic tissues. The gSE (Figure 1e) presented the three fundamental meristems and the suspensor (Figure 1f). A reduction of the suspensor was observed in the embryogenic stages that followed. The elongation of the gSE was originated the oSE (Figure 1g), the oSE presented parenchyma with abundant amyloplasts (Figure 1i). The embryogenic stages that followed were the scSE (Figure 1j) and the colSE (Figure 1m), both with vascular cells, reserve parenchyma and a defined axis. The colSE was made evident by the presence of the coleoptile (Figure 1ñ). Late embryogenesis was demonstrated by the presence of polarity and tissue differentiation. However, the identification of the late embryogenic stages was difficult due to the abundance of embryos with aberrant morphologies (fused, doubled over the axis, with over-expression or suppression of structural components).

The cellular-histogenic differentiation made it possible to create a roadmap of the somatic embryogenesis of *T. domingensis* (Figure 2), that helped establish the sequence and the degree of maturity of the somatic embryo generated by the embryogenic lines of *T. domingensis* based on the morphohistological information obtained (Figure S1).

Discussion

The somatic embryogenesis of *T. domingensis* presented stages of proembryogenesis and early and late embryogenesis. However, it recorded an asynchronous process during the phases of induction, proliferation and embryogenic maturation. The formation of embryos that started in the inductive stage may be explained considering that the redox effect of the ascorbic acid is an enhancer of the embryogenic process (Dan 2008; Becker *et al.* 2014). However, the effect of

ascorbic acid on *T. domingensis* needs to be optimised in order to standardise the quality and number of produced SE.

In the case of emerging aquatic plants, somatic embryogenesis has varied in relation to the genotype, the explant, the culture medium and the culture technique used (Rogers 2003; Burris *et al.* 2009). In the species under study, it was possible to observe the embryogenic adeptness of the caulinar basis of the germinate and its potential in the production of embryogenic calli when stimulated by 2,4-D. Comparatively, the zygotic embryo of the Indica variety of *Oryza sativa* recorded a 35% formation of calli in 1.5 mg L^{-1} of 2,4-D with a minimum amount of necrotic material in a MS medium (Meneses *et al.* 2005). Germinates of *T. angustifolia* and inflorescences of *Phragmites australis* have produced embryogenic calli at concentrations below 2,4-D (Lauzer *et al*. 2000; Rogers 2003; Burris *et al.* 2009). In other species and explants, the yellow callus has been embryogenic at greater concentrations (Verdeil *et al*. 2001; Burris *et al.* 2009; Vega *et al*. 2009). The embryogenic callus cultured with 2 mg L^{-1} of 2,4-D and moved to light conditions was more efficient in the massification of *T. domingensis* SE (von Arnold *et al.* 2002; Elhiti *et al.* 2013). In two *Phalaenopsis* spp species, SE presented very low percentages with 70% and 90% oxidation over long periods of light (Gow *et al.* 2009). However, it is necessary to improve the process of maturation of the *T. domingensis* embryo in order to be able to increase the frequency of embryos of the best embryogenic line.

Parallel to the morphogenetic process, the histological study showed that the cellular organisation and embryogenic differentiation of *T. domingensis* are compatible with the descriptors cited for aquatic monocotyledons such as *Panicum virgatum* (Burris *et al.* 2009), *Oryza sativa* (Bevitori *et al*. 2014; Vega *et al*. 2009) and *Phragmites australis* (Máthé *et al.* 2000), and terrestrial monocotyledons such as C*ocus nucifera* and *Musa* sp. (Strosse *et al.* 2006; Saenz *et al*. 2006).

The 2,4-D influenced the transition of the meristematic cell to an embryogenic cell and its resulting development towards a SE. The meristematic and embryogenic cells of the *T. domingensis* callus evolved to form nodules of meristematic tissue and proembryogenic masses. These histological characteristics have defined the proembryogenesis stage of *Oryza sativa* (Bevitori *et al.* 2014; Vega *et al.* 2009), *Cocus nucifera* (Saenz *et al.* 2006) and *Musa* sp. (Strosse *et al.* 2006).

The stages of early and late embryogenesis of *T. domingensis* coincided with the globular, oblong, scutellar and coleoptilar sequential stages of the zygotic embryo in monocotyledons (Quiroz-Figueroa *et al.* 2006; Forestan *et al*. 2010) and with the stages of development reported by Dodeman (1997), Filonova *et al*. (2000), Quiroz-Figueroa *et al.* (2006) and von Arnold *et al.* (2002).

The observation of the suspensor in *T. domingensis* was a key point to determine the unicellular origin of the SE and its degree of development (Quiroz-Figueroa *et al.* 2006). The gSE presented a radial development plan with three fundamental tissues typical of a spermatophyte (Winkelmann 2016). The model species *Zea mays* and *Arabidopsis thalliana* have reported stages of transitory development or of cellular expansion, rather than of differentiation (Forestan *et al.* 2010; Radoeva & Weijers 2014). The oSE of *T. domingensis* was characterised as a transition stage between the gSE and the scSE (Forestan *et al.* 2010; Smertenko & Bozhkov 2014). The cotyledonary structure with reserve parenchyma rich in amyloplasts made it possible to confirm that the degree of development reached by the SE of *T. domingensis* was of the scutellar type. In the case of the SE of *O. sativa* during the scutellar stage, protoderm changes in the epidermis and the vascular bundle may be observed, indicating that the next stage of development is starting (Bevitori *et al.* 2014). In *T. domingensis*, the scSE presented vascular cells in some cases,

suggesting its advance towards a colSE, with both embryogenic stages differentiated only by the coleoptile-radicle bipolarity of the last one, although the coleorhiza and the plumule were not observed - two basic structures of a mature embryo in monocotyledons (Winkelmann 2016; Forestan *et al.* 2010). The high morphological variability of the SE made it possible to distinguish between a normal embryo and an abnormal or aberrant embryo resulting from the lack or over-expression of one or more structural elements that form it, particularly during the late stages (Hoenemann *et al.* 2010). In the case of the date palm, the problem of the production of aberrant embryos in the routine propagation through SE and the change to seedling were solved by applying a period of drying in polyethylene glycol (El Dawayati *et al.* 2012).

Conclusions

In the present study were defined the optimal culture conditions and morphohistological path of *Typha domingensis*, that culminated in the formation of mature somatic embryos. In the stages of embryogenic induction and proliferation, 2,4-D in low concentrations, the somatic embryo showed an indirect and unicellular embryogenic route. The multistage monitoring of the cellular-histogenic differentiation made it possible to create a roadmap of the somatic embryogenesis of *T. domingensis* that helped establish the sequence and the degree of maturity of the somatic embryo. However, the embryogenic structures presented asynchrony and the presence of abnormal embryos. The model of embryogenic development for this species will be useful to deepen the reproductive metabolism for different biotechnological applications.

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426 Table 1. Percentage of cultures with proliferation of embryogenic products of *Typha*

427 *domingensis*. Phase 2 of somatic embryogenesis.

Level	Dependent variable	% of cultures			
		Embryogenic line			
		BC0	YC1	YC2	YC3
Explant	Yellow callus	8.33[b]	75.75[a]	86.11[a]	75.00[a]
	Brown callus	86.11[a]	9.09[b]	11.11[b]	25.00[b]
	oSE	0.00	8.33	16.66	13.88
	scSE	0.16	0.00	0.08	0.41
Medium	Yellow callus	16.66	45.45	33.33	27.77
	Brown callus	8.33	0.00	0.00	0.00
	oSE	0.00	16.66	13.88	5.55
	scSE	8.33	0.00	0.00	0.00
	Suspended cells	8.33[b]	75.00[a]	66.66[a]	75.00[a]
Σ	SE	8.49	24.99	30.62	19.84

428 SE=somatic embryo, Σ SE=sum of SE adhered to the explant and suspended in the culture

429 medium. Averages with same literals were not different ($p<0.05$).

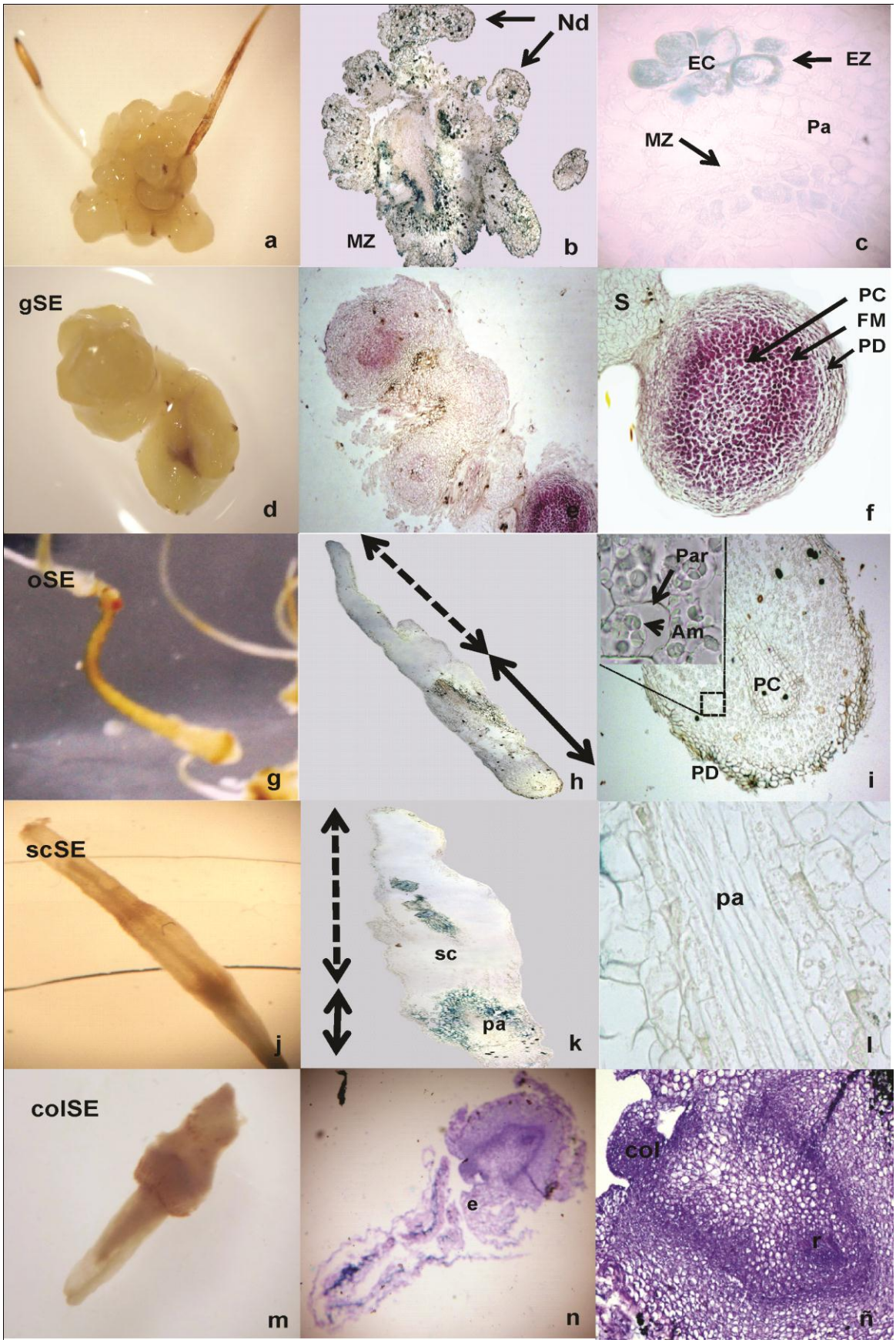
430 Table 2. Percentages of cultures with embryogenic products of *Typha domingensis* in the phase of

431 embryogenic maturation.

Level	Dependent variable	% of culture							
		Embryogenic line							
		BC0L	YC1L	YC2L	YC3L	BC0D	YC1D	YC2D	YC3D
Explant	Yellow callus	0.00	33.33[bc]	26.66[bc]	66.66[ab]	0.00	66.66[ab]	60.00[ab]	93.33[a]
	Brown callus	93.33[a]	39.99[cd]	40.00[cd]	0.00	86.66[ab]	20.00[cd]	46.66[bc]	0.00
	oSE	0.00	0.00	6.66	16.66	0.00	20.00	13.33	6.66
	scSE	0.00	0.00	6.66	33.33	0.00	0.00	0.00	0.00
Medium	Yellow callus	0.00	20.00[bc]	60.00[ab]	75.00[a]	0.00	60.00[ab]	26.66[abc]	73.33[a]
	Brown callus	33.33	0.00	0.00	41.66	6.66	13.33	0.00	0.00
	White callus	0.00	0.00	0.00	0.00	0.00	0.00	20.00	20.00
	oSE	0.00	20.00	6.66	25.00	0.00	20.00	0.00	0.00
	scSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.33
	Suspended cells	0.00	60.00[b]	80.00[ab]	100.0[a]	0.00	100.0[a]	100.00[a]	100.0[a]
Σ	ES	0	20	19.80	74.99	0	40.00	13.33	19.99

432 SE=somatic embryo, Σ SE=sum of SE adhered to explant and suspended in the culture medium.

433 Averages with same literals were not different ($p<0.05$).



434

Figure 1. Embryogenic differentiation of *Typha domingensis*.

Yellow callus: a) morphology (8x), b) cross-section (toluidine blue, 200x), c) meristematic and embryogenic region (toluidine blue, 400x); gSE: d) over yellow callus, e) cross-section (hematoxylin-eosin, 200x), f) radial pattern made by three meristems: protoderm, fundamental and procambium; oSE: g) over yellow callus of 56 days, h) longitudinal section showing the suspensor connected to calli (arrow with the letter x) and oSE (arrow with the letter y), i) tissue differentiation, reserve parenchyma cells (spherical and birefringent amyloplast) and procambium; scSE: j) suspended in the medium, k) cross-section (toluidine blue, 200x), l) scSE with procambium and some vascular cells; colSE; m) suspended in the medium, cross-section (toluidine blue, 200x) high histo-differentiation in the region near the embryo, along the scutellum formed by reserve parenchyma cells and defined axis with meristem of apex and root, ñ) detail of coleoptile and apical and radicular meristem.

MZ: meristematic zone. EZ: embryogenic zone. EC: embryogenic cells. gSE: globular SE. oSE: oblong SE. scSE: scutellar SE. colSE: coleoptilar SE. FM: fundamental meristem. PD: protoderm. PC: procambium. S: suspensor. Pa: parenchyma. Rep: reserve parenchyma. Am: amyloplast. col: coleoptile. pa: procambial axis. esc: scutellum. r: radicula. e: embryo.

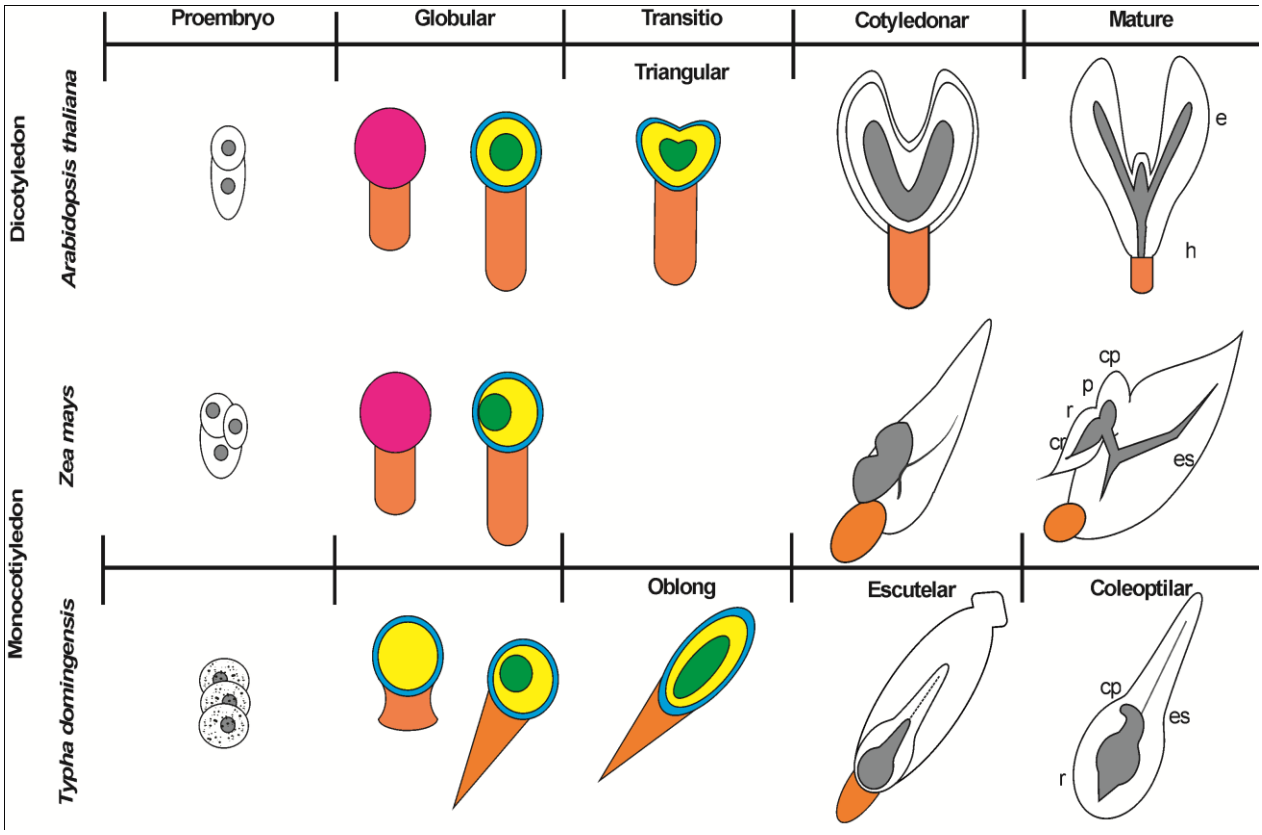


Figure 2. Histogenic model of the process of somatic embryogenesis of *Typha domingensis* compared with two model species: *Arabidopsis thaliana* a dicotyledon and *Zea mays* a monocotyledon. The illustrations are not to scale. Symbols: e-epicotyl, h-hypocotyl, cp-coleoptile, cr-coleorhiza, p-plumule, r-radicle. Colour code: yellow-fundamental tissue, green-procambium, blue-protoderm, orange-suspender, pink-zygote.

Supplements

Figure S1. Map of the embryogenic lines of *Typha domingensis* that sums up the morphological development of the somatic embryo.

Table S1. Table of significance.