Pak. J. Bot., 40(3): 1179-1184, 2008.

PLANT REGENERATION FROM LEAF EXPLANTS OF *TIGRIDIOPALMA MAGNIFICA* (*MELASTOMATACEAE*)

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Abstract

An efficient micropropagation system using leaves as explants has been established for *Tigridiopalma magnifica* C. Chen. It was found that adventitious bud organogenesis occurred from the leaf explants cultured on Murashige & Skoog (MS) medium with appropriate supplements of plant growth regulators. Up to 7.6 adventitious buds formed per leaf explant after a 40-day culture on MS medium containing 2.0 $mg.l^{-1}$ 6-benzylaminopurine (BA) and 0.1 $mg.l^{-1}$ thidiazuron (TDZ). During 30-day subculture, the proliferation rate of adventitious bud in cluster was 5.7 on MS medium supplemented with 2.0 $mg.l^{-1}$ BA and 0.5 $mg.l^{-1}$ 1-naphthaleneacetic acid (NAA). The half-strength MS medium supplemented with 0.5 mg l^{-1} indole-3-butyric acid (IBA) was best for rooting. When micropropagated plantlets with well-developed root systems were transferred to planting pots containing a mixture of sand, sieved peat and perlite (1:1:1; v/v) in greenhouse conditions, 86.0% of the plantlets survived. The regeneration protocol in this study provides a basis for germplasm conservation and large-scale multiplication of *T. magnifica*.

Introduction

Tigridiopalma magnifica C. Chen is a perennial herbaceous plant in the *Melastomatacea* family and is endemic to the Guangdong province of China (Chen, 1979; Zhang & Miao, 1984). It is of great horticultural value as an ornamental plant because of its huge leaves (30-50 cm) with reddish villi and because of its tolerance of shady surroundings, it can be used indoors as well as in gardens (Wang *et al*., 2004; Zeng, 2005).

Its popularity has led to illegal collection, which has in turn led to the increasing rarity of the species, which is also threatened by habitat destruction. It is listed as Endangered (EN) in China Species Red List by Chinese Government now (http://www.chinabiodiversity.com/redlist/search/index.shtm) (Ye *et al*., 2002). The problem is further exacerbated by the fact that seeds are seldom produced and are very difficult to germinate. Vegetative propagation of auxiliary buds was successful but is too slow for large-scale propagation (Zeng, 2005).

An efficient micropropagation method could make the plants widely available, meeting the demands of the horticultural trade and hobbyist, without further damage to the natural population. Availability of large numbers of plants would reduce the incentive to remove plants from the wild by reducing the commercial value of wild collected material. Micropropagation could also be used for conservation of germplasm. To our knowledge, there have been no reports of regeneration of *T. magnifica* through tissue culture to date. In this study, we established a system for high-frequency, *In vitro* multiplication of *T. magnifica* from leaf explants.

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Materials and Methods

Plant material and sterilization: Wild plants of *Tigridiopalma magnifica* C. Chen were harvested from their natural habitat at Mountain Ehuangzhang, YangChun City, Guangdong province and maintained in a greenhouse at South China Botanical Garden, as an explant source. Fifteen-, 30-, and 60-day-old leaves were surface sterilized by dipping in 70% ethanol for 30 seconds, then immersed in 0.1%(w/v) $HgCl_2$ solution for 6 min., followed by five rinses with sterile distilled water.

Regeneration of plantlets: The sterilized leaves were cut into about 0.8 x 0.8 cm sections and cultured on Murashige & Skoog (Murashige & Skoog, 1962) either alone or supplemented with the following plant growth regulators: BA (0.5, 1.0, 2.0, and 4.0 mg.l^{-1}); NAA (1.0,2.0,4.0 mg.l^{-1}); TDZ (0.1,0.2,0.5); or BA (1.0,2.0 mg.l^{-1}) in combination with TDZ (0.1, 0.2 mg.l^{-1}) or with NAA (0.2, 0.5 mg.l^{-1}). After 40 days in culture, the regenerated adventitious buds in cluster and calli from explants were transferred onto MS medium with BA (0.5, 1.0, 2.0, 5.0 mg.l^{-1}), or BA (1.0, 2.0 mg.l^{-1}) in combination with NAA (0.2, 0.5, 1.0 mg.l^{-1}) for adventitious bud proliferation and growth.

After a several subcultures, the regenerated adventitious buds were cultured on a medium with a low concentration of plant growth regulators (1.0 mg.l^{-1} BA combined with 0.2 mg.l^{-1} NAA) for proliferation and growth and transferred 1-2 times, so as to have strong adventitious buds for root induction.

The adventitious buds were divided into single buds for rooting on half-strength MS solid medium, supplemented with either IBA (0.2,0.5,1.0 mg.l^{-1}) or NAA (0.2,0.5,1.0 mg.l^{-1}).

Micropropagated plantlets with well-developed root systems were removed from *In vitro* culture, rinsed in water to remove any medium, and transferred to planting pots containing a mixture of sand, sieved peat and perlite (1:1:1v/v) under greenhouse conditions. Survival rates of transplanted plantlets were investigated after 1 month.

Culture conditions: All cultures incubated in 250ml conical flasks containing 40 ml medium. The medium was adjusted to pH 5.8 with 1N NaOH and 1N HCl before autoclaving at 121°C for 20 min., at 1.06 kg cm^{-2}. All cultures were incubated in a controlled-environment growth room at 25±2°C with a 16-h photoperiod under cool white light (40-50 µmol $m^{-2}s^{-1}$).

Data analysis: The design of all experiments was a completely random. Each experiment was repeated three times and consisted of five explants per flask and 10 replicate culture flasks. The data were analyzed using one-way ANOVA followed by Duncan's multiple range test.

Results and Discussion

Shoot initiation and multiplication: Leaf explants from greenhouse plants of *T. magnifica* (Fig. 1A), were used to induce adventitious bud regeneration on MS medium in the presence and absence of plant growth regulators. 15-d-old leaf explants of *In vitro* culture produced phenolic exudates from the cut ends and the surrounding medium browned severely during the first week, and did not show adventitious bud or root organogenesis. 60-d-old leaf explants had low frequency of organogenesis (Data not shown). 30-d-old leaf explants were suitable for tissue culture. Adventitious bud

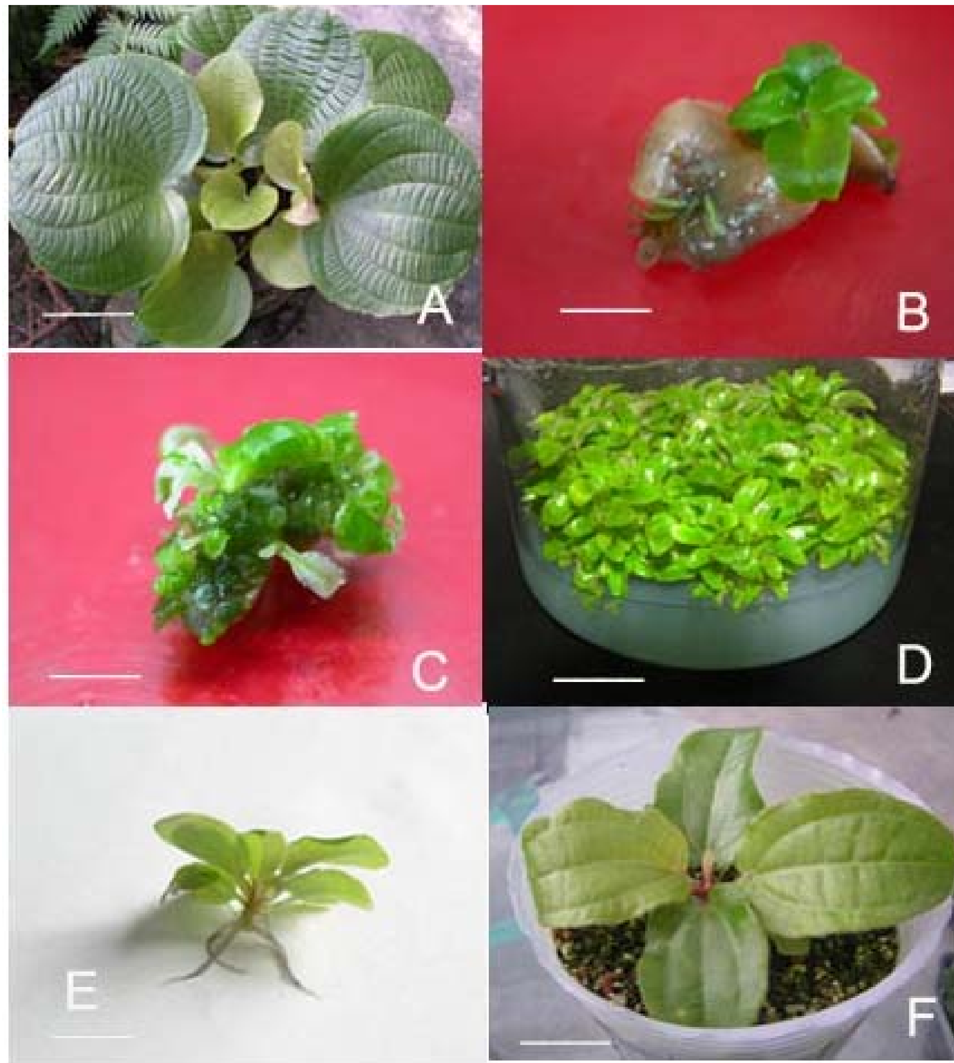

Fig. 1. Plant regeneration from leaf explants of *Tigridiopalma magnifica*.
A. Specimen of *T. magnifica* growing in greenhouse condition (bar=150 mm), B. Shoots and roots induced from a leaf explant on MS medium with 2.0 mg l^{-1} NAA (bar=10 mm), C. Shoot organogenesis induced by the combination of 2.0 mg l^{-1} BA and 0.1 mg l^{-1} TDZ from leaf explants after 40 d (bar=10 mm), D. Shoot proliferation on MS medium with 2.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA (bar=10 mm), E. Rooting of regenerated shoos on half-strength MS medium supplemented with 0.5 mg l^{-1} IBA (bar=10 mm), F. Plantlets after 2 mo. of transplantation (bar=20 m).

organogenesis of *T. magnifica* was not induced in the absence of plant growth regulator. Only roots were induced on MS medium with 1.0 mg.l^{-1} NAA, and roots and adventitious buds were simultaneously induced on different parts of the same explants in the presence of 2.0 mg.l^{-1} NAA (Fig. 1B). Only adventitious buds were induced in the presence of 4.0 mg.l^{-1} NAA. On MS medium supplemented with 4.0 mg.l^{-1} BA or 0.5 mg.l^{-1} TDZ, few

adventitious buds and lots of calli were induced. Of all media tested, the highest frequency of adventitious bud formation (88%) was obtained using of 2.0 mg.l^{-1} BA combined with 0.1 mg.l^{-1} TDZ. Every leaf explants produced an average of 7.6 adventitious buds after 40-d of culture (Fig. 1C). However, the presence of high BA (4.0 mg.l^{-1}) or TDZ (0.5 mg.l$^{-1)}$ inhibited adventitious bud induction promoted formation of calli (Table 1). This observation confirms findings in *Carica papaya* (Reuveni *et al.*, 1990) and *Houttuynia cordata* (Chakraborti *et al.*, 2006).

Table 1. Effect of different levels of combinations of plant growth regulators on adventitious bud regeneration from leaf explants of *Tigridiopalma magnifica* after 40 days of culture.

Plant growth regulators (mg l^{-1})			Percentage (%) explants producing buds	Number of buds per explant
BA	NAA	TDZ		
0			0 h	0h
0.5			12 ± 10gh	1.50 ± 1.3g
1.0			36 ± 13f	3.40 ± 0.4f
2.0			58 ± 15cd	4.70 ± 06cde
4.0			38 ± 15ef	4.17 ± 0.6e
	1.0		0h	0h
	2.0		12 ± 10gh	1.52 ± 1.3g
	4.0		40 ± 13ef	4.22 ± 0.4e
		0.1	30 ± 25f	2.84 ± 1.5f
		0.2	46 ± 21de	4.84 ± 0.6cde
		0.5	16 ± 16g	1.52 ± 1.4g
1.0		0.1	46 ± 14de	4.58 ± 0.6de
1.0		0.2	66 ± 19bc	5.36 ± 0.6cd
2.0		0.1	88 ± 14a	7.60 ± 0.5a
2.0		0.2	78 ± 15ab	6.32 ± 0.7b
2.0		0.2	68 ± 14bc	5.41 ± 0.4c
2.0		0.5	60 ± 13cd	4.76 ± 0.3cde

Each value represents the means ± SE of 30 replications.
Values followed by the same letter within columns are not significantly different at $p \leq 0.05$.

Table 2. Effect of different levels of combinations of BA and NAA on adventitious bud proliferation of *Tigridiopalma magnifica* after 1 mo. of culture

Plant growth regulators (mg l^{-1})		Proliferation rate of adventitious buds	Height of adventitious buds (cm)
BA	NAA		
0.5		1.76 ± 0.2h	1.35±0.14c
1.0		3.09 ± 0.3g	0.93± 0.18de
2.0		4.16 ± 0.3c	0.60 ± 0.09f
5.0		3.66 ± 0.3ef	0.40 ± 0.08g
1.0	0.2	3.46 ± 0.3f	1.80 ± 0.18a
1.0	0.5	3.84 ± 0.3de	1.61 ± 0.15b
1.0	1.0	4.06 ± 0.2cd	1.37 ± 0.15c
2.0	0.2	4.46 ± 0.4b	1.05 ± 0.11d
2.0	0.5	5.66 ± 0.5a	0.92 ± 0.12e
2.0	1.0	4.56 ± 0.4b	0.68 ± 0.15f

Each value represents the means ± SE of 30 replications.
Values followed by the same letter within columns are not significantly different at $p \leq 0.05$.

Table 3. Effect of different levels of NAA and IBA on rooting of *Tigridiopalma magnifica* after 1 mo. of culture.

Plant growth regulators (mg l^{-1})		Rooting percentage (%)	Number of roots per adventitious bud	Root length (mm)
NAA	IBA			
0.0	0.0	50 ± 17.0c	2.90 ± 0.8c	3.94 ± 1.0c
0.2	0.0	76 ± 18.4b	3.61 ± 0.4bc	5.70 ± 0.7b
0.5	0.0	68 ± 16.9b	3.11 ± 0.6bc	3.56 ± 0.5c
1.0	0.0	40 ± 21.0c	2.93 ± 1.3c	2.14 ± 0.9d
0.0	0.2	70 ± 17.0b	3.76 ± 0.84b	6.57 ± 0.9a
0.0	0.5	96 ± 8.4a	4.78 ± 0.5a	6.89 ± 0.6a
0.0	1.0	82 ± 11.4ab	5.02 ± 0.6a	5.57 ± 0.5b

Each value represents the means ± SE of 30 replications.
Values followed by the same letter within columns are not significantly different at $p \leq 0.05$.

In general, the stimulation of tissue growth to form adventitious roots and adventitious buds depends on the relative ratios of auxin to cytokinin in the culture medium (Skoog & Miller 1957), but in our experiment, adventitious roots could not be induced on media supplemented with 2.0 mg.l^{-1} BA in combination with 0.2 or 0.5 mg.l^{-1} NAA, or with a high concentration NAA (4.0 mg.l^{-1}).

Calli with regenerated adventitious buds were subcultured onto media with 2.0 mg.l^{-1} BA and 0.5 mg.l^{-1} NAA and could differentiate further to produce many adventitious buds. The regenerated adventitious buds in clusters had an adventitious bud proliferation rate of 5.7 on the same new medium after 30 days (Fig. 1D). BA in combination with NAA can promote proliferation and growth (Table 2). This agrees with results for other plant species (Beena *et al.*, 2003; Prakash *et al.*, 2004; Loc *et al.*, 2005).

After several subcultures, the regenerated adventitious buds were cultured onto a medium containing 1.0 mg.l^{-1} BA and 0.2 mg.l^{-1} NAA. The proliferation rate was 3.5 and the height of the adventitious buds used for rooting was about 1.8 cm.

Rooting and transplanting: The individual regenerated adventitious buds were excised from the subcultured adventitious bud cluster and inoculated into rooting media. Rooting occurred on half-strength MS solid medium in the absence of plant growth regulators, however, the rooting percentage was low (50%) and the roots were relatively slim and fragile. In contrast, normal and healthy roots were observed on half-strength MS solid medium containing low concentration NAA and IBA. On half-strength MS medium supplemented with IBA or NAA, IBA was shown to be more effective for root induction than NAA (Table 3). Similar differential effects of auxin on rooting have also been reported in *Steria rebaudianum* and *Arctium lappa* (Shen, 1995; He *et al*., 2006). In high concentrations of NAA (0.5-1.0 mg.l^{-1}) and IBA (1.0 mg.l^{-1}), the rooting percentages were low and the adventitious roots induced were few, short, sturdy and had no root hairs. The best rooting frequencies of 96% were achieved using media containing 0.5 mg.l^{-1} IBA (Table 3) and plantlets had healthy roots (Fig. 1E). Three hundred well-developed root systems were transferred to planting pots containing a mixture of sand, sieved peat and perlite (1:1:1;v/v) in greenhouse conditions, 86.0% of the plantlets survived after 30 days (Fig. 1F). Compared to the source plants, the *In vitro* raised plants did not show any phenotypic variation in later growth.

In conclusion, our study provides an efficient regeneration system for the first time for rapid clonally propagation and germplasm conservation of *T. magnifica*.

Acknowledgment

The authors are thankful for the financial support of Guangzhou Key Technology Research and Development Program grant (NO. 2003F1-E0021, 2007B20704004) and thank Grace Prendergast of Royal Botanic Gardens, Kew, UK, for reviewing the manuscript.

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(Received for publication 10 March 2008)