Micropropagation to Conserve the Endangered Gabal Elba Dragon Tree (*Dracaena ombet* Heuglin ex Kotschy & Peyr)

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Abstract. Plant tissue culture offers opportunities for the rescue and conservation of endangered plant species. Here, we report the successful *in vitro* propagation of *Dracaena ombet*, an endangered plant. Several physical and chemical seed treatments were evaluated to develop a propagation approach. Germination of *D. ombet* seeds was monitored for 16 weeks by placing them onto Murashige and Skoog (MS) medium. Maximum seed germination (20%) was recorded when seeds were soaked-scarified, whereas all other treatments did not result in seed germination. Fragmented (longitudinally bisected) and intact *in vitro* shoots were cultured onto MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP) and indole butyric acid (IBA) to induce axillary shoots. Longitudinal fragmentation of explants had a greater effect than the intact explants for shoot proliferation when cultured onto medium containing plant growth regulators. Fragmented shoots cultured onto MS medium supplemented with 2 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA treatment resulted in the highest amount of axillary shoots (seven shoots per explant). The intact shoots had the highest axillary shoots (1.8 shoots per explant) when cultured onto a medium supplemented with a combination of 1 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA. One hundred percent rooting was obtained using half strength MS medium supplemented with 0.5 or 1 mg·L⁻¹ IBA. With full strength MS medium, a maximum rooting of 60% was obtained with 1 mg·L⁻¹ IBA or naphthalene acetic acid (NAA) addition. The plantlets were acclimatized to *ex vitro* conditions with a 95% survival rate. This study offers a simple method for *in vitro* propagation of *D. ombet*, which is valuable to enable conservation of this endangered species.

The genus *Dracaena* includes 113 species (The Plant List, 2018) of woody stemmed foliage plants in the tropics (Bailey, 1949). *Dracaenas* are evergreen shrubs or trees most frequently characterized by long linear leaves

of significant decorative and horticultural values. Most species are grown as potted indoor plants and propagated for commercial purposes (Jones and Luchsinger, 1986; Vinterhalter and Vinterhalter, 1997). Besides

their ornamental value, some species including *Dracaena arborea* and *D. mannini* (Okunji et al., 1996), *D. cochinchensis* (Nong, 1997), *D. draco* (Mimaki et al., 1999), and *D. loureiri* (Ichikawa et al., 1997) also possess several medicinal properties and are used as an herbal remedy in traditional medicine.

The Gabal Elba dragon tree, also known as the Nubian dragon tree (*Dracaena ombet* Heuglin ex Kotschy & Peyr.; Asparagaceae) is an evergreen tree capable of reaching heights of 2 to 4 m. The branches are dichotomous, short, and spreading, with thick, rigid, and sword-shaped leaves. The leaves are clustered as rosettes at the ends of the dichotomous branches (Bari, 1968). The mature fruits are edible and eaten by local people as a supplement to their meagre diet, and the resin obtained from the trunk is used in traditional medicine [International Union for Conservation of Nature (IUCN), 1998]. Steroidal saponins from *D. ombet* (El-amin et al., 2002; Moharram and El-Shenawy, 2007) possess analgesic and anti-inflammatory properties (Moharram and El-Shenawy, 2007). *Dracaena ombet* is distributed in the coastal mountainous regions of the Red Sea, mainly in Gabal Elba, Egypt (Ghazali et al., 2008), with scattered populations in Saudi Arabia, Sudan, Ethiopia, Somalia, and Djibouti (IUCN, 1998). It has experienced population declines throughout its range and scattered individuals remain in inaccessible areas (Bos, 1997; El-Azzouni, 2003; Friis and Lawesson, 1993; Kamel et al., 2015). The subpopulations on the Red Sea Hills and Gabal Elba in Egypt and Sudan are particularly threatened (El-Azzouni, 2003). On the basis of the assessment of the World Conservation Monitoring Center (1998) using a now-outdated set of criteria (Version 2.3), *D. ombet* has been categorized as endangered in the IUCN Red List of Threatened Species (IUCN, 1998). In northern Sudan, *D. ombet* populations have completely vanished from Erowit, the only area where they are known to have existed in that country (El Azzouni, 2003). Overgrazing, overcutting, droughts, and attack by parasitic pests or diseases have contributed to the decline. Scanning and transmission electron microscopy revealed the association of pathogenic fungal species in leaf spots of *D. ombet* (Baka and Krzywinski, 1996). Field-based observations suggest that 80% of the *D. ombet* population in Gabal Elba may soon be extinct, making it critically endangered (Kamel et al., 2015).

Conventional propagation of the genus *Dracaena* is achieved through cuttings (Vinterhalter and Vinterhalter, 1997), but some species such as *D. draco* and *D. ombet* are mainly propagated by seeds. Although the seed production in *D. ombet* may be high, many seeds do not germinate. Moreover, vegetative propagation of *D. ombet* is difficult. Ghazali et al. (2008) attempted to propagate *D. ombet* using the newly vegetative axillary buds, but no rooting occurred, and the propagation failed. *In vitro* propagation methods have been developed for a few

ornamental *Dracaena* sp., including *D. surculosa* (Miller and Murashige, 1976; Liu et al., 2010), *D. deremensis* (Badawy et al., 2005; Blanco et al., 2004; Debergh, 1976), *D. fragrans* (Debergh, 1975, 1976; Debergh and Maene, 1981; Lu, 2003; Vinterhalter, 1989; Vinterhalter and Vinterhalter, 1997), *D. sanderiana* (Aslam et al., 2013; Beura et al., 2007), and *D. marginata* (Chua et al., 1981; El-Sawy et al., 2000). However, propagation of ornamental *Dracaena* sp., still relies on imported cuttings as a commercial practice.

An alternative approach is to propagate and conserve *D. ombet* through tissue culture techniques. Tissue culture facilitates the rapid production of propagules from several species that are difficult to propagate using conventional techniques as well as the conservation of endangered and threatened plant species (Fay, 1992; Sarasan et al., 2006). Although *D. ombet* is an endangered species of economical and medicinal importance, to our knowledge, tissue culture techniques have not been used to propagate and conserve this species. The aim of this study was to develop in vitro propagation methods for *D. ombet* to conserve this critically endangered species.

Materials and Methods

Plant material, seed treatment and in vitro culture. Fully ripe fruits of *D. ombet* were collected from trees growing at Medina (lat. 24°28'7.00" N, long. 39°36'51.01" E), Saudi Arabia (Fig. 1A and B). The seeds were manually removed from the fruit (Fig. 1C), and the small and injured seeds were excluded. One hundred fresh seeds were weighed (6.21 g), washed in tap water, and subjected to physical and mechanical treatments. Physical treatment was performed by soaking the intact seeds in sterile distilled water for 24 h at ambient temperature (25 °C). Mechanical scarification treatment involved manual scarification with a cutting blade. All seeds were surface sterilized by rinsing in 70% (v/v) ethanol for 30 s followed by 0.15% (w/v) HgCl₂ containing one drop of Tween 80 for 5 min under constant hand agitation. The seeds were then washed with sterile distilled water and rinsed in 30% Clorox solution containing 5.2% sodium hypochlorite and a few drops of Tween 20 for 10 min. They were then washed three times with sterile distilled water for 5 min each. The seeds (one seed per culture vessel) were inoculated in glass test tubes plugged with plastic caps (24 × 200 mm) each con-

Fig. 1. In vitro seed germination and shoot proliferation of *Dracaena ombet*. (A) In situ *D. ombet* tree, (B) *D. ombet* fruits, and (C) *D. ombet* seeds. (D and E) Germinated seed after 10 and 20 d of culture on Murashige and Skoog (MS) medium without plant growth regulators. (F and G) Intact and longitudinal fragmented shoot tips at 0 d of culture on shoot proliferation medium. (H and I) Shoot proliferation of intact and longitudinal fragmented shoot tips after 8 weeks of culture on MS medium supplemented with 2 mg·L⁻¹ 6-benzylaminopurine and 0.5 mg·L⁻¹ indole-3-butyric acid.

taining 20 mL of MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar-agar. The pH of the medium was adjusted to 5.7 before autoclaving for 20 min at 121 °C. There were 25 seeds in each treatment, and intact seeds without preculturing treatments were considered the control. The cultures were incubated at 25 ± 2 °C under dark conditions and monitored for 16 weeks. Germinated seeds were transferred to light conditions under a 16-h photoperiod provided by cool white fluorescent tubes at 35 μmol·m⁻²·s⁻¹ photosynthetic photo flux density (PPFD). Repetitive subcultures of the shoot tips and nodes of *D. ombet* seedlings onto MS medium without plant growth regulators (PGRs) for 6 months produced sufficient stock of shoots for further experiments.

Axillary shoot proliferation. Both intact and fragmented (longitudinally bisected using scalpel) shoot tips of *D. ombet* (1.5–2 cm; Fig. 1F and G) were cultured onto MS medium supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP; 0, 0.5, 1, 2, and 3 mg·L⁻¹), and IBA (0, 0.5, and 1.0 mg·L⁻¹). All media were supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar-agar. The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 min. The cultures were incubated at 25 ± 2 °C under a 16-h photoperiod provided by cool-white fluorescent tubes at 35 μmol·m⁻²·s⁻¹ PPFD. The number of axillary shoots, length of the main shoot, and fresh weight per explant were recorded from 10 shoots after 8 weeks of culturing.

In vitro rooting and acclimatization. Shoots (2.5–4 cm) were used for rooting in full or half strength MS basal medium supplemented with 0, 0.5, or 1.0 mg·L⁻¹ IBA or NAA. Rooting parameters including rooting percentage, number of roots, length of the main root and fresh weight were recorded after 4 weeks of culturing, and from 10 plantlets. The micropropagated plantlets

were dipped (for a few seconds) in a fungicide solution (0.5 g·L⁻¹ Aromil-Plus 50 WP; Mobedco-Vet, Amman, Jordan), and transplanted into a culture plastic box (25 × 15 × 15 cm) filled with a sterilized mixture of peatmoss and perlite (1:1). The plantlets were grown in a growth chamber (Model KBWF 720; Binder, Tuttlingen, Germany) for 4 weeks before transplanting to plastic pots (10-cm diameter) in the greenhouse. The environment in the growth chamber was adjusted to a temperature of 25 ± 2 °C, 50% to 60% relative humidity and 100-μmol·m⁻²·s⁻¹ PPFD (16-h photoperiod under white fluorescent lamps).

Experimental design and data analysis. The experiments were performed using a completely randomized design. Data expressed as percentages were arcsine transformed before the analysis (Compton, 1994). All data were subjected to Tukey's range test and analysis of variance using the SAS program Version 6.12 (SAS Institute Inc., Cary, NC).

Results and Discussion

Seed germination and establishment of in vitro culture of *D. ombet*. Germination of *D. ombet* seeds were monitored for 16 weeks and only 20% (5 of 25 seeds) of soaked-scarified seeds germinated. Following a subculture of the germinated seeds, healthy seedlings developed within 2 weeks (Fig. 1D and E). Nonscarified and nonsoaked seeds did not germinate, indicating a physical dormancy of *D. ombet* seeds. However, the low germination percentage obtained using mechanical scarification indicate that *D. ombet* not only possesses physical dormancy but also physiological dormancy. A field survey conducted by Ghazali et al. (2008) indicated that *D. ombet* seeds seem to possess a long dormancy and could remain below ground for long periods until the climatic conditions are favorable, thus, enabling its survival. Detailed information on seed

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germination and viability in this species is lacking, which calls for further research. Germination of *D. ombet* in situ is achieved with difficulty, and as a result, natural regeneration of this species is endangered.

Axillary shoot proliferation. Shoot proliferation, shoot length, and fresh weight of *D. ombet* explants were significantly influenced by the explant type and concentration of the PGRs used in the treatments except for shoot length was not influenced by IBA treatment (Table 1; Fig. 1H and I). Fragmentation of explants had a greater effect than intact explants for shoot proliferation when cultured onto a medium containing the PGRs. Various combinations of BAP and IBA were tested for induction of axillary shoots. Our results indicated that MS medium containing 2 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA had the highest rate of shoot proliferation (seven shoots per explant) compared with all other combinations. The intact shoots showed very low proliferation of axillary shoots with a maximum of 1.8 shoots per explant when cultured onto a medium supplemented with a combination of 1 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA. Successful shoot proliferation using BAP in combination with auxin has been reported in other *Dracaena* species, including *D. deremensis* (Singh et al., 2001), *D. fragrans* ‘Massangeana’ (Aziz et al., 1996), and *D. marginata* ‘Tricolour’ (El-Sawy et al.,

2000). The synergistic increase in shoot proliferation as a result of the BAP and auxin combination has been reported in woody plant species, including, *Arbutus unedo* (2 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ NAA; El-Mahrouk et al., 2010), *Conocarpus erectus* (2 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA; Dewir et al., 2018), and *Lessertia frutescens* (0.5 mg·L⁻¹ BAP and 0.1 mg·L⁻¹ NAA; Shaik et al., 2010).

The shoots of *D. ombet* cultured in MS medium without the PGRs had the least number of axillary shoots at 1 and 1.2 for intact and fragmented explants, respectively. These results indicate that the intact shoots of *D. ombet* possess high apical dominance that hinders shoot proliferation despite their culture in a medium containing high levels of BAP (3 mg·L⁻¹). The longitudinal fragmentation of *D. ombet* shoot tips enhanced the shoot proliferation capacity of the explants probably through overcoming the apical dominance and enabling the growth of axillary buds. Shoot multiplication of *Hylocereus undatus* was achieved after subjecting to longitudinal cut or decapitation to eliminate apical dominance (Mohamed-Yasseen, 2002). The technique involving the fragmentation of shoot apices was used for in vitro propagation of *Vitis vinifera*, through the formation of multiple adventitious shoots (Barlass and Skene, 1978). Improved multi-

plication rates of *Vanilla planifolia* ‘Andrews’ was also achieved through fragmentation of the base of shoot clusters (Gonzalez-Arnavo et al., 2009).

In vitro rooting and acclimatization. Varying medium strength significantly influenced rooting of *D. ombet* (Table 2). The best rooting medium was half-strength MS supplemented with 0.5 or 1 mg·L⁻¹ IBA; as these two treatments induced rooting in 100% of the explants with an average of 2.6 and 3.8 roots per explant, and average root lengths of 9.3 and 9 cm, respectively (Table 2; Fig. 2A). Addition of NAA at 0.5 or 1 mg·L⁻¹ also resulted in 100% rooting, but the roots were stunted and thick (Fig. 2B) compared with that of the roots induced by IBA (Fig. 2C). At full strength MS, a maximum rooting of 60% was achieved with the addition of 1 mg·L⁻¹ IBA or NAA. No rooting occurred in the full-strength MS medium lacking auxins, whereas the half-strength MS medium resulted in 10% rooting. Thus, a reduced salt concentration stimulated rooting of *D. ombet* microshoots, significantly influenced the rate of rooting and the average number of roots, but had no significant effect on fresh weight. In vitro rooting of *Dracaena* plants including *D. fragrans* ‘Massangeana’ (Aziz et al., 1996) and *D. deremensis* (Blanco et al., 2004) was achieved in MS medium lacking the PGRs. Other species such as *D. sanderiana* (Aslam

Table 1. Effect of explant type and BAP and IBA concentrations on shoot multiplication and growth of *Dracaena ombet* after 6 weeks in culture.

Treatments		Shoots (no./explant)	Length of the main shoot per explant (cm)		Fresh wt per explant (g)
Explant type	BAP (mg·L ⁻¹) IBA (mg·L ⁻¹)				
Intact shoot tip	No PGRs (control)	1.0 e ^z	7.9 abcd		0.31 g
	0.5 0.0	1.0 e	6.8 bcde		0.28 g
	0.5 0.5	1.0 e	9.6 a		1.08 defg
	0.5 1.0	1.0 e	7.8 abcd		0.39 g
	1.0 0.0	1.0 e	9.3 ab		0.35 g
	1.0 0.5	1.8 de	4.0 fghi		0.65 g
	1.0 1.0	1.4 e	5.6 cdefg		0.58 g
	2.0 0.0	1.4 e	6.0 cdef		0.71 g
	2.0 0.5	1.2 e	5.1 efghi		0.93 efg
	2.0 1.0	1.4 e	7.8 abcd		1.13 defg
	3.0 0.0	1.0 e	4.9 efghi		0.63 g
	3.0 0.5	1.0 e	7.7 abcd		0.97 efg
	3.0 1.0	1.4 e	4.9 efghi		1.31 defg
Fragmented shoot tip	No PGRs (control)	1.2 e	5.3 defgh		0.38 g
	0.5 0.0	2.2 de	3.3 defgh		0.79 fg
	0.5 0.5	3.2 cd	8.1 abc		1.07 defg
	0.5 1.0	4.2 bc	6.5 cdef		2.25 cd
	1.0 0.0	5.4 b	5.4 defgh		2.11 cde
	1.0 0.5	5.6 b	6.0 cdef		3.51 ab
	1.0 1.0	4.6 bc	6.7 cde		3.79 ab
	2.0 0.0	5.4 b	3.9 fghi		2.70 bc
	2.0 0.5	7.0 a	5.3 defgh		3.12 abc
	2.0 1.0	4.4 bc	4.9 efghi		3.04 abc
	3.0 0.0	4.6 bc	2.6 i		3.15 abc
	3.0 0.5	2.4 de	3.1 ghi		3.91 a
	3.0 1.0	2.2 de	2.9 hi		1.97 cdef
Significance					
Explant type (A)		**	**		**
BAP concentration (B)		**	**		**
IBA concentration (C)		*	NS		**
A × B		**	*		**
A × C		NS	NS		NS
B × C		*	**		NS
A × B × C		*	*		**

^zValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

BAP = 6-benzylaminopurine; IBA = indole-3-butyric acid; PGRs = plant growth regulators.

Table 2. Effect of MS medium salt strength and IBA and NAA concentrations on rooting of *Dracaena ombet* after 4 weeks in culture.

Treatments			Rooting %	Roots (no./plantlet)	Length of the main root per plantlet (cm)	Fresh wt per plantlet (g)
MS medium salt strength	Auxin type	Auxin concn (mg·L ⁻¹)				
Full	No auxins (control)		0 e ^z	0.0 c	0.0 c	0.35 b
	IBA	0.5	0 e	0.0 c	0.0 c	0.33 b
		1.0	60 b	1.6 abc	3.0 b	0.67 b
	NAA	0.5	40 c	2.5 ab	3.9 b	0.60 b
		1.0	60 b	2.3 ab	4.4 b	0.96 a
Half	No auxins (control)		10 d	1.0 c	0.5 c	0.44 b
	IBA	0.5	100 a	2.6 ab	9.3 a	0.42 b
		1.0	100 a	3.8 a	9.0 a	0.66 b
	NAA	0.5	100 a	3.6 a	4.2 b	0.73 ab
		1.0	100 a	3.0 ab	3.2 b	0.63 b
Significance						
Medium strength			**	**	**	NS
Auxin type			*	NS	*	NS
Auxin concentration			*	NS	NS	NS

^cValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

IBA = indole-3-butyric acid; NAA = naphthalene acetic acid.

Fig. 2. In vitro rooting and acclimatization of *Dracaena ombet*. (A) In vitro plantlet of *D. ombet* after 4 weeks of culture. (B) Plantlets of *D. ombet* rooted on Murashige and Skoog (MS) medium containing naphthalene acetic acid. (C) Plantlets of *D. ombet* rooted on MS medium containing indole-3-butyric acid. (D and E) Acclimatization of *D. ombet* plantlets in a mixture of peatmoss and perlite (1:1) after 4 and 8 weeks, respectively.

et al., 2013; Beura et al., 2007), *D. sanderiana* 'Virescens' (Tian et al., 1999), and *D. marginata* (El-Sawy et al., 2000) required auxin supplement in the rooting medium. For in vitro rooting of *D. marginata*, IAA, IBA, and NAA were applied at 2 mg·L⁻¹, and NAA induced the highest rooting percentage (80%) compared with IBA (50%) and IAA (48%) (El-Sawy et al., 2000). Low rooting ability is a limiting factor in the micropropagation of woody plant species (Dewir et al., 2016; Nemeth, 1986). Improved rooting under reduced salt levels has been suggested to be due to the reduction in nitrogen content rather than a change in ψ_s (Hyndman et al., 1982). Lowering the salt strength in the medium to half and the addition of 0.5 to 1 mg·L⁻¹ IBA yielded the highest rooting percentage for *D. fragrans* (Singh et al., 2001). Our results indicate that lowering the salt strength in the medium to half resulted in 100% rooting of *D. ombet*, regardless of the concentration

of IBA or NAA used. These results confirm previous findings on in vitro rooting of woody plant species such as *Cassia angustifolia* (Agrawal and Sardar 2007), *Conocarpus erectus* (Dewir et al., 2018), and *Syzygium cordatum* (Dewir et al., 2011), wherein the rooting was dependent on medium strength and the type and concentration of auxins. Regenerated plantlets of *D. ombet* were acclimatized to ex vitro conditions (Fig. 2C and D), and a 95% survival rate for the plantlets was achieved after 8 weeks.

In conclusion, an efficient micropropagation protocol for *D. ombet* was developed. Fragmentation of shoot tips resulted in the highest rate of shoot proliferation (seven shoots per explant) in MS medium supplemented with 2 mg·L⁻¹ BAP and 0.5 mg·L⁻¹ IBA. Root proliferation occurred in 100% of the explants in half strength MS medium supplemented with 0.5 or 1 mg·L⁻¹ IBA or NAA and successfully acclimatized to ex

vitro conditions. Germplasm conservation through plant tissue culture techniques therefore provides means to conserve this endangered plant.

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