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Induction of somatic embryogenesis in *Tecomella undulata* (Sm.) Seem using a pistillate explant

Sasan Rastgoo*

Department of Horticultural Science, Faculty of Agriculture, Persian Gulf University, Borazjan - 75611 58808, Bushehr Province, Iran

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Desert teak (*Tecomella undulata* (Sm.) Seem) a multipurpose ornamental tree native to the arid and semi-arid tropics has entered the endangered plant category mainly as a result of the species' ineffective seed reproduction system. The tree usually reproduces through a few root suckers in old stands. Conventional methods of plants multiplication could not offer a viable practice for its mass multiplication. Low adventitious rooting of the cuttings has been the principal cause of failure in its vegetative propagation. Hence, the present research was planned and conducted to evaluate the feasibility of somatic embryogenesis in this species, the pathway that bypasses the need for rooting stage by developing bipolar embryos. Ovary explant was cultured in modified Murashigue & Skoog (MS) medium supplemented with different auxins and cytokinins. The results showed α-naphthalene acetic acid (NAA) was superior in inducing embryogenic callus. NAA ranging 5.4-21.5 µM could induce the highest embryogenic calli which exhibited developing pro-embryogenic masses (PEM) and globular somatic embryos. The calli which were induced by the use of 2,4-dichlorophenoxyacetic acid (2,4-D) were poor in quality and showed no morphogenesis potency. Individual application of Thidiazuron (TDZ) and N^6-benzyladenine (BA) induced good callogenesis at low concentrations but the calli were non-embryogenic with both. The proliferation of embryogenic calli was the best in a hormone-free medium. However, the media containing 40.5 and 54 µM NAA alone could induce somatic embryos along with callus proliferation. Low BA-contained medium (0.9-4.44 µM) led to recurrent somatic embryogenesis. Neither BA and GA_3, nor the elevating sucrose concentration could cause further development and maturation of the somatic embryos induced during previous stages (callogenesis and callus proliferation). More research is required to optimize the maturation stage. The findings of the present study can be useful for future studies in the micropropagation of this recalcitrant specieslationships in Indian mustard under heat stress and the differential remobilization efficiencies in the advanced breeding lines.

Keywords: Desert teak, Embryogenic callus, Ovary explant, Pro-embryogenic masses, Rohida

Desert teak [*Tecomella undulata* (Sm.) Seem], a woody perennial of family Bignoniaceae, is an evergreen ornamental tree that thrives under hot arid and semi-arid climates over a region extending from the Arabian Peninsula, southern Iran, and Pakistan to northwest of India. Besides elegant crown, the tree bears a waterfall of charming, mesmerizing yellow, orange flowers from early March till late May. It is well suited for the landscape of arid regions in tropics and sub-tropics. This multipurpose tree is drought and wind-resistant, a biomass producer, also holds the tremendous potential of medicinal value, and has been traditionally used in various diseases like syphilis, leukoderma, blood disorders, etc.[1]. Leaf, bark, wood, and roots of desert teak have high pharmaceutical properties[2-4]. In the south of Iran, the tree is now heading towards extinction because of several reasons such as over-exploitation for its timber and destruction of its small populations during urban, agricultural, and industrial expansion, but the biggest challenge is the tree's failure in seed reproduction. Being hard-to-root in cutting propagation has aggravated the situation. The natural but inefficient regeneration tool is the root suckers which are produced on old stands in a few numbers. The tree has been designated as "threatened" and "endangered" in India[1].

Micropropagation offers a breakthrough in clonal mass production of recalcitrant species. Intact plants potentially can be regenerated from *in vitro* cultured cells, tissues, and organs through either organogenesis or embryogenesis. Both regeneration pathways are capable of regenerating plants either directly or indirectly (callus-mediated regeneration)[5]. In *Tecomella undulata*, almost the entire past attempts have been based on utilizing organogenesis pathway in which nodal or internodal segments taken from

*Correspondence:
E-Mail: snrastgoo@yahoo.com; rastgoo@pgu.ac.ir

mature tree stands or *in vitro* propagules, and/or the callus tissue derived from such explants were employed as starting materials[6-9]. Miserably, those endeavors have not yielded a viable regeneration protocol yet. The most important hindrance has been the extremely low capability of microshoots in adventitious rooting which resulted in poor regeneration efficiency.

Somatic embryogenesis (SE) is an asexual process where bipolar embryos that can develop into whole plants are produced from somatic cells. It is an alternative pathway for mass cloning of elite, endangered, or high demanding genotypes as it has various advantages over organogenesis[10]. Adventive embryogenesis was reported for a few arid zone woody species, some recent works have been in *Alnus glutinosa*[11], *S. album*[12], *Terminalia chebula*[13] and *Picea* spp.[14]. Based on a thorough literature review, no published report was found on micropropagation of *T. undulata* via SE. Thus, initiating research in this context was seen as critically necessary. Success in SE is confined to defined genotypes, developmental states, and explant types[7]. One of the key prerequisites for somatic embryo formation by external inducement is an explant with a source of totipotent cells[5,15]. Most of researches on SE in tree species have used mature vegetative explants. However, considerable reports are showing higher potency of reproductive organs like the ovary in SE[16-18]. Earlier, usage of ovary explant for *in vitro* regeneration has been reported in woodies like *Salix*[19] and *Azadirachta indica*[20].

The induction of embryogenic tissues (ET) is mainly dependent on employing plant growth regulators (PGRs) in the culture media as external inducers. During SE somatic cells change to embryonic cells in a physiological sequence that is firmly regulated by a fine balance of PGRs[21]. Auxins and cytokinins are the major PGRs in this context. The 2,4-Dichlorophenoxyacetic acid (2,4-D), α-naphthaleneacetic acid (NAA), 6-benzylaminopurine (BA), and thidiazuron (TDZ) are the most common PGRs frequently have been used for initiation of SE and proliferation of embryogenic tissues[22]. Hence, in the present study, we have made an attempt to evaluate *in vitro* morphogenetic response of ovaries of *Tecomella undulata* to different PGRs and to find out the potential of somatic embryogenesis in this species. The effect of sucrose concentration in the culture medium on advancing the development of the regenerants was also examined.

Materials and Methods

Culture media preparation

The study was carried out in the Plant Tissue Culture Laboratory of the Department of Horticultural Science, Faculty of Agriculture, Persian Gulf University, Iran in 2020. The study was performed in four experiments. To induce embryogenic callus (EC), MS medium[23] modification No.1 (half strength of MS macroelements, full strength of microelements and vitamins) was used as the basal medium in the first two experiments fortified with various concentrations of auxins; NAA (0.5-54 µM) and 2,4-D (0.45-22.5 µM) and cytokinins; TDZ (0.45-9 µM) and BA (22.2-66.6 µM). Experiment 3 was conducted on the proliferation of EC using different auxins; NAA (0-54 µM), IBA (0 and 12.3 µM), and IAA (0 and 14.3 µM) with different combinations, and in the fourth experiment, somatic embryo development and maturation were followed by using MS medium modification No.2 (full strength of MS macroelements and microelements and Gamborg's B5 vitamins) equipped with gibberellic acid (GA_3) (0.0, 1.45 and 2.9 µM) and BA (0.9, 4.44 and 22.2 µM). Sucrose level of the medium was also tested at 30 and 70 g L^{-1} when GA_3 was the sole source of PGR in the medium. The prepared media were autoclaved at 121°C and 1.1 atm pressure for 20 min before culture initiation.

Explant preparation and culture

For the first two experiments, inflorescences having unopened, unpollinated flowers at the balloon stage, were harvested from six years old stands growing at the landscape of the Faculty of Agriculture, Persian Gulf University, at beginning of March till the first half of April in 2020. Individual unopened flowers were separated, surface-sterilized through immersing into 96% ethanol for 15 s. Thereafter, pistils were dissected inside a functioning Laminar Air Flow Cabinet, and style and stigma were cut off and discarded (Fig. 1). The isolated whole

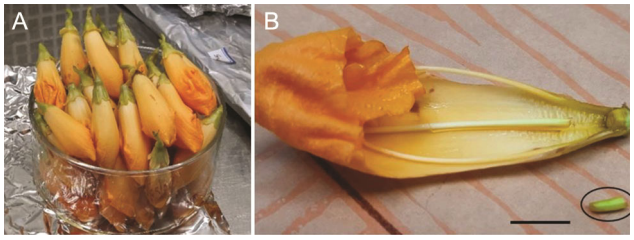

Fig. 1 — Explant preparation. (A) Surface-sterilized blossoms at balloon stage ready for ovary extraction; and (B) 4 mm long ovary explant dissected from a surface-sterilized bloom. (Bar = 5 mm).

ovaries were cultured horizontally on the prepared media. For the proliferation of embryogenic callus, the NAA-induced embryogenic calli obtained through the first experiment were used as the initiating explants, and for obtaining mature somatic embryos the fourth experiment was performed using embryogenic proliferated materials during the third experiment. In each experiment, each treatment replicated thrice and in each replication fifteen explants were cultured.

Experimental design, record of observations, and statistical analysis

Experiments were laid out in a completely randomized design (CRD) with five replications and five explants/treatment/replication. To record experimental observations, explants were investigated in terms of growth, development, and proliferation of callus, organ, Pro-embryogenic masses (PEMs), and somatic embryo, 8 weeks after culture initiation. Data on percentages of callogenesis, explant survival, proliferated explants, and recurrent callus proliferation were analyzed by one-way ANOVA. The percentage data were subjected to arcsin transformation before analysis by one-way ANOVA. The mean comparison was performed using Duncan's new multiple range test (DMRT) at 5% level. Callus quality, texture, and colour were also evaluated visually and recorded descriptively.

Results

Induction of embryogenic callus using NAA and 2,4-D

The first experiment with auxins showed significant superiority of NAA over 2,4-D in callus induction (Table 1). A hundred percent callogenesis was achieved with 2.7 µM NAA whilst callogenesis was at least 80% with other NAA levels. 2,4-D could induce a maximum of 80% callogenesis at 0.45 µM. Increasing 2,4-D beyond 0.45 µM decreased callogenesis drastically so that the callus production was ceased at ≥13.5 µM. The quantity of NAA-induced callus manifested a rising trend with an increase in the concentration up to 16.1 µM. Quantity of 2,4-D-induced callus was assessed as poor at 0.45 µM, dropped to the scale of very poor when the concentration increased up to 9 µM. In terms of callus texture and color, all NAA levels, as well as 2,4-D at 0.45 µM, caused light green friable callus. Such calli were recognized potent for SE. Those inductive levels of 2,4-D (above 0.45 µM) resulted in the formation of friable callus but brownish, usually unsuitable materials to be used for further development.

Along with callogenesis, some stages of SE also occurred only with NAA media. NAA at 5.4 and 10.8 µM could induce PEMs at rates of 40 and 20 percent, respectively, exhibiting few somatic embryos at globular stage, while 21.5 µM induced PEMs revealing a larger number of globular embryos (Fig. 2A and B). The highest level of NAA caused adventitious root induction with no visible SE (Fig. 3). The 2,4-D media made no progress neither in organogenesis nor in SE. Overall, comparing the two applied auxins, the most outstanding outcomes were obtained with NAA that could cause satisfactory ECs accompanied with the initiation of SE.

Induction of embryogenic callus using BA, TDZ, and high NAA

To know about the impact of cytokinins (CKs) on *in vitro* response of ovary explant, BA and TDZ were incorporated into the medium individually. NAA was also tested at higher concentrations to find out the better perception of the explant response. BA induced callus formation at a rate of 47 to 94 percent, the highest percentage was obtained at 44.4 µM (Table 2). Similarly, TDZ induced callusing at all concentrations was applied. By an increase in TDZ, a significant decline was observed in callogenesis. A hundred percent callogenesis was achieved with 0.45 µM TDZ. NAA applied at concentrations as high

Table 1 — Regeneration from Desert teak's ovary explant on MS medium modification No.1 supplemented with NAA and 2,4-D 8 weeks after culture

	Auxins (µM)	Callo-genesis* (%)	**Callus quantity	Callus texture	Callus colour	***SE/ Organogenesis
NAA	0.5	80^{bc}	+++	friable	Light green	nil
	2.7	100^{a}	+++	friable	Light green	nil
	5.4	80^{bc}	+++	friable	Light green	40% PEMs with few GEs
	10.8	92^{ab}	++++	friable	Light green	20% PEMs with few GEs
	16.1	92^{ab}	+++++	friable	Light green	nil
	21.5	92^{ab}	++++	friable	Light green	20% PEMs with numerous GEs
	27.0	88^{b}	++++	friable	Light green	40% rhizogenesis
2,4-D	0.45	80^{bc}	++	friable	Light green to yellow	nil
	2.3	64^{cd}	+	friable	Brownish green	nil
	4.5	40de	+	friable	Brownish	nil
	9.0	28^{ef}	+	friable	Brownish	nil
	13.5	0^{f}	-			
	18.0	0^{f}	-			
	22.5	0^{f}	-			

[*Mean having same letter(s) show no significant difference at $P \leq 0.05$ by DMRT. **Callogenesis rate. - no callus; + very poor; ++ poor; +++ moderate; ++++ good; +++++ Excellent; ***PEMs: Pro-embryogenic masses; GEs: Globular embryos]

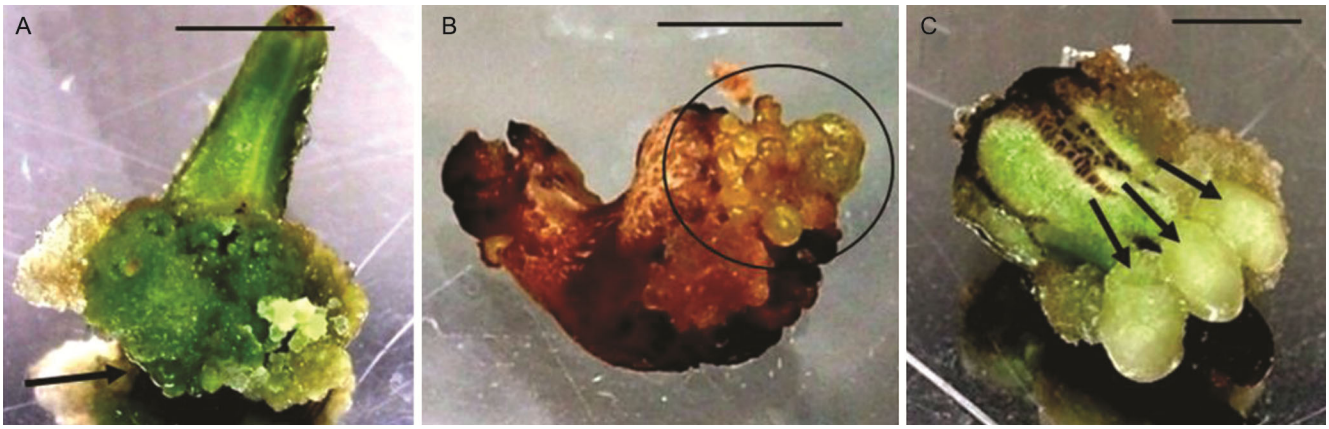

Fig. 2 — Morphogenesis of ovary explants eight weeks after culture initiation in NAA supplemented media. (A) Regeneration of interconnected somatic embryoids from a healthy friable EC produced at the basal end of the explant in the medium with 21.5 µM NAA; (B) A cluster of globular somatic embryos at the ovary basal end in the medium with 21.5 µM NAA; and (C) Development of nodular callus at the ovary stylar end in the medium supplemented with 5.4 µM NAA. (Bars = 5 mm).

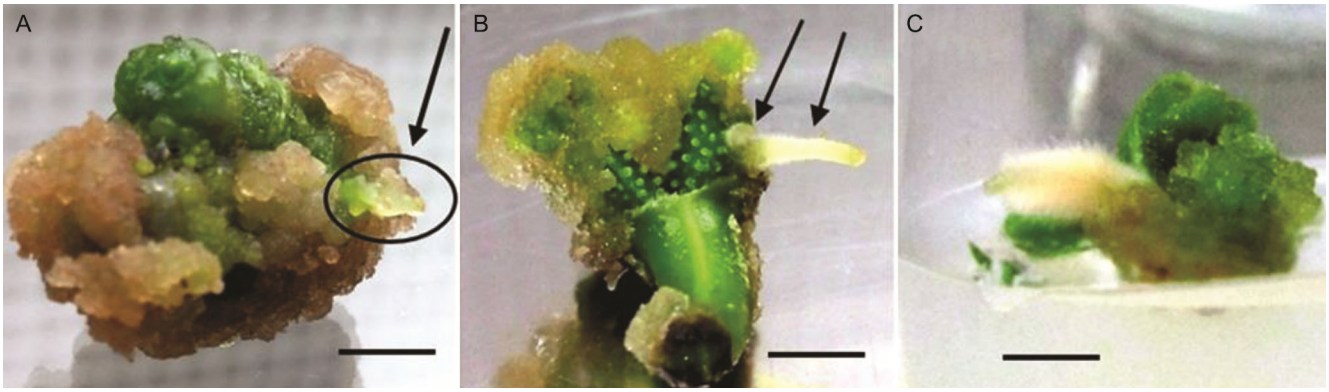

Fig. 3 — Adventitious rhizogenesis from ovary tissue within a few weeks after culture initiation in the medium containing 27 µM NAA. (A) A hair-free double root structure newly formed 4 weeks after culture initiation; (B) A slightly hairy double root structure six weeks after culture initiation; and (C) A profusely hairy single root eight weeks after culture initiation. (Bar = 5 mm).

Table 2 — Callus regeneration from desert teak's ovary explant on MS medium modification No.1 in response to BA, TDZ and high NAA eight weeks after culture

Growth regulators (µM)		Callogenesis* (%)	**Callus quantity	Callus texture	Callus colour
BA	22.2	73.3 b	++++	Compact	Light brown
	44.4	94.4 a	+++	Compact	Light brown
	66.6	47.2 c	++	Compact	Dark brown
TDZ	0.45	100 a	+++++	Compact	Brownish green
	2.3	77.8 b	++++	Compact	Brownish green
	4.5	72.2 b	+++	Compact	Brownish green
	9.0	53.3 c	++	Compact	Dark brown
NAA	27	36.4 cd	+++	Compact	Light brown
	40.5	20.0 d	+++	Compact	Brownish green
	54	54.2 c	++	Compact	Brownish green

*Mean having the same letter show no significant difference at $P \leq 0.05$ by DMRT. **Callogenesis rate; - no callus; + very poor; ++ poor; +++ moderate; ++++ good; +++++ Excellent]

as 27 to 54 µM yielded the poorest callus induction compared to both CKs used. Raising BA from 22.2 to 66.6 µM reduced callus quantity drastically. Also, the same trend occurred with TDZ. While in the first experiment the highest callus quantity was achieved in the range of 10.8 to 21.5 µM NAA, here the callus quantity reduced to the scale of moderate even poor by the rise in NAA level to 27 µM and more.

The regenerated calli by all three PGRs exhibited compact texture (Table 2). The common feature observed with all three PGRs was that the higher concentration of the PGR in the medium, the darker the callus color became. Overall, the medium fortified with 0.45 µM TDZ was concluded as the best inductive treatment for callogenesis. Nevertheless, neither the CKs treatments nor the high NAA media could induce EC since there was no indication of formation of whether PEMs or pre-embryos.

Proliferation of embryogenic callus (EC) and formation of somatic embryos

The NAA-induced ECs transferred onto a PGR-free medium and also onto the media containing different auxins. The results showed the highest explant survival rates in the PGR-free medium (80%) and also the media containing 40.5 µM NAA with and without 12.3 µM IBA (77.7% and 66.7%, respectively) (Table 3). The percentage of proliferated explants recorded the highest values with 40.5 and 27 µM NAA, each combined with 12.3 µM IBA (77.7% and 56.3%, respectively). The highest score for the quantity of proliferated callus was obtained with NAA alone (40.5 µM) as well as with NAA+IBA (27+12.3) µM. Callus texture was friable in the control and the medium with 27 µM NAA alone whereas the remaining media proliferated compact callus.

Apart from callus proliferation, several embryogenic morphogenesis was also observed. The control treatment protruded several individual globular and heart-shaped somatic embryos in a few explants (Fig. 4A). All the media contained NAA alone exhibited the development of PEMs and revealed numerous globular and heart-shaped somatic embryos (Fig. 5B). The strongest demonstration of SE was observed with 54 µM NAA that induced and developed a high number of globular and heart-shaped embryos, up to 34 somatic embryos per explant, in 85% of the survived and proliferated cultures. Combinations of IBA and IAA with NAA showed an inhibitory effect on the induction and development of somatic embryos in the proliferated ECs. Therefore, it can be inferred, cautiously, both IBA and IAA might hurt somatic embryo

Table 3 — The proliferation of ovary-derived callus and induction of somatic embryo on MS medium modification No.1 supplemented with different concentrations and combinations of auxins eight weeks after culture initiation

Growth regulators (µM)			*Explant survival (%)	*Proliferating explant (%)	**Callus quantity	Callus texture	Callus colour	***Somatic embryogenesis
NAA	IBA	IAA						
0	0	0	80.0^{a}	53.2^{bc}	+++	friable	Light green to light yellow	20% of explants with numerous yellow GEs
27	0	0	55.5^{bc}	27.7^{cd}	++	friable	Green to lemon yellow	75% of explants with PEMs and numerous yellow GEs
40.5	0	0	66.7^{ab}	44.0^{bcd}	++++	compact	Green to lemon yellow	75% of explants with PEMs and numerous green GEs
54	0	0	25.0^{d}	25.0^{d}	+++	compact	Light green to brownish	85% of explants with PEMs and numerous green globular and heart-shaped embryos
27	12.3	0	64.5^{b}	56.3^{ab}	++++	compact	Light green	nil
40.5	12.3	0	77.7^{ab}	77.7^{a}	+++	compact	Light green	nil
54	12.3	14.3	44.3^{cd}	44.3^{bcd}	+++	Granular compact	Light green	nil

[*Mean having same letter(S) show no significant difference at $P \leq 0.05$ by DMRT. **Callogenesis rate; - no callus; + very poor; ++ poor; +++ moderate; ++++ good; +++++ Excellent; *** PEMs: Pro-embryogenic masses, GEs: Globular embryos]

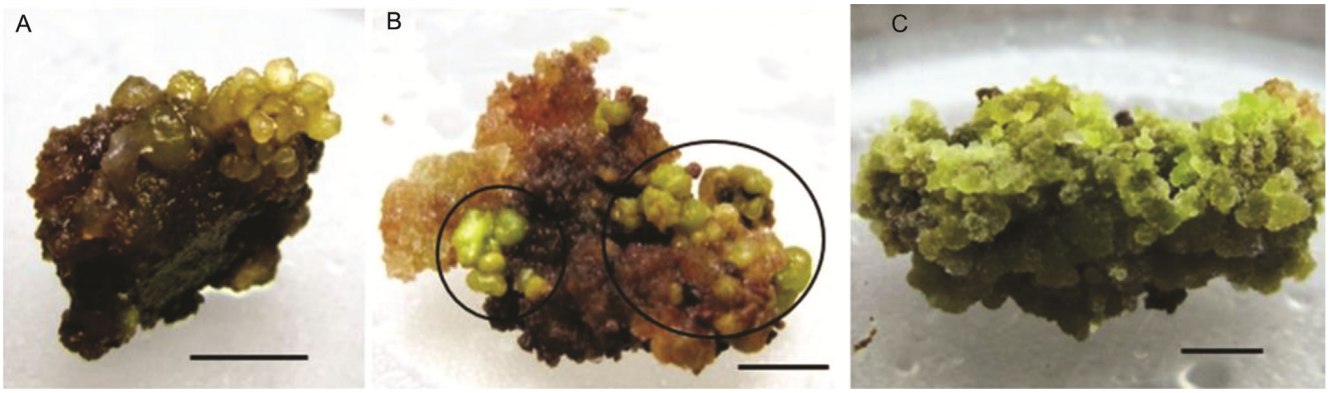

Fig. 4 — Various developmental stages of ovary-derived ECs from experiment 1 when transferred onto the proliferation media with or without auxins. (A) Formation of numerous pre-embryos, globular and heart-shaped somatic embryos 8 weeks after transfer of EC onto a hormone-free medium (Control); (B) Formation of numerous well-developed globular and heart-shaped somatic embryos 8 weeks after transfer of EC onto the medium containing 54 µM NAA; and (C) Proliferation and formation of compact, granular callus in MS modification No.1 medium containing a combination of 27 µM NAA and (12.3+14.3) µM IBA+ IAA (Bar = 5 mm).

formation in this species under the conditions prevailing in this experiment.

Development and maturation of pro-embryogenic masses and somatic embryos

The proliferated ECs carrying embryonic structures obtained during experiment 3 were evaluated after transfer onto the medium devoid of PGR (control), and also onto the media supplemented with GA_3 and BA at different concentrations, individually. The effect of sucrose concentration was also tested at two levels; the standard and high dose (30 and 70 g L^{-1}) when GA_3 was used as the sole source of PGR. According to Table 4, the media containing 30 g L^{-1} sucrose whether PGR-free or with GA_3 (1.45 and 2.9 µM) could not advance the SE path, yielded no promising outcomes. Regardless of GA_3 concentration, elevating sucrose level to 70 g L^{-1} not only did not improve further development of the EC but also paralyzed the explant response entirely that led to gradual degeneration of the explants. Fortifying the media with BA at low concentrations (0.9 and 4.44 µM) led to recurrent callus proliferation and induction of SE in 25% of the cultured ECs (Fig. 5A). Raising BA level to 22.2 µM though maintained callus proliferation status (Fig. 5B) but impaired SE.

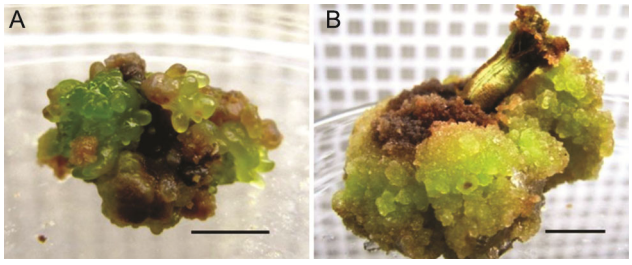

Fig. 5 — Different developmental patterns of ovary-derived EC and PEMs when transferred onto the MS modification No.2 medium supplemented with BA ten weeks after explant transfer. (A) Formation of numerous pre-embryos and secondary somatic embryos of different developmental stages (globular, heart-shaped, and torpedo) in the medium containing 0.9 µM BA; and (B) Profuse proliferation of compact granular EC in the medium containing 22.2 µM BA (Bar = 5 mm).

Discussion

Various PGRs mainly auxins and cytokinins (CKs) were used for callus induction in tree species[24]. Auxins are the key plant PGRs that control *in vitro* morphogenesis including SE[14]. In general, the most common route for inducing SE is through initial exposure of cultures to a medium containing higher doses of auxins, which in most cases leads to the formation of EC, followed by subsequent transfer to a medium either devoid of auxin, with a drastic reduction of auxin concentration or supplemented with optimal levels of CKs[25-27]. However, some species tend to express SE even on auxin-contained medium, the same medium used as their embryogenesis induction medium[27,28].

In this research, two famous auxins (NAA and 2,4-D) and two widely used cytokinins (BA and TDZ) were employed as the medium supplements for embryogenic callus (EC) induction from un-pollinated ovary explants of desert teak. The results revealed that NAA performed the best in terms of inducing EC compared to 2,4-D in the concentration ranges that were used. NAA could induce ECs bearing embryonic structures at low to moderate concentrations. Previously, a research on *Tecomella undulata* reported good callogenesis from internodal explants using NAA and 2,4-D individually while no indication of SE they obtained[29]. Apart from genotypic differences, comparatively, it seems the explant type used in that study had a determining role

Table 4 — Response of ovary-derived EC and PEMs ten weeks after transfer onto MS medium modification No.2 supplemented with different levels of sucrose, GA_3 and BA

Sucrose (g l^{-1})	GA_3 (µM)	BA (µM)	*Recurrent EC proliferation (%)	**Callus quantity	Callus texture	Callus colour	***Somatic embryogenesis
30	0		6.7^{bc}	++	compact	Light green	nil
30	1.45		6.7^{bc}	+++	compact	Light green	nil
30	2.9		0^{c}	-			nil
70	0		0^{c}	-			nil
70	1.45		0^{c}	-			nil
70	2.9		0^{c}	-			nil
30		0.9	26.7^{ab}	++++	compact	Light green to brownish	25% of explants with numerous secondary pre-embryos
30		4.44	28.3^{a}	+++++	compact	Light green to brownish	25% of explants with numerous secondary pre-embryos
30		22.2	40.0^{a}	+++++	compact	Light green	nil

[*Mean having the same letter show no significant difference at $P \leq 0.05$ by DMRT. **Callogenesis scale; - no callus; + very poor; ++ poor; +++ moderate; ++++ good; +++++ Excellent]

in the lack of embryo production. There has been a dominant assumption for a long time that auxin is produced exclusively in young growing parts and is delivered to action sites. However, Robert *et al.*[10] highlighted the importance of local auxin biosynthesis for reproductive organ and embryo development processes. According to them, local auxin biosynthesis occurs in very few cells in specific developmental windows in stamens, gynoecia, ovules, and embryos. These recently identified sources of auxin influence the flow of auxin within the reproductive tissues and embryos, and subsequently the formation of auxin gradients which play a key role in organ morphogenesis. With these facts, ovary explants may be richer in auxin content compared to other vegetative tissue-originated explants such as mature internodal segment explants which were used by Patel & Patel[29]. Previously, NAA has been cited as a promoting PGR for the expression of SE in other species such as *Fraxinus mandshurica*[30] and *Picea abies*[14]. There are also supporting reports regarding the positive influence of 2,4-D in inducing SE in woody species like *Carica papaya*[31], and *Camellia oleifera*[32]. The discrepancy between these results and our findings refers to the differences in species genetic makeup, explant type, the season of explant collection, mother tree developmental stage and its growing conditions, and other factors that can influence explant endogenous phytohormones contents. The emergence of adventitious roots in the cultured ovaries was unexpected morphogenesis which was observed in the medium containing a high level of NAA (27 µM). Occurrence of adventitious rooting during SE induction using an auxin-fortified medium has also been reported earlier in several woody species such as *Dalbergia sissoo*[33], *Koelreuteria paniculata*[34], and *Olea europaea*[35]. This phenomenon uncovers the fact that non-optimal concentration(s) of the auxin used in the culture medium can divert, partially or totally, the SE path within the explant.

Neither the cytokinins (BA and TDZ) nor the high NAA which were used in the second experiment of the study could yield EC. Somatic embryos mostly originate from soft friable callus or granular, white, or yellow calli[21] which was not observed in this experiment. In *Tecomella undulata*, previously, profuse callusing with fewer shoot buds per explant was observed by Chhajer & Kalia[9] who tried to propagate it through *in vitro* organogenesis using vegetative explants in BA-contained media. TDZ, a substituted phenylurea compound with strong cytokinin-like activity, originally used as a cotton defoliant has received remarkable attention as a potent regulator of explant morphogenesis in *in vitro* regeneration systems[21]. There are numerous reports showing employment of TDZ for SE in many woody species such as white ash, eastern black walnut, *Rubus*, and *Vitis vinifera*[36] *Azadirachta indica*[37], and blueberry (*Vaccinium corymbosum* × *V. angustifolium*) cultivars[38].

Chhajer & Kalia[9] found that TDZ is unsuitable for shoot multiplication of *T. undulata* as it led to the proliferation of excessive callus, a morphogenic response that was also observed in the present study. Higher levels of CKs are known to induce programmed cell death in cell cultures[39]. Cytokinins are also known to enhance the biosynthesis of ethylene[40] thereby applying them at relatively high levels can adversely affect the growth of cultures and lead towards aging. Testing lower concentrations of CKs might bear promising results in future studies.

The results obtained with the callus proliferation experiment revealed that the PGR-free medium was concluded the best choice for the proliferation of friable EC. This outcome is consistent with the results of a report on *Cnidium officinale*[28] but it is opposite to the results of the study carried out on *Camellia oleifera*[35] where the hormone-free medium was found as the best for the development of globular embryos to cotyledonary stage embryos, not for callus proliferation. In the meantime, looking for induction of SE led to the medium containing 54 µM NAA alone. However, the medium with 40.5 µM NAA could also yield a reasonable output as it showed admissible rates of explant survival and callus proliferation and a satisfactory percentage of embryo development. Earlier, NAA was found as a suitable PGR for the proliferation of embryogenic tissue in some other species such as *Picea abies*[14] and *Fraxinus mandshurica*[30]. In the meantime, opposite findings have also been reported earlier[11]. Such contradictory results can be justified by the endogenous hormonal balance of the explant used which in turn is under the influence of genetic, developmental, and various external factors.

To fulfill the SE process under *in vitro* conditions, the early-stages somatic embryos must be developed

to the cotyledonary stage followed by the stage of embryo maturation. Such matured embryos can germinate on either plain medium (hormone-free) or hormone-fortified media, mainly depending on the genotype. In the fourth experiment aimed at embryo development, the application of GA_3 in the culture medium influenced the development of the embryonic structures negatively. There are also reports indicating inhibitory effects of GA_3 on somatic embryo formation and development in some other systems[41,42]. However, the stimulatory effect of GA_3 in somatic embryo induction, formation or germination has also been reported in other species like *Medicago sativa*[43], and *Cocos nucifera*[44]. While a supporting report of Rudus *et al.*[43] theorized that the level of endogenous GAs is presumably sufficient for callus induction and growth but not optimal for the induction and particularly for the differentiation of embryos, an opposing report by Hutchinson *et al.*[41] concluded that the presence of GAs during both the induction and expression phases of SE was significantly detrimental to somatic embryo formation. It seems species genetic make-up as well as explant type-dependent endogenous hormonal balance especially intrinsic content of gibberellins would ultimately determine the need or no need for using exogenous gibberellins in the developmental phases of SE process. In this study, it was realized that an increase in carbohydrate source of the medium (sucrose) to 70 g L^{-1} suppressed the growth and development of PEMs and the early-stage somatic embryos. This result is contradictory to Dennis Thomas's report[45] that showed a beneficial influence of 200 mM (~70 g L^{-1}) sucrose in the embryogenesis response of *Tylophora indica*. Unlike GA_3, the media supplemented with low concentrations of BA (up to 4.44 µM) positively affected the growth and development of ECs and PEMs. Therefore, the encouraging conclusion drawn was that for keeping SE capability in the ECs, the optimum medium is the one having sucrose at 30 g L^{-1} and BA in the range of 0.9-4.44 µM. This conclusion agrees with results obtained by Corredoira *et al.*[11] with *Alnus glutinosa* and Liu *et al.*[30] with *Fraxinus mandshurica.* Substitution of sucrose by other carbohydrate sources or use of osmotic agents have also been proposed as effective alternatives for advancing the SE process and maturation of somatic embryos for some species not responding satisfactorily with sucrose[46,47].

Conclusion

The present research was performed on induction of *in vitro* somatic embryogenesis (SE) in desert teak, a highly recalcitrant woody species. α-Naphthalene acetic acid (NAA) at low concentrations (5.4 to 21.5 µM) was the best inducer of Embryogenic callus (EC) in ovary explant. NAA up to 40.5 µM was a suitable stimulator for the proliferation of the EC which had been induced by itself. However, a PGR-free medium was also found satisfactory in this respect. N^6-benzyladenine (BA) acted positively at 0.9 and 4.44 µM on embryo development though was unable to develop the embryos to the cotyledonary stage. The promising findings achieved here can be a good backbone for future research works. To the best of our knowledge, the present study is the first research undertaken on SE in *Tecomella undulata*. Also, in this species, it was for the first time that potency of ovary explant for *in vitro* regeneration was evaluated. Nevertheless, much more research works are still necessary to be conducted for optimizing a perfect protocol for SE in this precious endangered ornamental species.

Conflict of Interest

Author declares no competing interests.

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