ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 21(2) 2022, 77–86
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.2.7
ORIGINAL PAPER
Accepted: 6.10.2021

In vitro SEED GERMINATION AND SHOOT PROLIFERATION OF BAT FLOWER (*Tacca chantrieri* Andre)

Ni Made Armini Wiendi ✉, Rezky Yuanikha Nur Sushanty, Citra Aluna Sagita, Krisantini

Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Meranti Street, IPB Darmaga Campus, Darmaga, Bogor 16680, Indonesia

ABSTRACT

Tacca chantrieri Andre, or bat flower, is a species from Taccaceae family that has unique inflorescences consisting of large, dark-colored bracts with long whiskers, making it suitable as ornamental pot plants. *T. chantrieri* leaves and rhizomes contain phytochemicals that have been reported to have medicinal properties. *T. chantrieri* is increasingly hard to find in their native habitat; their seeds have a very slow and low germination rate, whereas propagation *in vivo* by division takes a long time. A protocol is presented to optimize seed germination and *in vitro* propagation of *T. chantrieri* from West Borneo, Indonesia. We have developed a method to improve *in vitro* germination of freshly harvested *T. chantrieri* seeds. Pre-sowing treatment with 5 mg L^{-1} of GA_3 for 5 × 24 h and addition of GA_3 at 5 mg L^{-1} to the MS medium significantly promoted earlier and final germination up to >90% within 10 weeks after sowing compared to without GA_3, or immersing in GA_3 for shorter durations. MS media supplemented with indole acetic acid (IAA) at 0.5 mgL^{-1} and benzyl adenine (BA) at 1 or 2 mgL^{-1} was effective to induce shoot proliferation from *in vitro* germinated seeds; 7–10 shoots were produced after 12 weeks of culture. Shoot proliferation from basal shoot explants were best on MS supplemented with IAA at 0.5 mgL^{-1}. The results of this study have provided a basis for further mass propagation efforts of *T. chantrieri*.

Key words: bat flower, cytokinin, auxin, Taccaceae

INTRODUCTION

Tacca chantrieri, also known as bat flower, or 'bunga kelelawar' in Indonesian, is a perennial belong to Taccaceae family. *T. chantrieri* has unique inflorescences consisting of large, dark-colored bracts with long whiskers; make it an attractive flowering ornamental plant. In Europe, *T. chantrieri* is a high value potted plants for its exotic dark-purple blooms with wing and uniquely long filaments, surrounded by large dark green foliages. *Tacca chantrieri* in the northern hemisphere flowers in spring to early fall. The phytochemical content of *T. chantrieri* rhizomes and their potential uses as herbal have been reported, e.g. *T. chantrieri* rhizomes have been used as traditional herbs in China [Yokosuka et al. 2002] and Thailand [Charoensub et al. 2008]. Rhizomes of the species contain saponin spirostanol, which is regarded as being effective against leukemia [Yokosuka et al. 2002]. Rhizomes of *T. chantrieri* contain taccalonolide AJ and taccabulin A, two distinct microtubule active compounds that have synergistic effects against proliferative cancer cells [Risinger et al. 2013]. Evelynin, a new benzoquinone-type *retro*-dihydrochalcone, was isolated from the roots and rhizomes of *Tacca chantrieri; e*velynin has antiproliferative activities against a range of cancer cells at low micromolar concentrations [Peng et al. 2010].

✉ nmarmini@gmail.com

Most studies have reported the phytochemical content of *T. chantrieri* rhizomes, and limited studies have been conducted on its culture and propagation. He at al [2002] and Krisantini et al. [2017] reported a low and slow seed germination of *T. chantrieri* seeds *in vivo*. Charoensub et al. [2018] reported micropropagation of *T. chantrieri* by culturing sterile seedlings *in vitro*, but no studies have been reported on the seed viability and germination of seeds from different fruit maturity. Seeds collected from the ripe fruits have reached their physiological maturity and will produce vigorous seedlings [Murrinie et al. 2019]. Seed germination increases along with the ripening of the fruits, even though Cruz-Tejada et al. [2018] reported that in several tropical species there were no differences between the ripe and overripe stages of fruits in affecting seed germination. Therefore, in this study we evaluated germination of the seeds collected from ripe and overripe fruits of *T. chantrieri*.

Gibberellic acids, particularly GA_3, GA_4, and GA_7, are effective in breaking the dormancy and promoting seed germination of *Solanum* sp. [Gisbert et al. 2011], *Pistacia lentiscus* [Yildirim et al. 2018], *Penstemon digitalis* [De Mello et al. 2009], and loquat [Al-Hawezy 2013]. GA actions might be related to changes in the seed membrane properties or to weakening of the endosperm layer [Gisbert et al. 2011] which leads to seed germination.

In vitro propagation allows large scale production of crops which otherwise have low chances of germinating and growing. Cytokinin in combination with auxin can induce high regeneration potential; e.g. 9.0 µM benzyl adenine (BA) and 0.5 µM indole-3-acetic acid (IAA) resulted in >80% of shoot proliferation of *Pyrus elaeagrifolia* [Aygan and Dumanoglu 2015]. In *Vigna subterranean* a high number of shoots per explant from cotyledons were obtained on medium supplemented with 3–5 µM of BAP combined with synthetic auxin 0.01–0.1 mg L^{-1} NAA [Kone et al. 2007]. These reports highlighted that the optimum ratio of cytokinin to auxin for shoot proliferation might vary with plant species. Development of multiple shoots from an explant in tissue culture propagation is a desired growth character to allow further multiplication.

The aims of this work are to improve germination of *T. chantrieri* from West Borneo and to develop its propagation method *in vitro*. Specifically, our studies are to answer the following questions: (1) what are the germination rates of *T. chantrieri* seeds from ripe and over ripe fruits?; (2) can GA_3 treatment improve germination of *T. chantrieri* seeds?; and (3) can IAA and BAP addition to MS media improve shoot proliferation of the *in vitro* germinated seedlings?

MATERIALS AND METHODS

Plant material, culture conditions and establishment. *Tacca chantrieri* for this experiment was originated from West Borneo that has been preserverd, maintained and propagated at Bogor Botanical Garden, West Java. Mature plants of *T. chantrieri* were purchased from Bogor Botanical Garden nursery in 2015. The plants were grown under shade structure at the Department of Agronomy and Horticulture nursery in Darmaga, (6°35'3.3"S, 106°43'51.8"E), IPB University, Indonesia, until flowering and produced fruits. Seeds were washed with sterilized water, disinfected in fungicide (Dithane® M-45, a.i. mancozeb) and bactericide (Agrept® 20 WP, a.i. streptomycin), immersed in 30% chlorox (active ingredient NaClO 5.25%) for 5 min, and washed three times with sterilized water prior to sowing to the MS medium containing 30 g·L^{-1} sucrose and 7 g·L^{-1} agar. All chemical used were of analytical grade (Sigma Chemical Co. USA). The pH of all media was adjusted to 5.8 with 0.1 N NaOH before autoclaving (121°C for 20 min). Plant growth regulators (PGRs) were added to the medium prior to adjustment of pH and sterilization. Unless otherwise described, all cultures were maintained in a 24-h photoperiod at 23 ±2°C with photosynthetic photon flux density of ±134 lux (15 W, 59 lumen W^{-1}) provided by cool white fluorescent lamps.

Effect of fruit maturity on seed germination. Three separate studies on seed germination were conducted. The first experiment tested the germination rates of seeds from ripe and overripe fruits. Seeds each from ripe and overripe fruits (Fig. 1 B) were sown on 30 mL MS media in 100 mL flasks 3 days after harvesting the fruits. Ten seeds each were sown per flask, and each treatment was replicated nine times. Seed germination was recorded weekly for 14 weeks after sowing.

Effect of different GA_3 concentration on germination. The second experiment tested whether addi-

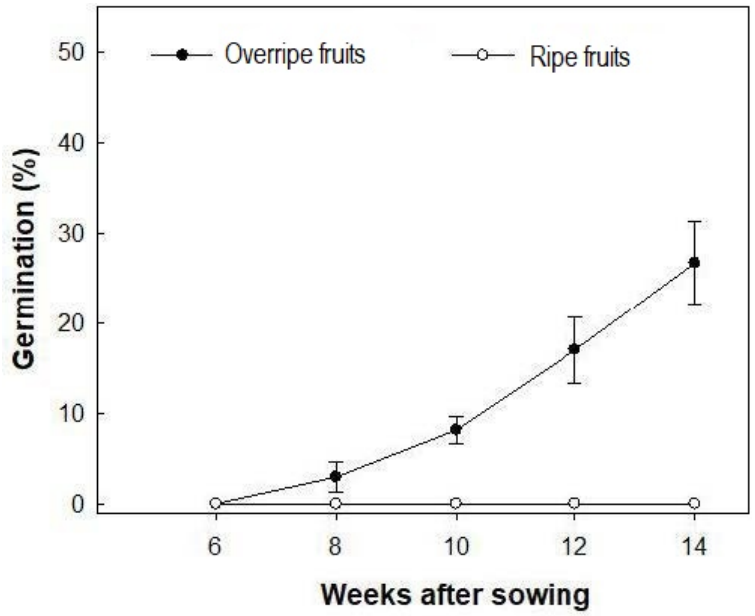


Fig. 1. Germination of *T. chantrieri* seeds collected from ripe and overripe fruits

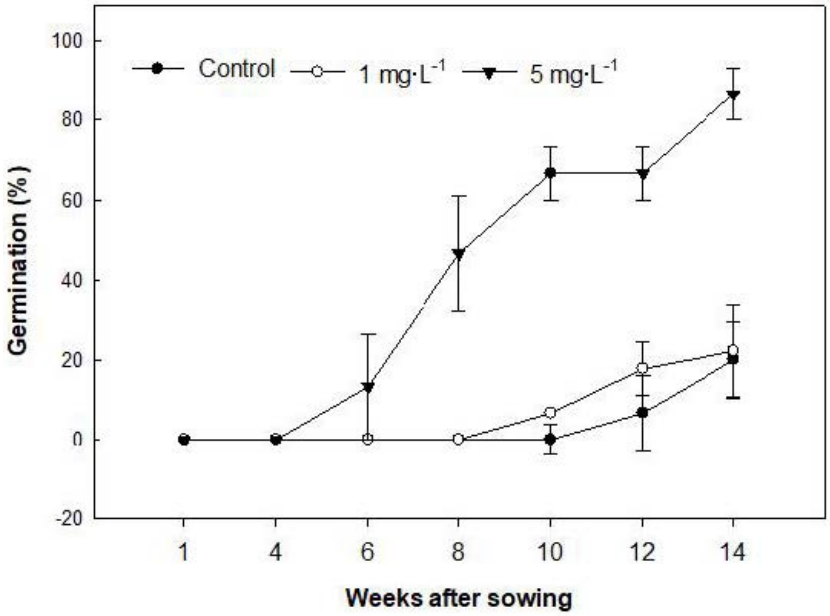


Fig. 2. *T. chantrieri* seed germination in MS media without and with GA_3 supplementary

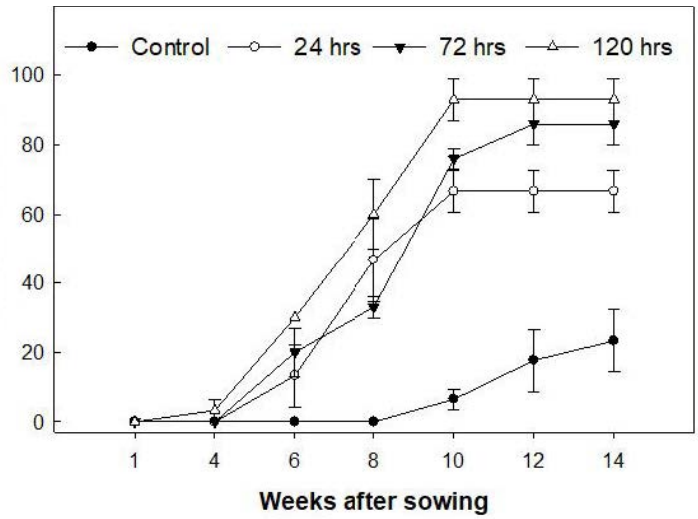
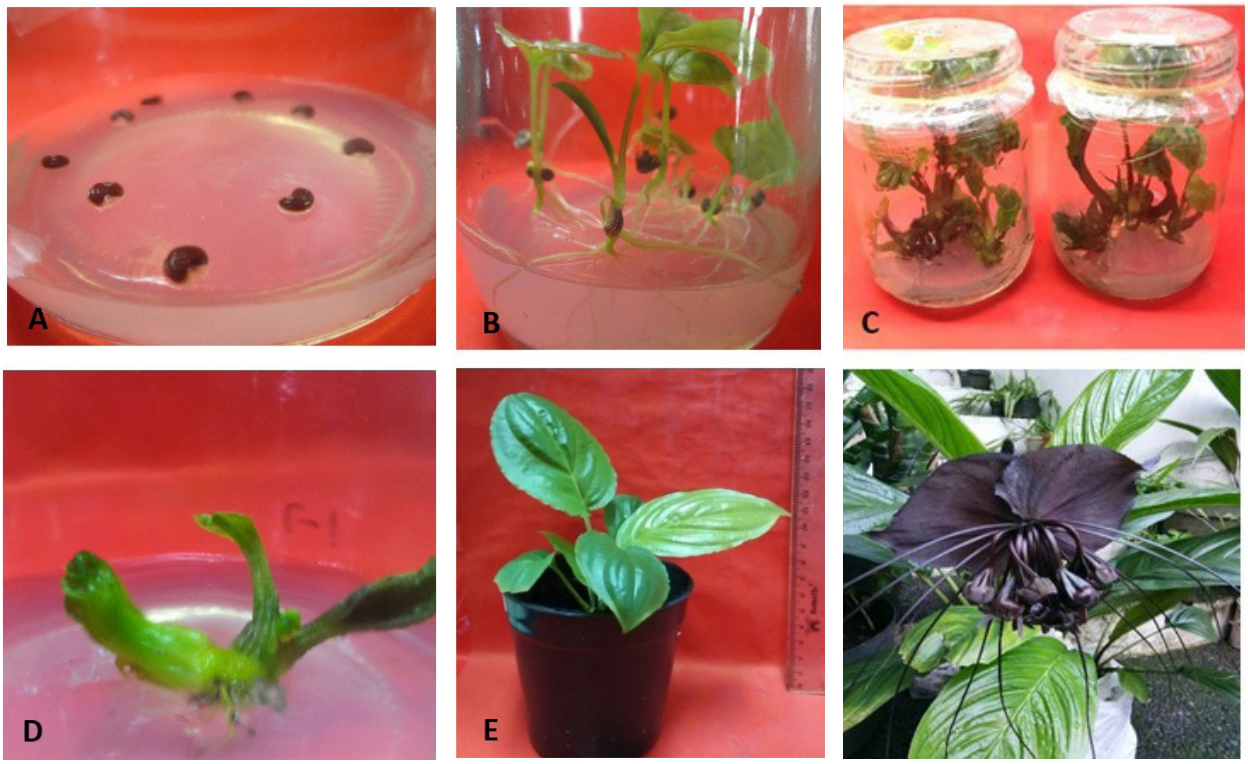


Fig. 3. *In vitro* germination of *T. chantrieri* seeds from overripe fruits after treatment with 5 mgL^{-1} of GA_3 solution for 1, 3 and 5 days prior to sowing



Germination seeds without PGR; (B) germinated seeds after incubation of seeds in 5 $mg \cdot L^{-1}$ of GA_3 for 5 days; (C) individual seedlings on MS medium supplemented with IAA at 0.5 $mg \cdot L^{-1}$ and BA at 2 $mg \cdot L^{-1}$; (D) basal shoot explant on MS medium supplemented with IAA at 0.5 $mg \cdot L^{-1}$ and BA at 3 $mg \cdot L^{-1}$; (E) acclimatized plants at 4 weeks after acclimatization; (F) acclimatized plants after transplanted to individual pots at 12 weeks after acclimatization

Fig. 4. *In vitro* culture of seeds and development of *T. chantrieri* plantlets

tion of GA_3 in the MS media at 1 and 5 mg L^{-1} affect seed germination. MS media without GA_3 was used as control. Ten seeds were sown on each 30 mL media in 100 mL flasks, and each treatment was replicated three times.

Effect of duration of seed imbibition in GA_3 solution on germination. The third experiment tested the effects of imbibition duration in 5 ppm GA_3 on seed germination, i.e. 1, 3, or 5 × 24 hours, prior to sowing on the MS media. Ten seeds were sown on each 30 mL media in 100 mL flasks, and each treatment was replicated three times.

The effects of GA_3 on seed germination experiments were repeated at least twice using the similar number of seeds and replications under the same environment. Percentage of germinated seeds was measured weekly from the 4th to the 12th week after sowing.

Shoot proliferation. To study shoot proliferation of seedlings, *in vitro* germinated seeds were transferred individually to MS medium and supplemented with auxin IAA at 0.25 mgL^{-1} or 0.50 mgL^{-1} in combination with N^6-benzyladenine (BA) at 1 mgL^{-1}, 2 mgL^{-1}, or 3 mgL^{-1}. MS hormone-free media was set as control. Each treatment consists of 9 replications, with 1 seedling per flask. Experiments were conducted in a culture room with environment as described for the germination study. Shoot proliferation of the *in vitro* germinated seedlings was measured weekly over a period of 12 weeks.

The basal shoots from *in vitro* germinated seedlings were used as explants and grown on MS medium supplemented with IAA at 0.5, 1, or 1.50 mgL^{-1} in combination with N^6-benzyladenine (BA) at 3 mg L^{-1}, 4 mg L^{-1}, or 5 mg L^{-1}. Each treatment repeated five times with 3 explants per flask. Proliferation was measured for 12 weeks based on the number of shoots developed per explant. All cultures were maintained in a culture room with the environmental condition described above.

Rooting and acclimatization. Shoots that developed from proliferation study were rooted on MS medium supplemented with IAA at 0.1, 0.5, and 1 mgL^{-1} in combination with N^6-benzyladenine (BA) at 1 mg L^{-1}, 2 mg L^{-1}, or 3 mg L^{-1}. Plantlets that have grew up to >20 mm in height having a minimum of one fully expanded leaf and two roots were transferred out of the flasks. Agar medium attached to the roots were gently washed out with running tap water prior to planting into 15-cm pots containing a mixture of sterilized compost, coir and burnt rice hulls (1 : 1 : 1 by volume) under shade. During the first 7 days after transfer each pot was covered with plastic sheets to maintain humidity. Plantlets were watered twice a day and fertilized with MS solution weekly. The final number of explant survival was counted on the 4th and 8th week after acclimatization.

Statistical analysis. Data were subjected to analysis of variance using SAS 9.1.3. Significant differences between treatments were further separated by Fisher test at $\alpha = 0.05$.

RESULTS

Effect of fruit maturity on seed germination. Only seeds from overripe fruits that can germinate. The first germination was recorded at 8 weeks after sowing with a maximum germination of <30% at 14 weeks (Fig. 1). None of the seeds from ripe fruits can germinate within 14 weeks of study. Therefore, the next experiments only used seeds collected from overripe fruits.

Effect of different GA_3 concentration on germination. Addition of GA_3 at 5 mg L^{-1} to MS media significantly promoted earlier germination compared to media containing 1 mg L^{-1} GA_3 or media without GA_3. Seeds on MS + 5 mg L^{-1} GA_3 started germinating at 6 weeks after sowing and germination reached 90% at 14 weeks after sowing (Fig. 2, 4A, 4B). Seeds germinated on the media without GA_3 or with GA_3 concentration of 1 mg L^{-1} germinated slowly, only ca. 20% seeds from these two treatments germinated at 14 weeks after sowing (Fig. 2).

Effect of duration of seed imbibition in GA_3 solution on germination. Having determined that addition of 5 mg L^{-1} of GA_3 on MS media significantly improved germination, we examined if duration of seed imbibition in GA_3 solution prior to sowing affects *T. chantrieri* seed germination. Imbibing seeds in GA_3 solution for 5 × 24 hours was effective in promoting earlier germination, i.e. at 4 weeks after sowing, and increased germination percentage of *T. chantrieri* seeds by 25% over hormone-free media (Fig. 3 and 4 B). Seeds immersed in 5 mgL^{-1} GA_3 for 5 × 24 hours had the final germination of 92% at 10 weeks after sowing, compared to only 5% in the control, 67% with

Table 1. Growth responses of *T. chantrieri* seedlings on MS supplemented with various concentrations of indole-acetic-acid (IAA) and benzyl-adenine (BA) after 12 weeks of the culture

Treatment	Responsive seedling (%)	Shoot number	Root number
Control	0	1.00 a	3.33
IAA 0.25 mg L^{-1}			
BA mg L^{-1}			
1	66.67	4.33 ab	4.33
2	53.33	2.00 a	2.33
3	66.67	4.00 ab	4.33
IAA 0.50 mg L^{-1}			
BA mg L^{-1}			
1	66.67	9.67 b	3.00
2	100.00	7.33 b	3.33
3	66.67	2.67 a	3.67
		*	ns

Means followed by the different letters within one column are significantly different according to Fisher test at 5% (*)

Table 2. Growth responses of *T. chantrieri* shoot explants treated with various concentrations of indole-acetic-acid (IAA) and benzyl-adenine (BA) at 8 weeks after culture

PGR	Survived culture (%)	Explants that formed callus (%)	Explants that formed new shoots (%)	Shoot number
IAA mg L^{-1}				
0.5	62.89	53.89a	69.67	7.03a
1.0	77.78	82.67b	67.00	4.72ab
1.5	65.33	53.67a	63.00	4.21b
	ns	*	ns	*
BA mg L^{-1}				
3	75.33	57.89	71.11	6.16
4	61.67	72.44	68.67	5.12
5	69.00	59.89	59.89	4.73
	ns	ns	ns	ns
IAA × BA	ns	ns	ns	ns

Means followed by the different letters within one column are significantly different according to Fisher test at 5% (*)

the 1 × 24 hours, 87% with the 3 × 24 hour immersion in GA_3 solution (Fig. 3).

Shoot proliferation. *Tacca chantrieri* seedlings on the MS media supplemented with IAA and BA showed significant increase in the number of shoot, whereas seedlings on the MS medium without PGR only grew one main shoot that grew taller and larger (Tab. 1). Seedlings on MS supplemented with IAA 0.5 mg L^{-1} and BA at 1 mg L^{-1} or 2 mg L^{-1} started developing shoots at 4 weeks after culture establishment (data not shown), and at 12 weeks had developed significantly more new shoots (>7) than those on the other media (2–4; Fig. 4c; Tab. 1). The maximum responsive seedling (100%) was obtained from MS supplemented with 0.50 mg L^{-1} and BA at 2 mg L^{-1} (Tab. 2).

In the second subculture, basal shoot explants developed new shoots with addition of IAA and BA to the MS medium (Tab. 2). The control explants were excluded from statistical analysis as they only formed callus. The percentage of survived culture were similar across treatments (Tab. 2). Explants in the MS media containing 1 mg L^{-1} of IAA formed the highest percentage of callus (82.67%) whereas explants in the media containing 0.5 mg L^{-1} of IAA produced the highest number of new shoots (7.03; Tab. 2). BA concentrations did not affect the percentage of explants that formed callus or shoots, and the number of shoots (Tab. 2).

Rooting and acclimatization of *T. chantrieri*. Addition of IAA from 0.1 to 0.5 mg L^{-1} to the MS medium did not increase the number of roots per plantlet, and explants on MS with IAA at 1 mg L^{-1} had fewer root number compared to control (Tab. 3). Increasing the BA concentrations from 1 to 3 mg L^{-1} progressively reduced the root number (Tab. 3). After transfer to the *ex vitro* environment >97% plants survived after 8 weeks, and the percentage of survived plants were similar across treatments (Tab. 3, Fig. 4e and 4f).

DISCUSSION

This study demonstrated that only seeds from overripe fruits that can germinate. Pre-sowing treatment with 5 mg L^{-1} of GA_3 for 5 × 24 h, and sowing the seeds on the MS media supplemented with GA_3 at 5 mg L^{-1} significantly promoted earlier and high fi-

Table 3. Root number and root length of *T. chantrieri* treated with various concentrations of indole-acetic-acid (IAA) and benzyl-adenine (BA) on MS media at 33 weeks after culture and survived explants after acclimatization

PGR	Root length (cm)	Root number	Survived explants at	
			4 weeks after acclimatization (%)	8 weeks after acclimatization (%)
Control	2.7 ±1.5	11.1 ±5.5 a	100	100
IAA (mg L^{-1})				
0.1	2.3 ±1.2	10.1 ±5.9 a	100	97.2
0.5	1.7 ±0.7	9.8 ±5.4 a	100	97.2
1	1.9 ±1.1	8.9 ±5.4 b	100	100
	ns	ns	ns	ns
BA (mg L^{-1})				
1	1.9 ± 0.9	12.1 ± 6.2 a	100	97.2
2	1.9 ± 1.1	9.7 ± 6.0 b	100	97.2
3	2.1 ± 1.2	7.4 ± 3.7 c	100	100
	ns	**	ns	ns
IAA – BA	ns	ns	ns	ns

Means followed by the different letters within one column are significantly different according to Fisher test at 5% (*) or at 1 % (**)

nal germination (>90%) within 10 weeks after sowing compared to without GA_3, or immersing in GA_3 for shorter durations of 1 and 3 days (Fig. 2). Previous studies had reported *T. chantrieri* germination of only 10–20% *in vivo* after 4 months [Krisantini et al. 2017], and He et al. [2002] reported a germination rate of 12% after 8 months. Failure of seeds to germinate can be caused by several reasons; seeds could be produced by unhealthy mother plants [Narayanaswamy and Siddaraju 2011], seeds are physiologically immature [Ayyub et al. 2007], seeds do not have fully developed embryo [Forbis et al. 2002, Lafon-Placette and Kohler 2014]. The source and age of the seeds used in the previous report by He et al. [2002] were not specified. *T. chantrieri* seeds in the current study were sown within three days after harvesting the fruits from the well-maintained stock plants grown under a shaded structure. We hypothesized that the viability of *T. chantrieri* seeds decreases quickly over time, which perhaps had differentiated the results of our study from the previous studies reporting poor germination of *T. chantrieri* seeds. This hypothesis, however, needs further confirmation, as no study has reported seed viability and germination rates of *T. chantrieri* seeds of different maturity stages, or duration of seed storage after harvest. The optimum growing environment of the stock plants in this study had likely to result in good quality seeds.

Gibberellic acids, particularly GA_3, GA_4, and GA_7, can break seed dormancy of a number of crops including *Solanum* sp. [Gisbert et al. 2011], *Myrica rubra* [Chen et al. 2008], *Penstemon digitalis* [De Mello et al. 2009] and *Brassica tournefortii* [Mahajan et al. 2018]. GA action on germination promotion was related to enhanced mobilization of food reserves in the seeds [Subedi and Bhattarai 2006], whereas GA effects on seed germination in *Myrica rubra* [Chen et al. 2008] were through promotion of embryo growth. It is possible that a similar system occurs in *T. chantrieri*. The germination protocol using GA_3 from this study has provided a significant improvement for *T. chantrieri* germination *in vitro*, which could be useful for the future studies on *T. chantrieri* propagation and could be tested to other Tacca species.

Auxin and cytokinin interacted in regulating plant development, including meristem formation [Su et al. 2011]. It is important to have an optimal ratio of auxin to cytokinin during cell division and differentiation of plant tissues [Bashan and de-Bashan2010]; the change of auxin to cytokinin ratio could affect shoot proliferation [Clayton et al. 1990]. BA has been widely used for *in vitro* shoot induction of different crops including legumes [Aasim et al. 2010]. Benzyl-adenine is a relatively stable compound and inexpensive compared to e.g. zeatin and isopentenyladenine [Bashan and de-Bashan 2010]. Depending on the crop species and cultivars, BA in combination with IAA was more effective for *in vitro* shoot regeneration compared to BA and a synthetic auxin NAA [Aasim et al. 2010]. However, the effective type of cytokinin and its optimal concentration might vary with the crops and culture [Park et al. 2008]. pOur study demonstrated that media with IAA at 0.5 mg L^{-1} combined with BA at 1 or 2 mg L^{-1} had more shoot proliferation compared to the other treatments (Tab. 1). When basal shoots from the germinated seedlings were cultured on MS containing 0.5, 1 and 1.5 mg L^{-1} of IAA, explants on IAA at 0.5 mg L^{-1} had a higher percentage of proliferation and number of shoots per explant than IAA at higher concentrations (Tab. 2) whereas increasing BA concentrations from 3 to 5 mg L^{-1} did not affect shoot proliferation (Tab. 2).

At rooting stage, addition of IAA to the MS media in our system did not increase root formation whereas adding IAA at 1 mg L^{-1}, BA at 2 or 3 mg L^{-1} significantly reduced root formation over control (Tab. 3). The survival of the plantlets after acclimatization across treatments were similar; at 8 weeks after acclimatization the survival rate was around 97% (Tab. 3). The acclimatization results in this study are comparable with the success rate of the other experiments conducted in *T. leontopetaloides* [Cepkova et al. 2015]. No morphologically abnormal growth was observed, and the plants developed new leaves within 8 weeks after transferring to the *ex vitro* environment. These results showed that the *in vitro* propagation protocol developed in this study resulted in viable plants.

CONCLUSIONS

Only seeds collected from overripe fruits that can germinate. *T. chantrieri* seed germination can be promoted by imbibing the seeds in the 5 mg L^{-1} of GA_3 solution for 5 days, followed by sowing into MS me-

dia supplemented with 5 mg L^{-1} GA_3. MS media supplemented with indole acetic acid at 0.5 mg L^{-1} and benzyl adenine at 1 or 2 mg L^{-1} was effective to induce shoot proliferation of *in vitro* germinated seeds; 7–10 shoots were produced after 12 weeks of culture. Rooted plantlets were successfully acclimatized with 97–100% survival. The present protocol to propagate *T. chantrieri* have provided a basis for further propagation studies of this species.

ACKNOWLEDGMENTS

The authors wish to thank The Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, for their supports throughout our study. We acknowledge Dr Christian Forstner of The University of Queensland, Australia, for English editing of this manuscript.

REFERENCES

Aasim, M., Hussain, N., Umer, E.M., Zubair, M., Hussain, S.B., Saeed, S., Rafique, T.S., Sancak, C. (2010). *In vitro* shoot regeneration of fenugreek (*Trigonella foenum-graecum* L.) using different cytokinins. Afr. J. Biotech., 9, 7174–7179.

Al-Hawezy, M.S.N. (2013). The role of the different concentrations of GA_3 on seed germination and seedling growth of loquat (*Eriobotrya japonica* L.). J. Agric. Vet. Sci., 4(5), 3–6. https://doi.org/10.9790/2380-0450306

Ajisyahputra, N.R., Palupi, E.R., Wiendi, N.M.A., Mulyono, J., Krisantini (2017). Evaluation of growth, flowering and seed morphology of bat flower *Tacca chantrieri* Andre. J. Trop. Crop Sci., 64–69. https://doi.org/10.29244/JTCS.4.2.64-69

Aygan, A., Dumanoglu, H. (2015). *In vitro* shoot proliferation and *in vitro* and *ex vitro* root formation of *Pyrus elaeagrifolia* Pallas. Front Plant Sci., 6, 225–239. https://doi.org/10.3389/FPLS.2015.00225.

Ayyub, C.M., Ziaf, K., Pervez, M.A., Amjad, M., Rasheed, S., Akkhtar, N. (2007). Effect of seed maturity and storability on viability and vigour in pea (*Pisum sativum* L.) seeds. In: Proceedings of International Symposium of Horticultural Industry in Pakistan 28th–30th March 2017. Institute of Horticultural Science, University of Agriculture, Faisalabad, Pakistan.

Bashan, Y., de-Bashan, L.E. (2010). How the plant growth-promoting bacterium *Azospririllum* promotes plant growth – a critical assessment. Adv. Agron. 108, 77–135. https://doi.org/10.1016/S0065-2113(10)08002-8

Cepkova, P.H., Vitamvas, J., Ciehmannova, I., Kisilova, J., Cusimamani, E.F., Milella, L. (2015). Simplified *in vitro* propagation protocol for *Tacca leontopetaloides* (L.) Kuntze and assessment of genetic uniformity of regenerated plantlets. Emir. J. Food Agric., 27(10), 736–743. https://doi.org/10.9755/ejfa.2015.06.350

Charoensub, R., Thiantong, D., Phansiri, S. (2008). Micropropagation of bat flower plant *Tacca chantrieri* Andre. Kasetsart J. Nat. Sci., 42, 7–12.

Chen, S.Y., Kuo, S.R., Chien, C.T. (2008). Roles of gibberellins and abscisic acid in dormancy and germination of red bayberry (*Myrica rubra*) seeds. Tree Physiol., 28(9), 1431–1439. https://doi.org/10.1093/TREEPHYS/28.9.1431

Clayton, P.W., Hubstenberger, J.F., Phillips, G.C. (1990). Micropropagation of members of the Cactaceae Subtribe Cactinae. J. Amer. Soc. Hort. Sci., 115(2), 337–343. https://doi.org/10.21273/JASHS.115.2.337

Cruz-Tejada, D.M., Acosta-Rojas, D.C., Stevenson, P.R. (2018). Are seeds able to germinate before fruit color ripening? Evidence from six Neotropical bird-dispersed plant species. Ecosphere, 9(6), 1–18. https://doi.org/10.1002/ecs2.2174

De Mello, A.M., Streck, N.A., Blankenship, E.E., Paparozzi, E.T. (2009). Gibberellic acid promotes seed germination in *Penstemon digitalis* cv. Husker Red. Hort. Sci., 44(3), 870–873. https://doi.org/10.21273/HORTSCI.44.3.870

Forbis, T.A., Floyd, S.K., Queiroz, A. 2002. The evolution of embryo size in Angiosperms and other seed plants: implications for the evolution of seed dormancy. Evolution, 56(11), 2112–2125. https://doi.org/10.1111/J.0014-3820.2002.TB00137.X

Gisbert, C., Prohens, J., Nuez, F. (2011). Treatments for improving seed germination in eggplant and related species. Acta Hortic., 898, 45–51. https://doi.org/10.17660/ACTAHORTIC.2011.898.4

He, H., Lan, Q., Zhang, Y. (2002). *In vitro* propagation of *Tacca chantrieri.* J. Agric. Biol. Sci., 21(2), 108–110.

Koné, M., Patat-Ochatt, E.M., Conreux, C., Sangwan, R.S., Ochatt., S.J. (2007). *In vitro* morphogenesis from cotyledon and epicotyl explants and flow cytometry distinction between landraces of Bambara groundnut [*Vigna subterranea* (L.) Verdc], an under-utilised grain legume. Plant Cell Tiss. Org. Cult., 88, 61–75. https://doi.org/10.1007/s11240-006-9179-y

Krisantini, Wiendi N.M.A., Palupi E.R. (2017). Evaluation of horticultural traits and seed germination of *Tacca chantrieri* 'André. Agric. Nat. Res., 51(3), 169–172. https://doi.org/10.1016/J.ANRES.2016.12.006

Lafon-Placette, C., Kohler, C. (2014). Embryo and endosperm, partners in seed development. Curr. Opin.

Plant Biol., 17, 64–69. https://doi.org/10.1016/J.PBI.2013.11.008

Mahajan, G., Mutti, N.K., Jha, P., Walsh, M., Chauhan, B.S. (2018). Evaluation of dormancy breaking methods for enhanced germination in four biotypes of *Brassica tournefortii*. Sci. Reports, 8(1), 17103. https://doi.org/10.1038/S41598-018-35574-2

Murrinie, E.D., Yudono, P., Purwantoro, A., Sulistyaningsih, E. (2019). Effect of fruit age and postharvest maturation storage on germination and seedling vigor of wood apple (*Feronia limonia* L. Swingle). Asian J. Agric. Biol., special issue, 196–204.

Narayanaswamy, S., Siddaraju, R. (2011). Influence of spacing and mother plant nutrition on seed yield and quality of sweet corn (*Zea mays* var. *rugosa*). Mysore J. Agric. Sci., 45(2), 296–299.

Park, S.Y., Kim, Y.W., Moon, H.K., Murthy, H.N., Choi, Y.H., Cho, H.M. (2008). Micropropagation of Salix pseudolasiogne from nodal explants. Plant Cell Tiss. Org. Cult., 93, 341–346.

Peng, J., Jackson, E.M., Babinski, D.J., Risinger, A.L., Helms, G., Frantz, D.E., Mooberry, S.L. (2010). Evelynin, a cytotoxic benzoquinone-type retro-dihydrochalcone from *Tacca chantrieri*. J. Nat. Prod., 73(9), 1590–1592. https://doi.org/10.1021/NP100350S.

Risinger, A.L., Peng, J., Rohena, C.C., Aguilar, H.R., Frantz, D.E., Mooberry, S.L. (2013). The bat flower: a source of microtubule destabilizing and stabilizing compounds with synergistic antiproliferative actions. J. Nat. Prod., 76(10), 1923–1929. https://doi.org/10.1021/NP4005079.

Su, Y.H., Liu, Y.B., Zhang, X.S. (2011). Auxin cytokinin interaction regulates meristem development. Mol. Plant, 4(4), 616–625. https://doi.org/10.1093/MP/SSR007

Subedi, C.K., Bhattarai, T. (2006). Effect of gibberellic acid on reserve food mobilization of maize (*Zea mays* L. var Arun-2) endosperm during germination. Himalayan J. Sci., 1(2), 99–102. https://doi.org/10.3126/HJS.V1I2.205

Yildirim, H., Calar, N., Onay, A. (2018). An effective protocol for in vitro germination and seedling development of lentisk (*Pistacia lentiscus* L.). Acta Sci. Pol. Hortorum Cultus, 17(4), 3–13. https://doi.org/10.24326/asphc.2018.4.1

Yokosuka, A., Mimaki, Y., Sashida, Y. (2002). Steroidal and pregnane glycosides from the rhizomes of *Tacca chantrieri*. J. Nat. Prod., 65(9), 1293–1298. https://doi.org/10.1021/np020094l