



Original Article

Evaluation of horticultural traits and seed germination of *Tacca chantrieri* 'AndréKrisantini,^{*} Ni Made Armini Wiendi, Endah Retno Palupi

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ABSTRACT

Tacca chantrieri André is a perennial plant belonging to the Taccaceae family. *T. chantrieri* is known as the 'bat flower' or 'bat plant' due to its unique black bracts that resemble bats. It has the potential to be commercialized as an indoor, flowering, ornamental plant due to its unique flower morphology and shade tolerance. The distribution of *T. chantrieri* in its natural habitat has contracted due to land clearing and habitat destruction to the extent that it is now very hard to find in its traditional natural environment. The objectives of this study were to evaluate the morphological characteristics of *T. chantrieri*, to evaluate seed germination *in vivo* and *in vitro*, and *in vitro* culture of the plant using standard Murashige and Skoog (MS) media. Only 10% of the seeds germinated in 22 wk. An evaluation of the non-germinated seed showed that 42% of the seeds did not have embryos. *In vitro* culture using the standard MS media resulted in 3–7 new shoots growing from the basal parts of the seedlings after 22 wk, and each shoot further developed 4–7 shoots following transfer to MS media supplemented with indole acetic acid (IAA) at 0.25–0.75 mg/L and N⁶-benzyladenine (BA) at 1–2 mg/L. These results indicate that even though propagation protocols should be further developed, *in vitro* propagation using standard MS media supplemented with combinations of IAA and BA provides a more effective way to propagate *T. chantrieri* when compared to the conventional propagation techniques. This information will be useful for introducing *T. chantrieri* to the new ornamental plant market and to conservation efforts for this species.

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Introduction

Tacca chantrieri André is perennial plant belonging to the Taccaceae family. The *Tacca* genus has 10 species and is the only genus of the monocotyledon family of the Taccaceae (Zhang et al., 2005). *T. chantrieri* can be found growing in the understory of natural forests in the humid tropical regions of Southeast Asia (Zhang et al., 2005). From the corresponding author's personal observation, *T. chantrieri* leaves are broad and dark green in color, similar to those of the peace lily, *Spathiphyllum* (Araceae). *T. chantrieri* is also known as the 'bat flower' or 'bat plant', due to its large, dark-colored bracts that resemble bats, with lots of long whiskers or bracteoles growing beneath the bracts (Zhang et al., 2005). *T. chantrieri* has the potential to be introduced as a new ornamental plant in the market due to its unique and attractive floral displays and black, floral-colored bracts.

At least two native species have been recorded in Indonesia—*T. borneensis* and *T. palmata* (Quattrocchi, 2012), though

its natural distribution has contracted due to land clearing and habitat destruction (Zhao and Zhang, 2015). Published information on the species is mostly in relation to the phytochemical and medicinal properties of their rhizomes which have been used as a source of traditional medicine in China (Yokosuka and Mimaki, 2016) and Thailand (Steinrut et al., 2011). Rhizomes of the species contain saponin spirostanol, which is regarded as being effective against leukemia (Yokosuka et al., 2002). Compounds from the rhizome can reduce inflammation and can cure abscesses in the stomach and duodenum (Zhang et al., 2007). The attributes for introducing *T. chantrieri* as a potential new, potted, ornamental plant have not previously been assessed and reported.

Reports on *in vitro* propagation of the species are limited; for example, Charoensub et al. (2008) and He et al. (2002) for *T. chantrieri*, and Cepkova et al. (2015) for *T. leontopetaloides*. The current study provides a preliminary report on *T. chantrieri* propagation from seeds *in vivo* and *in vitro*, and its floricultural attributes, including growing period, flowering period and duration, flower-stem length, shape of flowers, time-to-anthesis, and plant growth habit. Low germination of *T. chantrieri* seeds has been reported (Charoensub et al., 2008). The plant growth regulator

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gibberellic acid (GA₃) was used in the current study to determine whether or not treatment by soaking with GA₃ would promote seed germination to facilitate stock plant establishment. Gibberellic acid (GA₃, GA₄, and GA₇) is effective in breaking the dormancy and increasing the seed germination of many plant species, including *Penstemon digitalis* (De Mello et al., 2009), and *Solanum* sp. (Gisbert et al., 2011). The study was extended to include the proliferation of *T. chantrieri* from seedlings grown *in vitro*. This information is potentially important in relation to the introduction of *T. chantrieri* as a new, ornamental plant for markets and in relation to the conservation of the species.

Materials and methods

T. chantrieri seeds were sourced from commercial nurseries in Thailand and Australia, while rhizomes were sourced from Queensland, Australia. The studies of *in vivo* seed germination, and plant growth and flowering were conducted at the Bogor Experimental Station, Leuwikopo, Indonesia while the study of *in vitro* seed germination was conducted in the Tissue Culture Laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia, in the period December 2014 to December 2015.

Evaluation of horticultural traits of *T. chantrieri*

The evaluation of *T. chantrieri* growth was based on 25 single-plant replications grown from rhizomes, using coir, burnt rice hulls and sand (equal proportions by volume) growth media in 20 cm diameter plastic pots. Plants from rhizomes were grown for 6 mth under 50% shade, hand watered three times a week and fertilized on a weekly basis with Seasol liquid fertilizer. The average day temperatures during the study period were in the range 25–30 °C and average night temperatures were in the range 23–25 °C.

Scoring was conducted on the natural flowering period, number of leaves and stem diameter at flower initiation, the number of flowers/inflorescence, the length of petioles and the bract size, at anthesis.

Seed germination *in vivo*

The growth media used for the *in vivo* germination study comprised coir and burnt rice hulls (1:1 on a volume basis). The growth medium was steamed at 80 °C for about 30 min and left to settle for 24 h prior to use. A sample of 300 seeds was sown onto the growth medium and placed under 50% shade. The growth medium was kept moist by watering once or twice a day. This experiment was repeated twice.

Scoring was conducted on the time-to-germination and the percentage of seeds germinated. Seeds were considered to have germinated if the radicle had protruded about 2 mm. *In vivo* germination experiments were conducted three times—in January, March and April 2015. To identify the presence of embryos in the seed, 60 non-germinated seeds were individually cut in half and examined under a microscope to determine the presence of an embryo. Pictures of the seed sections were taken using a digital camera (Model DP 25; Olympus; Tokyo, Japan).

Seed germination and proliferation *in vitro*

MS medium (Murashige and Skoog, 1962) was used for seed germination. The medium was autoclaved at 121 °C at 1.1 atm for 20 min and the pH was adjusted to 6.0. Flasks (100 mL volume) containing 30 mL of the medium were used in sowing the seeds.

The cultures were incubated at 22 °C under a 24 h light regime and at a photosynthetic photon flux density of ±134 lux (15 W, 59 lumen/W), provided by cool-white fluorescent tubes. Prior to sowing, the exterior of the seeds was cleaned twice with sterilized water, followed by immersion of the seeds in 30% Chlorox (active ingredient NaClO 5.25%) for 30 min and then in 10% Chlorox for 10 min.

The *in vitro* germination study was conducted in August 2015. In the first experiment 60 seeds were sown with and without soaking in GA₃ at 10 parts per million (ppm) for 24 h prior to sowing into the germination media. The second experiment was conducted in October 2015 by sowing 50 GA₃ treated seeds, with and without light. Germinated seedlings were grown for 8 wk prior to transfer to the proliferation MS media (Murashige and Skoog, 1962) which contained 30 g/L sucrose and 7 g/L agar supplemented with 0.1 mg/L indole acetic acid (IAA), 1.5 mg/L 2iP, 4 mg/L calcium pantothenate and 100 ml/L coconut water. The seedlings were cultured at 22 °C under the environmental conditions described above. Scoring of the seedling growth and development was conducted weekly over a period of 22 wk. The study was extended for another 12 wk to evaluate the shoot proliferation from the basal shoot explants collected from seedlings grown *in vitro*. The effects of N⁶-benzyladenine (BA) at 1 mg/L or 2 mg/L, in combination with the auxin IAA at 0.25 mg/L, 0.50 mg/L or 0.75 mg/L, on shoot proliferation were evaluated.

Results and discussion

Evaluation of the horticultural traits of *T. chantrieri*

T. chantrieri is a herbaceous evergreen with short stems bearing dark green, ovate to lanceolate foliage. The components of *T. chantrieri* that are often referred to as flowers are not true flowers, as these black-colored parts are bracts, which are actually modified leaves that surround the true flowers (Fig. 1).

For the *T. chantrieri* in this study, flowering occurred in the period from the end of March to mid-May, and then from September to mid-November. The inflorescence of *T. chantrieri* was near black in color; it had two large and two small bracts and numerous long, filiform bracteoles (Fig. 1). Anthesis occurred in the morning, starting with the florets at the centre of the inflorescence, with 1–2 florets opening each day, followed by florets on each side (Fig. 1). There were 18–20 florets per inflorescence. Young floral buds were green but turned dark on maturation (Fig. 1A). *T. chantrieri* inflorescence did not have an odor. Floral buds and

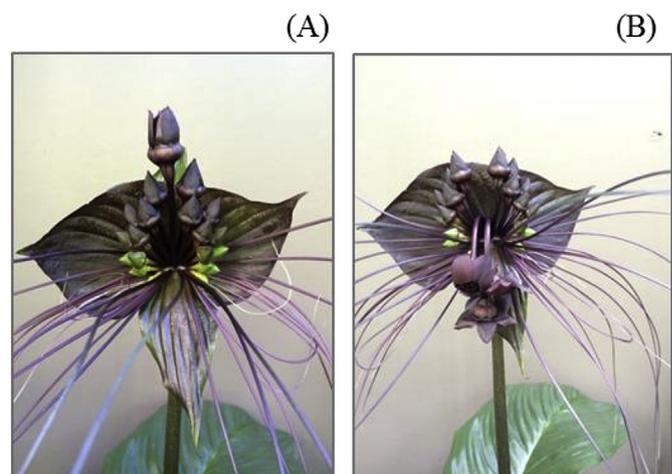


Fig. 1. *T. chantrieri* inflorescence before (A) and after (B) anthesis.

recently opened florets were erect but then bent downwards at the end of the day (Fig. 1B). The stalks of the inflorescence were longer than 40 cm (Table 1) and bent downward when the media dried out. For display purposes, the flower stalk needs support to keep the flowers erect.

T. chantrieri inflorescence lasted for 14–18 d, but after 9 d, the first florets that had reached anthesis dried out and wilted. However, the plants still looked attractive at this stage, as many more florets were still in the bud stage when the older florets had started to wilt.

T. chantrieri bracts were spread over 15–18 cm with numerous filiforms 30–40 cm in length. According to Zhang et al. (2005), *T. chantrieri* florets are predominantly self-pollinated and have several traits that promote autonomous self-pollination. None of the florets in this experiment produced fruit.

T. chantrieri is a shade-loving plant in its native habitat (Zhang et al., 2005). In addition to being adapted to low light conditions, *T. chantrieri* has numerous dark-colored flowers and broad bracts that rise up above the foliage, and hanging filiforms that make it attractive as a potential new, indoor flowering, potted plant. Its relatively large leaves and long flower stalks (Table 2) also make it suitable for planting into pots with a diameter of about 20 cm. As the market value of potted plants depends on the presence of inflorescence, further studies are needed to regulate uniform flowering of *T. chantrieri* beyond its natural flowering season such as has been achieved in peace lily (*Spatiphyllum*) according to Chen et al. (2015). Foliar spraying of GA₃ at 100 ppm is able to induce flowering and improve *Spatiphyllum* flower quality, so the

flowering time and optimal shipping dates can be scheduled with GA₃ application (Chen et al., 2015).

T. chantrieri seed germination *in vivo* and *in vitro*

T. chantrieri germination *in vivo* was found to be very low, with only 10% of the seeds germinating within 22 wk (Table 2). Low germination of *T. chantrieri* has also been previously reported, with 10% by Charoensub et al. (2008), 12% by He et al. (2002), and 12% in *T. integrifolia* by Meerow (n.d.).

The evaluation of the non-germinated seeds revealed that 42% ($n = 60$) of the *T. chantrieri* seeds did not have visible embryos (Fig. 2B). Invisible embryos might be due to rudimentary embryos in the seeds (Schutte and Knee, 2005; Herranz et al., 2013).

In addition to being under-developed, these embryos might have physiological dormancy (Baskin et al., 1995), and would require a lengthy time for development and maturation before the seeds can germinate (Schutte and Knee, 2005; Herranz et al., 2013).

T. chantrieri seeds have fibrous seed coats (Fig. 2A and B). Fibrous seed coats are semi-permeable, which allows the entry of water but blocks the entry of oxygen, and might retain germination inhibitors which prevent seeds from germination (Atwater, 1980).

The percentage seed germination *in vitro* was also very low, being just 10% after 22 wk (Table 2). Treatment of the seeds with GA₃ resulted in earlier germination compared to without GA₃, but the final percentage germination at 22 wk after germination was similar, being only 10% (Table 2). Therefore, the GA₃ treatment of

Table 1

Morphological characteristics of *T. chantrieri*.

Number of leaves at the first visible floral bud	Rhizome diameter at the first visible floral bud (mm)	Leaf length at anthesis (cm)	Leaf width at anthesis (cm)	Length of flower stalk at anthesis (cm)	Number of inflorescence/plant
5–8	20–36	18–34	9–16	42–53	1–2

Table 2

T. chantrieri seed germination *in vivo* and *in vitro*.

Condition/Treatment	Number of seeds sown	Number of seeds germinated (weeks after sowing)			Final germination (22 wk after sowing)
		8	16	20	
<i>In vivo</i> ^a	60	2	4	0	6 (10%)
<i>In vitro</i>					
Without gibberellin A ₃	30	0	1	2	3 (10%)
With gibberellin A ₃	30	3	0	0	3 (10%)
Light	30	2	3	1	6 (20%)
Dark	20	0	0	0	0

^a *In vivo* germination experiment was repeated three times with similar results.

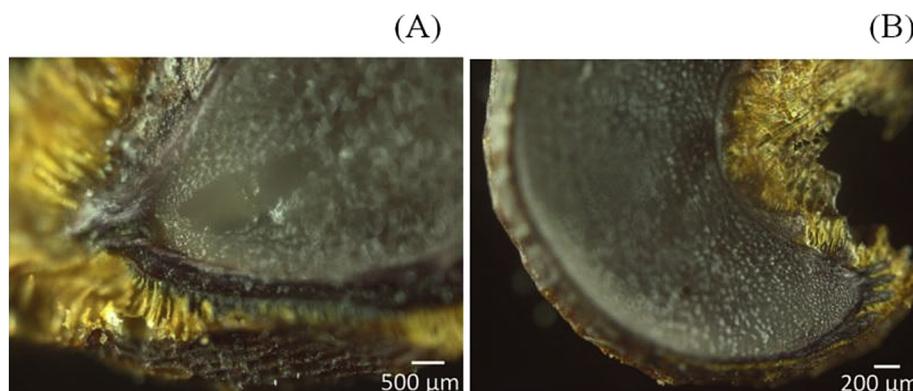


Fig. 2. *T. chantrieri* seed with embryo (A) and without embryo (B).



Fig. 3. *T. chantrieri* seedling *in vitro* aged 22 wk.

Table 3

Average (\pm SE, $n = 54$) *T. chantrieri* shoot proliferation *in vitro* on MS media (Murashige and Skoog, 1962) supplemented with various N^6 -benzyladenine (BA) and indole acetic acid (IAA) concentrations.

IAA concentration (mg/L)	BA concentration (mg/L)	Number of shoots per explant (weeks after culture)	
0.25	1	1	8
0.25	2	1	2.7 \pm 0.4
0.50	1	1	3.7 \pm 0.5
0.50	2	1	5.0 \pm 0.6
0.75	1	1	3.3 \pm 0.4
0.75	2	1	7.3 \pm 0.7
			6.7 \pm 0.6
			3.3 \pm 0.4
			3.7 \pm 0.5

T. chantrieri was only effective for increasing the speed of seed germination. It is possible that the effect of GA on *T. chantrieri* germination was through the direct effect of GA on embryo growth, as reported for *Arabidopsis* and tomato (Karssen et al., 1988).

T. chantrieri seeds failed to germinate in a dark environment (Table 2). Light is an important environmental factor for seed germination of many plant species (Plummer and Bell, 1995), but some species do germinate better in the dark. Sunlight converts phytochrome (a pigment which detects light quality) to the active form Pfr which stimulates GA biosynthesis (Toyomashu et al., 1998). The germination responses to light indicate that the phytochrome system probably operates in *T. chantrieri*.

Seeds that had germinated *in vitro* were transferred to the proliferation medium. Shoots started growing from the basal part of the seedlings at 12 wk after culture; after 22 wk, each seedling had grown 3–7 new shoots (Fig. 3). A further study to evaluate the proliferation of basal shoots from *in vitro* seedlings showed that 6–7 shoot formations were induced on the MS medium containing 0.5 mg/L of IAA in the presence of BA at 1 or 2 mg/L after 12 wk (Table 3). These results indicated that even though propagation protocols should be further developed, *in vitro* propagation using standard MS media supplemented with IAA and BA provided a more effective way to propagate *T. chantrieri* relative to the conventional propagation method.

T. chantrieri has the potential to be commercialized as a flowering indoor plant due to its unique flower morphology and shade tolerance. *In vivo* seed propagation of *T. chantrieri* from seed is not viable for commercial purposes due to its slow and low seed germination. *T. chantrieri* can be propagated *in vitro* by culturing sterile seedlings *in vitro*. This information is of potential value for the introduction of *T. chantrieri* into ornamental plant markets and also for the conservation of the species.

Conflict of interest

None declared.

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References

- Atwater, B.R., 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. *J. Seed Sci. Technol.* 8, 523–573.
- Baskin, C.C., Meyer, S.E., Baskin, J.M., 1995. Two types of morphophysiological dormancy in seeds of two genera (*Osmorhiza* and *Erythronium*) with an arctotertiary distribution pattern. *J. Bot.* 82, 293–298.
- Cepkova, P.H., Vitamvas, J., Ciehmannova, I., Ksililova, 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, 736–743.
- Charoensub, R., Thiantong, D., Phansiri, S., 2008. Micropropagation of bat flower plant *Tacca chantrieri* 'André. *Kasetsart J. Nat. Sci.* 42, 7–12.
- Chen, J., McConnell, D.B., Henny, R.J., Everitt, K.C., 2015. Cultural Guidelines for Commercial Production of Intiorscape *Spathiphyllum*. IFAS Extension Publication No ENH 958. Environmental Horticulture Department, University of Florida, Gainesville, FL, USA.
- 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, 870–873.
- Gisbert, C., Prohens, J., Nuez, F., 2011. Treatments for improving seed germination in eggplant and related species. *Acta Hort.* 898, 45–51.
- He, H., Lan, Q., Zhang, Y., 2002. *In vitro* propagation of *Tacca chantrieri*. *J. Agric. Biol. Sci.* 21, 108–110.
- Herranz, J.M., Copete, E., Ferrandis, P., 2013. Non-deep complex morphophysiological dormancy in *Narcissus longispatus* (Amaryllidaceae): implications for evolution of dormancy levels within section *Pseudonarcissi*. *Seed Sci. Res.* 23, 141–155.
- Karssen, C.M., Zagorski, S., Kepczynski, J., Groot, S.P.C., 1988. Key role for endogenous gibberellins in the control of seed germination. *Ann. Bot.* 63, 71–80.
- Meeerow, A.W. n.d. White batflower (*Tacca integrifolia*), an exciting new flowering foliage plant. IFAS Fort Lauderdale REC Plant Introduction. http://flrec.ifas.ufl.edu/hort/new_plants/tacca.html, 19 October 2014.
- Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15, 473–497.
- Plummer, J., Bell, D.T., 1995. The effect of temperature, light and gibberellic acid (GA_3) on the germination of Australian everlasting daisies (Asteraceae, Tribe Inuleae). *Aust. J. Bot.* 43, 93–102.
- Quattrocchi, U., 2012. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms and Etymology. CRC Press, Boca Raton, FL, USA.
- Schutte, B.J., Kneen, M., 2005. The effects of rudimentary embryos and elevated oxygen on seed dormancy of *Eryngium yuccifolium* Michx. (Apiaceae). *Seed Sci. Tech.* 33, 53–62.
- Steinrut, L., Itharat, A., Ruangnoo, S., 2011. Free radical scavenging and lipid peroxidation of Thai medicinal plants used for diabetic treatment. *J. Med. Assoc. Thai* 178–S182, Suppl. 7.
- Toyomashu, T., Kawaide, H., Mitsushashi, W., Inoue, Y., Kamiya, Y., 1998. Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. *Plant Physiol.* 118, 1517–1523.
- Yokosuka, A., Mimaki, Y., 2012. Phytochemicals of the Chinese herbal medicine *Tacca chantrieri* rhizomes. In: Sakagami, H. (Ed.), *Alternative Medicine*. <http://www.intechopen.com/books/alternative-medicine/phytochemicals-of-the-chinese-herbal-medicine-tacca-chantrieri-rhizomes/10> January 2016.
- Yokosuka, A., Mimaki, Y., Sashida, Y., 2002. Steroidal and pregnane glycosides from the rhizomes of *Tacca chantrieri*. *J. Nat. Prod.* 65, 1293–1298.
- Zhang, L., Barrett, S.C.H., Gao, J.Y., Chen, J., Cole, W.W., Liu, Y., Bai, Z.L., Li, Q.J., 2005. Predicting mating patterns from pollination syndromes: the case of sapromyophily in *Tacca chantrieri* (Taccaceae). *Am. J. Bot.* 92, 517–524.
- Zhang, L., Chen, J., Li, D.Z., Li, Q.J., 2007. Reproductive biology, mating system, and population genetics of devil flower: an autonomous selfing plant with showy floral display. *Flor. Ornam. Biotechnol.* 1, 115–124.
- Zhao, Y., Zhang, L., 2015. Phylogeographic history of the self-pollinated *Tacca chantrieri* (Dioscoreaceae) in the main land of southeast Asia. *Biochem. Syst. Ecol.* 58, 139–148.