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Full Length Article

Induction of Half-Sib Embryonic Callus and Production of Taxiod Compounds Therefrom in *Taxus chinensis* var. *mairei*

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Abstract

The half-sib population is an abundant of gene pool for selection breeding of lines or cultivars with high paclitaxel. The seeds, collected from the sampling tree of *Taxus chinensis* var. *mairei*, were exposed to hydropriming and incubated plantlets and which induced callus to screen stable and high paclitaxel content of cell lines. Seed dormancy was successfully broken with 100% germination rate by hydropriming for three to five days. Embryo germination was inhibited by illumination. The optimal medium for *in vitro* embryo germination was MS-modified media (BLG media) supplemented with 20 g/L sucrose, 5 g/L activated carbon and 7 g/L agar. The embryos were transferred to a 14 h photoperiod after seven days incubation in darkness. Seedling plantlets were obtained after fifty days *in vitro*. Embryonic calluses were induced with frequencies up to 73% and cultured in BLG medium supplemented with 2 mg/L 2,4-dichlorophenoxyacetic-D (2,4-D), 0.5 mg/L 6-benzyladenine (6-BA), 20 g/L sucrose and 7 g/L agar. The half-sib cell lines showed high variation levels of taxoids metabolite. Thus, a cell line with high paclitaxel content [60.87 mg/100 g fresh weight] was obtained. Our study provides a new strategy to achieve high seed germination rate and seedling plantlet growth of *T. chinensis* var. *mairei*. It is suitable for screening cell lines with high paclitaxel content, obtained from half-sib cells that initiated from the seedling plantlets of *T. chinensis* var. *mairei*. © 2019 Friends Science Publishers

Keywords: Callus; Paclitaxel; Taxoid-metabolite; *Taxus chinensis* var. *mairei*; Tube plantlet; Hydropriming

Introduction

Taxus chinensis var. *mairei*, a varietal of *T. chinensis* in the *Taxus* genus and Taxaceae family, is primarily distributed in Taiwan and southern China, especially in the Changjiang River Basin, the Henan Nanling Mountains, Shanxi province and Gansu province. Like the other plants in the *Taxus* genus, it contains diterpene alkaloids such as paclitaxel and has a number of beneficial properties (Ho *et al.*, 1997; Chang *et al.*, 2001; Wei *et al.*, 2013). The therapeutic effects of taxol and related taxoids, such as 10-deacetylbaccatin III and baccatin III, on various cancers (*e.g.*, ovarian, breast, and lung cancers) have been confirmed (Wani *et al.*, 1971; Flores *et al.*, 1993; Malik *et al.*, 2011). However, the scarcity of *Taxus* trees and their low contents of taxol have limited the supply of taxol in the region. Moreover, the plants reproduce slowly and suffer from excessive herbivory (Flores *et al.*, 1993). It is dire need to promote larger scale cultivation, which is necessary for the production of taxol. However, the production efficiency has been very low, and *Taxus* seeds have remained dormant due to immature embryos and seed inhibitors (Flores and Sgrignoli, 1991; Hilhorst and Karssen, 1992; Chee, 1994; Zarek, 2007).

Several studies have demonstrated that large scale suspension cultures are promising and stable alternatives to producing taxol and related toxoids (Son *et al.*, 2000; Mulabagal and Tsay, 2004; Sheikhpour *et al.*, 2014). In these cultures, the callus initiated from embryos or embryonic tissues grows rapidly, which is suitable for large scale cultivation and the efficient production of paclitaxel (Yukimune *et al.*, 1996; Ketchum *et al.*, 1999; Zhang *et al.*, 2000). The effective protocol of hunting for cell lines with stably high taxol yields and growth rates was the primary task for cell culture (Wang *et al.*, 2018). Flores *et al*. (1993) demonstrated that the immature embryos of *T. baccata* and *T. cuspidate* could be induced to form callus with embryogenic potential, and taxol and related taxanes were detected. Numbers of cell lines have been established from dissimilar species of *Taxus* genus (Liu *et al.*, 2016).

Half-sib mating, including polycross, topcross and open-pollination, is frequently used in the selection of cultivars, population growth and mining of new genetic marker (Alves *et al.*, 2014; Araghi *et al.*, 2014; Ocokoljić *et al.*, 2014; Spinelli *et al.*, 2014). The half-sib progeny of

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physic nut showed variable seed production (Spinelli *et al.*, 2014). Relatively low genetic variation, heritability of crude proteins and wide ranges of forage yield traits have been observed in half-sib mating in *Bromus inermis* (Araghi *et al.*, 2014). Phenotypic variations have also been observed among ten half-sib progenies of *Castanea sativa* in response to drought stress (Ciordia *et al.*, 2012). Zygotic embryos from a single *Taxus baccata* tree have been used as sources of cell lines that show diverse growth potential and taxane content without requiring a large number of adult trees (Mihaljević *et al.*, 2002). These factors indicate that the high production of paclitaxel and taxoids could be achieved.

There is an urgent need to develop an efficient protocol for screening high taxoid-metabolite-producing cell lines for paclitaxel production. The aim of this study was to develop a rapid and stable method to screen for high paclitaxel content cell line mutations from the half-sib embryonic callus of *T. chinensis* var. *mairei*.

Material and Methods

Plant Materials and Sterilization

Seeds of *T. chinensis* var. *mairei* were collected from a natural habitat (113º89'55", 28º26'32") in Liuyang region, Changsha city, Hunan Province, China, in November 2012. The seeds, which fully developed with red fruit coat and dark green seeds, were collected from the sampling tree. The seeds were either used immediately or refrigerated at 4°C. Seeds without outer coats were sterilized with 70% (v/v) ethanol for 30 s, rinsed 3-4 times with sterile water, immersed in 0.15% (w/v) mercuric chloride for 12 min and rinsed 6 times with sterile water. Then, they were used in further treatments.

Germination and Seedling Plantlet Culture

Efficacy of basal medium under darkness: The experimental design was completely randomized. To select the optimal medium, six basic media were tested as follows: BLG, B5, MS, WPM, SH and 1/2 MS. All the six basic media contained 20 g/L sucrose, 5 g/L activated carbon and 7 g/L agar. The embryos were incubated under darkness with three replications of 20 seeds for each. And the pH of these media was adjusted to 5.8 and the media were sterilized at 115°C at 105 kPa for 30 min. Final germination was taken after ten days and final seedling plantlet was calculated after 50 days later. The optimal medium was used in the following tests.

Efficacy of hydropriming: To promote embryo germination, the sterilized seeds were soaked in sterile distilled water for different time intervals (zero, one, three, five or seven days). The culture conditions were maintained as described previously (Afzal *et al.*, 2015).

Efficacy of light condition: The effect of light condition (a 14 h photoperiod with illumination at 100 μmol•m^{-2}•S^{-1} intensity or 24 h darkness) on embryo germination was investigated. The effects of the six basic media on seedling plantlets were also studied under the 14 h photoperiod light conditions. The embryos were incubated at 24°C. The percentages of embryo germination and seedling plantlets were recorded in triplicate for each treatment. Each replicate contained 10 dishes with 10 embryos per dish.

Embryonic Callus Induction

Efficacy of phytohormone concentration: After a 50 day incubation, the seedling plantlets were cut into 0.5 cm segments and incubated on solid MS medium supplemented with 0-2.0 mg/L 2,4-D and 0-0.5 mg/L 6-BA and transferred to fresh media each month. After incubation for 40 days, the frequency of callus induction was recorded. The segments were incubated at 24°C in darkness. Each treatment was performed in triplicate with two seedling plantlets per replicate.

Efficacy of different basic media supplemented with phytohormone: The effects on callus induction of two basic media (B5 and BLG) supplemented with two types of auxins (NAA and 2, 4-D) at two concentrations (1.0 and 2.0 mg/L) were compared. All treatments were supplemented with 0.5 mg/L 6-BA. The culture conditions were the same as mentioned earlier.

Half-sib Cell Line Construction

The earlier identified optimal medium was further used to construct the half-sib callus. All proliferating callus cell lines were used to determine the production levels of taxoids.

Isolation of Taxoid Compounds

The callus samples were ground with a mortar and a grinding rod in liquid nitrogen. The fine powder was weighed (1.5 g), placed in a 50 mL centrifuge tube and sonicated in 10 mL methanol® (Merck, USA) for 30 min at 20°C at 100 W power. The methanol extracts were then centrifuged at 1000 r/min for 15 min. The supernatant volumes were collected and transferred to a new centrifuge tube. The methanol extraction process was repeated. Six milliliters of each resultant supernatant volume was transferred to a 10 mL centrifuge tube and dried using a *Vacuum Concentration System*® (RC10-10, France). The residual powder was dissolved in 100 μL methanol and filtered through a 0.22 μm membrane (Whatman). This residue was analyzed by LC-ESI-MS and compared with 10-Deacetylbaccatin III® (Xili, China), Baccatin III® (Xili, China), Cephalomannine® (Xili, China) and Paclitaxel® (sigma, USA) chromatographic grade standards.

Determination of Taxoids

The determination of taxoids was performed using an Agilent 1290 HPLC system coupled with a 6530 Q-TOF accurate-mass spectrometer (Agilent Technologies, USA).

The Agilent 1290 HPLC system consisted of rapid resolution binary pumps, an auto-sampler a vacuum degasser a thermostatted column compartment and a tunable ultraviolet detector. A Unitary C18 column (2.1 mm×150 mm, 2.8 μm, Huapu, China) was used. The elution system consisted of deionized water (A) and acetonitrile (B). The gradient was as follows: 0.00-9.00 min, 20%-60% B; 9.00-15.00 min, 60% B; 15.00-15.10 min, 60%-95% B; 15.10-21.00 min, 95%; 21.00-21.10 min, 20% B and finally, 21.10-25.00 min, 20% B. The column flow rate was 0.2 mL/min and the injection volume was 5 μL with the column temperature maintained at 30°C.

Mass spectrometry was used in the positive electrospray ionization (ESI+) mode with the following conditions: 8 L/min nitrogen dry gas at 350°C; a 45 psi nebulizer pressure; 12 L/min sheath gas at 350°C; 4000 V capillary voltage; 0 V nozzle voltage; 230 V fragmentor voltage; 120 m/z - 1000 m/z mass range; and 10 μL/min reference ion flow velocity. Each sample was analyzed three times.

Statistical Analysis

The percentage of embryo germination (%) = number of embryo germination/total number of embryos×100; the percentage of seedling plantlets (%) = number of seedling plantlets/total number of embryos×100 and the percentage of callus induction (%) = number of seedling plantlets of callus/total number of seedling plantlets. All data were subjected to one-way analysis of variance (ANOVA) and the means of the various treatments were examined and compared at $P \leq 0.05$. All analyses were conducted using statistical software SPSS 13.0.

Results

Effect of Basic Medium on Embryo Germination and Seeding Plantlets

The germination was observed when the seed embryos were soaked in sterile water for two days on six basic media (BLG, B_5, MS, WPM, SH and 1/2 MS) (Fig. 1). On SH medium, only 3.33% of embryos germinated within 7 days (Fig. 1); these germinated embryos failed to develop further (Fig. 2e). Maximum embryo germination (83.33% - 90.00%) was observed in BLG, B_5, MS, WPAM and 1/2 MS after incubation for 7 to 10 days (Fig. 1). The highest rate of embryo germination was obtained on the BLG medium (90%), followed by the 1/2 MS medium. Moreover, the rate of embryo germination was higher than the rate of plantlet seedling for four basic media, B_5, MS, SH and 1/2 MS (Fig. 1). The seedling plantlets grew regularly with normal roots, stems and leaves only on the BLG and 1/2 MS media (Fig. 2). However, the germinated embryos did not develop into seedling plantlets on the BLG and 1/2 MS media. Long hypocotyls were observed in both B_5 (Fig. 2b) and MS media

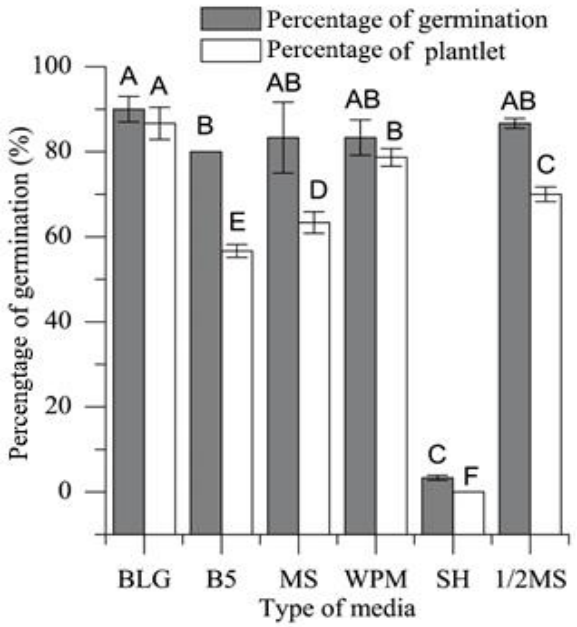

Fig. 1: Effects of different basic media on embryo germination and seedling plantlets in *T. chinensis* var. *mairei*

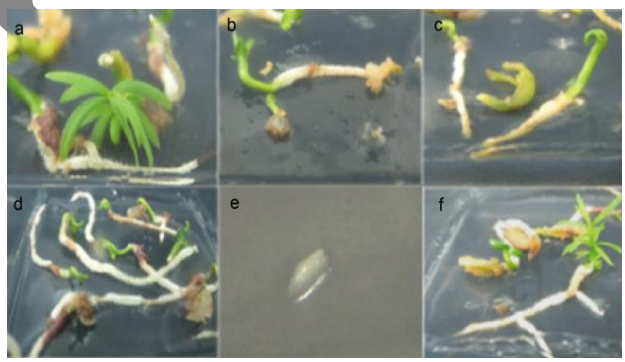

Fig. 2: Effects of different basic media on embryo growth in *T. chinensis* var. *mairei*. (a) - In-vitro culture on BLG for 50 days; (b) - in vitro culture on B5 for 50 days; (c) - in vitro culture on MS for 50 days; (d) - in vitro culture on WPM for 50 days; (e) - in vitro culture on SH for 50 days; (f) - in vitro culture on 1/2 MS for 50 days

(Fig. 2c) and a long main root was also observed in the WPM medium (Fig. 2d). Thus, the BLG basic medium was identified as the optimal medium for embryo germination and seedling plantlet growth.

Effect of Hydropriming on Embryo Germination

A higher percentage of embryo germination was obtained when the seeds were treated by hydropriming (Fig. 3). The rate of embryo germination reached a maximum of 100% after soaking in sterile water for 3-5 days at 24°C.

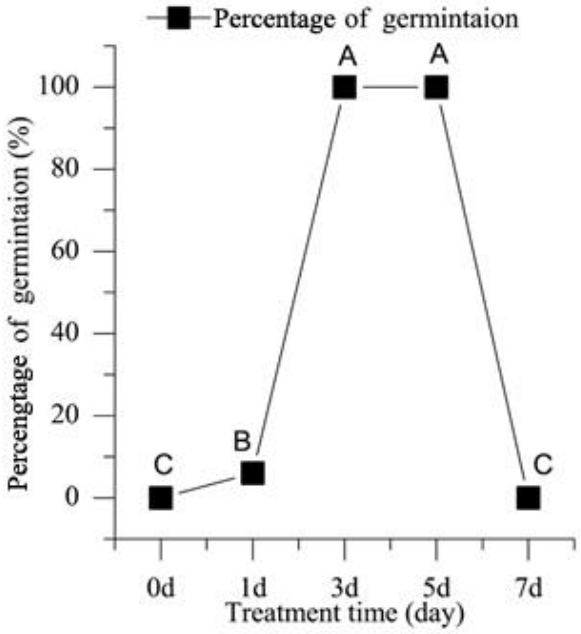

Fig. 3: Effects of sterile water soaking on embryo germination in *T. chinensis* var. *mairei*

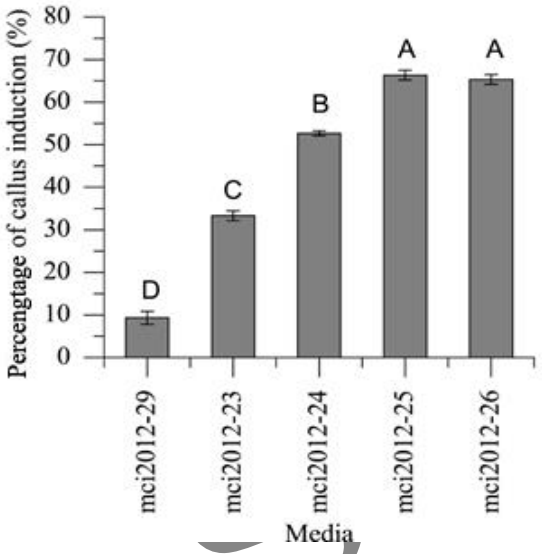

Fig. 4: Effects of different concentrations of plant regulators on in vitro callus induction ratio of *T. chinensis* var. *mairei.* mci2012-23, B5 plus 2,4-D 1.0 mg/L; mci2012-24, B5 plus 2,4-D 1.0 mg/L and 6-BA 0.5 mg/L; mci2012-25, B5 plus 2,4-D 2.0 mg/L and 6-BA 0.5 mg/L; mci2012-26, B5 plus 2,4-D 3.0 mg and 6-BA 0.5 mg/L

It was difficult to extract the embryos from the seeds without hydropriming; subsequently, no embryo germination was observed. However, after seven days, embryos extracted by hydropriming with sterile water had rotted and decayed. The results indicate that the embryonic inhibitors could be major factors causing seed dormancy in *T. chinensis* var. *mairei.*

Effect of Light Condition on Embryo Germination

The embryos used in the study could not germinate when incubated under a 14 h photoperiod, whereas embryos cultured in darkness were all observed to germinate (at a percentage of 100%). An initial incubation in darkness for seven days was critical for embryo germination.

Calluses were obtained on the B_5 medium containing plant regulators (2, 4–D or 2, 4-D and 6-BA) which could affect the induction of embryonic callus (Fig. 4 and 5g). On B_5 basic media supplemented with 20 g/L sucrose and 7 g/L agar, the few calluses that were induced from seeding plantlet segments showed no further proliferation (Fig. 4 and 5g). However, when supplemented with 2.0 mg/L 2,4-D and 0.5 mg/L 6-BA in the above condition medium, a higher callus induction percentage (66.33%) was obtained from segments of seeding plantlets (Fig. 4 and 5c). A lower frequency of callus induction with 1.0 mg/L 2, 4-D was observed (Fig. 4, 5a and b). Moreover, media supplementation with 3.0 mg/L 2, 4-D did not result in higher callus induction rates (Fig. 4 and 5d). To produce more embryonic calluses, the growth media should be supplemented with 2.0 mg/L 2, 4-D and 0.5 mg/L6-BA. In B_5 medium supplemented with 2, 4-D, a higher callus induction was observed when

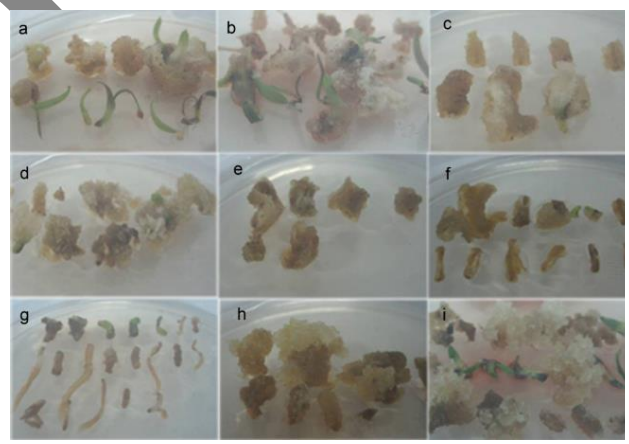

Fig. 5: Effects of different experiment factors on in-vitro callus induction of *T. chinensis* var. *mairei* for 40 days. (a) - Mci2012-23: B5 plus 2,4-D 1.0 mg•L-1; (b) - mci2012-24: B5 plus 2,4-D 1.0 mg/L and 6-BA 0.5 mg/L; (c) - mci2012-25: B5 plus 2,4-D 2.0 mg/L and 6-BA 0.5 mg/L; (d) - mci2012-26: B5 plus 2,4-D 3.0 mg and 6-BA 0.5 mg/L; (e) - mci2012-27: B5 plus NAA 1.0 mg/L and 6-BA 0.5 mg/L; (f) - mci2012-28: B5 plus NAA 2.0 mg/ and 6-BA 0.5 mg/L; (g) - mci2012-29: B5; (h) - mci2012-30: BLG plus 2,4-D 1.0 mg/L and 6-BA 0.5 mg/L; (i) - mci2012-31: BLG plus 2,4-D 2.0 mg/L and 6-BA 0.5 mg/L

compared with the calluses grown in medium supplemented with NAA; furthermore, A 2.0 mg/mL dose of 2, 4-D showed better results than a 1.0 mg/mL dose (Fig. 6).

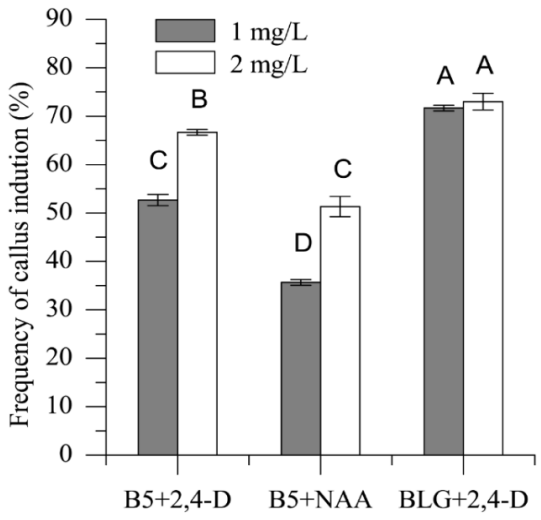

Fig. 6: Effects of different basic media and concentrations of plant growth regulator on the in vitro callus induction ratio of *T. chinensis* var. *mairei*. All the treatments were supplemented with 0.5 mg/L 6-BA, respectively

The growth potential and the quality of calluses in B_5 basic medium supplemented with 2, 4-D was better than those in medium supplemented with NAA (Fig. 5e and f). Calluses cultured in BLG basic medium supplemented with 2, 4-D grew more rapidly and showed similar embryonic callus structures (Fig. 5h and i). Furthermore, the frequency of callus induction in BLG medium with plant growth regulators was higher than that induced in B_5 medium (Fig. 6). Overall, the optimal medium for the callus induction of seeding plantlets of *T. chinensis* var. *mairei* was BLG medium supplemented with 2.0 mg/L 2,4-D, 0.5 mg/L 6-BA, 20.0 g/L sucrose and 7.0 g/L agar.

Half-Sib Cell Lines Construction

The BLG medium supplemented with 2.0 mg/L 2,4-D, 0.5 mg/L 6-BA, 20.0 g/L sucrose and 7.0 g/L agar was used as the optimal medium for the construction of half-sib cell lines. Three hundred fifty seeds from the same *T. chinensis* var. *mairei* mother plant were selected. Two hundred fifty-five embryonic calluses were obtained. The highly proliferating cell lines were used in the determination of toxoids.

The Selection of Taxoid-metabolizing Mutants in Half-sib of Cell Lines

The taxoid contents of the cell lines were detected by LC-ESI-MS. Four types of taxoids were detected in the cell lines derived from seedling plantlets. Significant variations were observed in taxoid levels for 100 cell lines; taxoids were also detectable in 26 of the half-sib cell lines. The paclitaxel concentration range in the half-sib cell lines was 0.00-60.78 mg/100 g FW (Table 1). The highest contents of paclitaxel

Table 1: The content of 4 compounds in different cell lines of *T. Chinensis* var. *mairei*

Cell lines number	Paclitaxel (mg/100g)	Cephalomannine (mg/100g)	BaccatinIII (mg/100g)	10-Deancetylbaccatin III (mg/100g)
2012-9	0.00	0.00	0.26	0.00
2012-16	4.40	0.41	1.74	1.00
2012-17	2.03	0.00	4.19	0.00
2012-23	1.62	0.00	0.00	0.00
2012-31	32.34	7.02	13.25	0.00
2012-32	53.30	3.10	3.05	0.00
2012-41	0.00	0.00	1.36	0.00
2012-54	4.99	0.00	0.29	0.00
2012-58	0.16	0.00	0.00	0.00
2012-78	4.59	2.68	2.02	0.00
2012-84	0.00	0.00	1.23	0.00
2012-86	1.39	0.00	0.33	0.00
2012-102	1.79	0.00	4.72	0.00
2012-104	4.46	2.15	0.62	0.00
2012-130	1.61	0.00	1.47	0.00
2012-145	2.21	2.16	3.40	0.00
2012-148	3.49	0.00	0.00	0.00
2012-150	1.99	1.79	3.71	0.00
2012-154	0.00	0.00	0.20	0.00
2012-182	29.54	0.00	0.00	0.00
2012-185	9.82	0.00	0.00	0.00
2012-196	5.97	0.00	0.00	0.00
2012-197	60.78	22.42	7.65	0.00
2012-215	1.14	0.00	3.05	0.00
2012-219	1.26	0.00	0.00	0.00
2012-222	0.00	0.00	0.32	0.00

(60.87 mg/100 g FW), cephalomannine (22.42 mg/100 g FW) and baccatin III (7.65 mg/100 g FW) were observed in cell line 2012-197, followed by cell line 2012-32. Only one cell line, 2012-16, was positive for the four measured taxoids, paclitaxel, deacetylbaccatin III, baccatin III and cephalomannine. The concentrations of paclitaxel in cell lines 2012-23, 2012-58, 2012-148 and 2012-196 ranged from 0.16 to 5.97 mg/100 g FW. Baccatin III was detectable in cell lines 2012-9, 2012-41 and 2012-222 with a range from 0.26 to 1.36 mg/100 g FW. These results indicated that high variations of taxoid metabolisms exist in the half-sib cell lines of *T. chinensis* var. *mairei* and that taxoid-metabolizing mutants could be obtained.

Discussion

The inhibitors in the endosperms and embryos of the *Taxus* genus cause seed dormancy. In immature embryos and endosperms, abscisic acid is a major inhibitor (Le Page-Degivry, 1968). Other inhibitors, including heptanoic acid, nonanoic acid and acetic acid, extracted from the middle spermoderm, endopleura and endosperm of *T. chinensis* var. *mairei* seed have inhibitory effects on germination (Zhang *et al.*, 2007). Furthermore, when water is unavailable to the embryos, a stronger germination inhibiting effect is observed (Qianhua, 2008). In our study, the embryos incubated on media without hydropriming could only achieve the lowest embryo germination rates. The results indicate that the embryonic inhibitors could be major factors causing seed dormancy in *T. chinensis* var. *mairei.*

Several studies have reported that seed dormancy could be broken by physical and chemical treatments (Flores and Sgrignoli, 1991; Chee, 1994; Zhiri *et al.*, 1994). It was evident that embryos isolated from seeds with running tap water for seven days resulted in 100% germination (Zhiri *et al.*, 1994). When the seeds of the *T. baccata* were soaked in distilled water at 4°C for at least two days followed by an incubation of the isolated embryo on MS media supplemented with 5 g/L activated charcoal, germination was observed (Zarek, 2007). Germination (55.8%) was also achieved in the seeds of *T. chinensis* var. *mairei* after soaking in 60% sulfuric acid solution for 3 h, followed by a treatment with a mixed solution of 100 mg/L GA_3 and 10 mg/L 6-BA (Qianhua, 2008). In present study, the hydropriming with sterile distilled water for three to five days at 24°C was an effective and simple method for embryo germination. Using this method, the embryonic inhibitors could potentially diffuse into the sterile water. The composition of the medium also proved to be another critical factor; 100% germination rates were obtained on BLG media supplemented with 20.0 g/L sucrose, 5.0 g/L activated carbon and 7.0 g/L agar with 3-5 days of hydropriming.

Light condition was another key factor in seed germination. Flores *et al*. (1993) suggested that a 14 h photoperiod could promote the embryo germination of *T. baccata* and *T. cuspidata* and their growth into seedlings. In our study, embryos cultivated in darkness for seven days achieved ideal germination rates. The inhibitors in seed coat and endosperm significantly arrested the seed germination of *T. chinensis* var. *marrei* (Le Page-Degivry, 1968; Zhang *et al.*, 2007). It is a promoter factor that these inhibitors released by hydropriming and removed the seed coat and endosperm accelerated the seed germination. And the darkness may avoid from the degradation of phytohormones, such as IAA and other beneficial germination substances and producing the ABA in embryos, which increase the higher seed germination of *T. chinensis* var. *mairei*. The seeds of different ecological groups with sensitivity to light may also account for this divergence (Costa *et al.*, 2018).

Our data demonstrated that different combinations of hormones and basic media caused different effects on callus induction among T. *chinensis* var. *mairei*. These results corroborated several other reports (Monacelli *et al.*, 1994; Jaziri *et al.*, 1996; Luo *et al.*, 1999). Genotype was considered a growth index of *Taxus brevifolia* (Gibson *et al.*, 1993). Embryo and juvenile tissues were suitable materials for callus induction, proliferation and regeneration (Mihaljević *et al.*, 2002; Datta *et al.*, 2006). In the present study, 9.33% of calluses could be induced from the plantlet segments on hormone-free B_5 basic media; however, these calluses did not exhibit any proliferation. Overall, the types and levels of hormones, the degree of development of the initial material and the type of basic medium significantly affected callus inductions of plantlets of *T. chinensis* var. *mairei*.

Due to genetic heterogeneity, the cell lines showed considerable variability in the production of secondary metabolites (Larkin and Scowcroft, 1981). Naik *et al.* (2012) suggested that different genotypes of *Bacopa monnieri* showed different accumulations of the triterpenoid secondary metabolite, saponin bacoside. Cell lines cultured under the same incubation conditions showed different capacities for paclitaxel production (Bruňáková *et al.*, 2004). The differences in biosynthetic activity among cultured cell lines were suggested to be major factors influencing paclitaxel production (Bonfill *et al.*, 2006). The paclitaxel contents of calluses from the same explants of the same mother plant or different mother plants showed significant variability (Bruňáková *et al.*, 2004). From 100 cell lines of half-sib progenies, twenty-six cell lines produced the taxoid metabolites, 10-deacetylbaccatin III, baccatin III, cephalomannine, and paclitaxel. High yields of paclitaxel and other taxoid metabolites were obtained from a number of cell lines from half-sibs of *T. chinensis* var. *mairei*; these cell lines may be valuable sources for suspension cultures.

Conclusion

Hydropriming with time intervals of three to five days was an efficiency method to overcome embryo germination barriers of *T. chinensis* var. *mairei*. The germination was inhibited by the light, while basic media had significant effects on the embryo germination and seeding plantlet growth of *T. chinensis* var. *mairei*. Our study provides a new strategy to achieve high seed germination rate and seeding plantlet growth of *T. chinensis* var. *mairei*.

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