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Micropropagation of *Romulea minutiflora*, *Sisyrinchium laxum* and *Tritonia gladiolaris* — Iridaceae with ornamental potential

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Abstract

Three species of the Iridaceae with ornamental potential were micropropagated with the intention of producing propagules more rapidly for possible commercialization. Shoot induction from *in vitro* germinated seedlings of *Romulea minutiflora* was obtained with 5.4 μM α-naphthaleneacetic acid (NAA) and 23.2 μM kinetin. Shoot explants formed corms best with 3.4 or 17 μM paclobutrazol, and one incidence of *in vitro* flowering was observed. *Sisyrinchium laxum* shoot explants produced more and healthier multiple shoots with *meta*-topolin (mT) than with 6-benzyladenine (BA). Rooting was best in control (no hormone) cultures, and addition of NAA and indole-3-acetic acid (IAA) inhibited root formation and growth of shoot explants, and formed short, stunted roots. Roots produced by indole-3-butyric acid (IBA) were morphologically most similar to those produced in control cultures. Liquid-shake culture of shoots did not lead to meristemoid formation, despite supplementation with various growth regulators (*m*T, GA_3 or paclobutrazol). Low temperature (10–20 °C) induced corm formation in *Tritonia gladiolaris* shoot cultures, while corm formation was completely inhibited above 20 °C. Increasing temperature from 10 °C to 15 °C and from 15 °C to 20 °C increased corm mass significantly. Paclobutrazol (3.4 μM), GA_3 (2.9 μM), NAA (5.4 μM) or methyl jasmonate (4.5 μM) could not induce corm formation at 25 °C, while at 15 °C, NAA and methyl jasmonate inhibited corm formation. These experiments successfully demonstrate the ease with which different genera of the Iridaceae can be multiplied in *in vitro* systems.
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Keywords: Auxins; Carbohydrates; Corm formation; Cytokinins; Shoot induction

1. Introduction

The Iridaceae is a large family with over 2000 species, many of which are endemic to South Africa and produce attractive flowers and foliage. Approximately 40 species and hybrids from 12 genera have been successfully micropropagated for the floricultural industry (Ascough et al., 2009a). Many species and genera thus remain horticulturally unexplored. Tissue culture procedures allow the rapid production of superior disease-free clonal material for nurseries and growers. Additionally, production of storage organs (corms, tubers or bulbs) during micropropagation can reduce the losses incurred during acclimatization and decrease the time to flowering (Ascough et al., 2008).

There are approximately 90 species of *Romulea* that are mainly confined to sub-Saharan Africa and the Mediterranean (De Vos, 1972; Manning and Goldblatt, 2001). The genus has its centre of diversity in the winter-rainfall zone of southern Africa, where 73 species are recognized (Manning and Goldblatt, 2001). Petals form a shallow cup, and flower colors include yellow, white, pink, red and blue. Many of the species are attractive in terms of their flower colour, shape and plant form (Manning and Goldblatt, 1996). Considered by many as undervalued horticulturally, these plants do well in pots (Manning et al., 2002).

Sisyrinchium is a large genus with well over 100 species, the phylogeny of which is poorly understood (Goldblatt et al., 1990). They are small plants with flattened stems, a short rhizome, with flowers usually blue or yellow (Baker, 1972). Although

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originating in the New World, some species, like *S. laxum* in South Africa, have become naturalized in other countries (P. Goldblatt, personal communication). Several species and hybrids are available for purchase from nurseries and online, indicating an increase in their popularity as garden subjects.

Tritonia is a genus of approximately 28 species (Manning et al., 2002). Several species (*T. crocata* and *T. deusta*) and hybrids are grown by the local nursery industry, and the plants are not particular about placement in the garden. They are hardy species that do well in rocky areas and are drought resistant. Plants occur in both the winter and summer rainfall areas, and flower colors are yellow to orange, and cream to white with a funnel shaped cylindrical perianth tube.

Our research laboratory has successfully micropropagated many geophytes with horticultural potential, including *Dierama latifolium* (Page and Van Staden, 1985), *Gladiolus flanaganii* (Dickens et al., 1986), *Sandersonia aurantiaca* (Finnie and Van Staden, 1989), *Gloriosa superba* (Finnie and Van Staden, 1989), *Gethyllis linearis* (Drewes and Van Staden, 1994), *Hypoxis colchicifolia* (Appleton and Van Staden, 1995), *Veltheimia brateata* (Taylor and Van Staden, 1997), *Scilla natalensis* (McCartan and Van Staden, 1998), *Babiana* (six species, McAlister et al., 1998), *Drimia robusta* (Ngugi et al., 1998), *Haemanthus* (three species, Rabe and Van Staden, 1999), *Tulbaghia simmleri* (Zschocke and Van Staden, 2000), *Crinum variabile* (Fennell et al., 2001), *Watsonia* (four species, Ascough et al., 2007), *Eucomis zambesiaca* (Cheesman et al., 2010) and *Albuca* (two species, Ascough and Van Staden, 2010). Despite this extensive collection of protocols, it is important to develop new protocols for noteworthy species and genera.

These attractive plants show potential for development as new horticultural products, and since no information is available on the micropropagation of these genera, we investigated tissue culture techniques in *Romulea minutiflora*, *Sisyrinchium laxum* and *Tritonia gladiolaris* (formerly *T. linneata*) as a means of speeding up plant production through multiplication and *in vitro* induction of storage organs. *Sisyrinchium laxum* was chosen as it has become naturalised in South Africa and seeds were readily available from plants growing at the University of KwaZulu-Natal. *Romulea minutiflora* was chosen because seeds were commercially obtainable. *Tritonia gladiolaris* was chosen because it has a widespread distribution in the summer-rainfall area so harvesting corms would not negatively impinge on wild populations.

2. Materials and methods

2.1. Plant material and general culture conditions

Tissue culture media were prepared a day before culture initiation using the formulation of Murashige and Skoog (1962) (MS) supplemented with 100 mg/L *myo*-inositol, 8 g/L agar (Agar no.1, Oxoid, Cambridge, UK) and 87.5 mM sucrose. The pH of the media was adjusted to 5.8 with KOH before adding agar. This was followed by autoclaving at 121 °C and 100 kPa for 20 min. Changes to this basic medium composition are detailed in corresponding experiments below. Unless otherwise stated, cultures were incubated in growth rooms kept at 25 ± 2 °C under a 16 h photoperiod with radiant flux density of approximately 20 μmol $m^{-2}s^{-1}$ provided by Osram® cool white fluorescent tubes (L 75 W 20×).

2.1.1. Romulea minutiflora Klatt

Romulea minutiflora seeds were obtained from Silverhill Nurseries, Kenilworth, South Africa. Seeds were surface decontaminated by placing them in a 1.75% NaOCl solution with a few drops of Tween 20® (Laboratory Consumables, Durban) for 15 min, after which they were rinsed three times with sterile distilled water (Ascough et al., 2007). Each seed was placed on 10 ml of 1/10th strength MS medium without sucrose in a 33 ml cylindrical glass culture tube, capped and sealed with Parafilm®. Root, leaf and hypocotyls (15 mm) were excised from seedlings greater than 30 mm in length (1–2 weeks after germination) and placed on media containing α-naphthaleneacetic acid (NAA) (0, 5.4, 26.9 or 53.7 μM) and kinetin (0, 2.3 or 23.2 μM).

Healthy shoots from responding explants were subcultured every two months for two rounds of multiplication (23.2 μM kinetin and 5.4 μM NAA) for use as explants for corm formation experiments. The medium (10 ml in 33 ml cylindrical tubes) was supplemented with 87.5, 175 or 262.5 mM sucrose or 5 g/L activated charcoal (AC, Sigma). The tubes were placed at 10, 20 or 25 °C. The effect of growth retardants on corm differentiation was also investigated by supplementing the culture medium with 3.4, 17 or 34 μM paclobutrazol, or 4.9 μM abscisic acid (ABA). After six months, corm induction frequency and corm size were evaluated.

2.1.2. Sisyrinchium laxum Otto ex Sims

Seeds of *S. laxum* were obtained from grassy areas adjacent to the Botany building at the University of KwaZulu-Natal Pietermaritzburg campus, South Africa (29°37′30″ S, 30°24′ 15″ E). Seeds were surface decontaminated by immersion in 70% (v/v) ethanol for 1 min, followed by 10 min in a 50% (v/v) commercial bleach solution with Tween 20®. Seeds were rinsed three times in sterile distilled water and placed on 1/10th MS medium without hormones or sucrose at 15 °C for germination. After three weeks, roots from the seedlings were carefully excised, and leaves trimmed to 3 cm. Four replicates each of four shoot explants were then transferred onto MS medium (40 ml in a 250 ml cylindrical glass jar) to investigate the effects of 6-benzyladenine (BA) and *meta*-topolin (*m*T) (0, 4.1, 8.3 or 20.7 μM) on shoot multiplication. After one month, the number of shoots per explant was recorded.

Shoots were multiplied on MS medium with 3.3 μM *m*T (two cycles each of 30 days), and transferred to 250 ml Erlenmeyer flasks containing 30 ml MS liquid medium supplemented with either 3.3 μM *m*T, 2.9 μM gibberellin acid (GA_3) or 3.4 μM paclobutrazol. Each treatment consisted of four flasks each containing four explants. Flasks were placed on a rotary shaker and rotated at 120 rpm within the same growth room. After three weeks, the number of shoots and roots were recorded.

In a third experiment, shoots were subcultured onto MS medium for rooting. The effect of NAA (0, 0.5, 2.7 or 5.4 μM), indole-3-acetic acid (IAA at 0, 0.6, 2.9 or 5.9 μM) and indole-3-butyric acid (IBA at 0, 0.5, 2.5 or 4.9 μM) on *in vitro* rooting was tested. Each jar contained four explants, with a total of four jars per treatment. Root morphology was observed after four weeks, and root length recorded. Plantlets were removed from culture vessels, agar was removed from the roots, and transferred to a mist-house (for *ex vitro* acclimatization) in trays filled with vermiculite. The day/night temperature in the mist-house was 22/15 °C, relative humidity between 80 and 90%, a misting interval of 15 min and a misting duration of 10 s. After 2 weeks, plantlets were transferred to pots with sterile potting soil (loam:compost:sand ratio of 3:1:1) in a temperature controlled greenhouse (average day/night temperature 23/14 °C, photoperiod during acclimatization was that of prevailing conditions during spring (12 h) with an average midday maximum radiant flux density of 450 $\mu mol m^{-2} s^{-1}$).

2.1.3. Tritonia gladiolaris (Lam.) Goldblatt and J. C. Manning

Flowering plants were collected in summer from Mount Gilboa (29°16′59.75″ S, 30°17′00.40″ E) and seeds obtained from those plants in the greenhouse. Seeds were decontaminated and germinated as described for *S. laxum* above. After seedlings had grown to 5 cm, they were subcultured onto MS medium containing 2.2 μM BA for shoot multiplication. When sufficient shoots were available, they were individually transferred to 33 ml cylindrical glass culture tubes containing 10 ml MS media and placed at 10, 15, 20, 25, 30 or 35 °C to determine the optimum temperature for corm formation. Other shoots were subcultured onto MS media containing 2.9 μM GA_3, 5.4 μM NAA, 3.4 μM paclobutrazol, 4.5 μM methyl jasmonate, or onto media without growth regulators (control). Explants were incubated at either 15 or 25 °C to determine if there were temperature-growth regulator interactions controlling corm formation. Seven to ten explants were used for each hormone treatment at each temperature, and the experiment was repeated four times. After three months, corm induction, corm mass and diameter were recorded.

2.2. Data analysis

Explants were always randomly assigned to the various treatments. Due to slow multiplication rates, corm induction experiments on *R. minutiflora* could not be repeated, and thus were not analysed for statistical differences. All other percentages were converted to proportions and arcsine transformed prior to analysis. All data were analyzed for significant differences using ANOVA and means were separated by Fischer's LSD at the 5% level of significance using Sigmaplot® 11.

3. Results and discussion

3.1. Romulea minutiflora

Hypocotyls were the only explants to respond *in vitro*, while root and leaf explants remained unresponsive. Shoots were induced on a medium with 5.4 μM NAA + 26.9 μM kinetin, while callus was observed on a medium containing 53.7 μM NAA and 26.9 μM NAA + 23.20 μM kinetin. All other hormone treatments did not induce any response. Similar results were observed in *Gladiolus carneus* (Jäger et al., 1998), six *Babiana* species (McAlister et al., 1998) and four species of *Watsonia* (Ascough et al., 2007) where regeneration was limited to sections containing the hypocotyl. The presence of the basal meristem in the hypocotyl region is likely the reason for the response of this explant type (Ascough et al., 2009b). As with other Iridaceae where cultures have been initiated from seed, both an auxin and cytokinin are required for shoot induction (Ascough et al., 2009a).

No significant differences in corm induction from shoot explants were observed either when changing the temperature or altering the sucrose concentration (Table 1). Corm mass, however, increased with increasing sucrose concentration (Table 1). This was true at both 10 and 20 °C. The addition of activated charcoal to media with 87.5 mM sucrose significantly increased corm mass under both temperature regimes. Addition of paclobutrazol at all tested levels (3.4, 17 or 34 μM) to the medium increased corm induction and size (Table 2). This is consistent with other Iridaceae where growth retardants reduce leaf elongation and promote storage organ formation (Madubanya et al., 2006; Ziv, 1989). Corm formation is advantageous since it eliminates the need for *in vitro* rooting and *ex vitro* acclimatization, and may prevent hyperhydricity that is often observed with high multiplication rates (Ascough et al., 2008). Further, a reduction in time to flower following first planting (often several growing seasons) has been observed in some species (Morán et al., 2003; Nhut, 1998).

Corms from this experiment were not harvested and weighed, but allowed to grow to determine if *in vitro* flowering could be observed. This occurred in one case (Fig. 1A), and despite repeated attempts to replicate this, further flowering was unfortunately not observed.

3.2. Sisyrinchium laxum

All concentrations of BA and *m*T promoted shoot multiplication compared to cultures without any hormones (Table 3). Although cultures with *m*T produced on average more shoots

Table 1
Effect of sucrose concentrations, temperature and activated charcoal (AC) on corm induction and growth in *Romulea minutiflora* after six months. Means in the same column with common letters are not significantly different (Fischer's LSD, $p \geq 0.05$).

	Treatment	Mean corm induction (percentage ± s.e.)	Mean corm weight (mg ± s.e.)
10 °C	87.5 mM sucrose	65.2 ± 9.2 a	108.7 ± 21.9 b
	175 mM sucrose	80.4 ± 6.9 a	279.0 ± 114.0 ab
	262.5 mM sucrose	81.9 ± 9.1 a	134.5 ± 19.6 b
	5 g/L AC	56.7 ± 5.6 a	285.2 ± 33.7 a
20 °C	87.5 mM sucrose	69.4 ± 4.6 a	152.0 ± 31.3 b
	175 mM sucrose	73.9 ± 3.9 a	227.0 ± 61.1 b
	262.5 mM sucrose	72.5 ± 10.3 a	273.7 ± 50.3 a
	5 g/L AC	63.8 ± 5.9 a	239.3 ± 45.0 a

Table 2
Percentage corm induction for *Romulea minutiflora* shoots cultured on media supplemented with growth retardants (Corm weights were not obtained as these corms were subcultured for *in vitro* flowering experiments).

Treatment		Basal swelling (%)	Corm induction (%)	Corms > 5 mm (%)	Potential maximum corm induction (%)
	Control	70	0	0	70
Paclobutrazol	3.4 μM	55	15	10	70
	17 μM	50	35	20	85
	34 μM	35	35	20	70
Abscisic acid	4.9 μM	45	20	5	65

than cultures with BA, conclusions must remain speculative since a high variation was observed. Morphologically, shoots produced in *m*T resembled more closely plants grown under natural conditions (long straight leaves, and allowed rooting), while the appearance of shoots produced in BA was abnormal, curled, twisted, stunted and without roots.

Shoots subcultured onto media containing various auxins (NAA at 0, 0.5, 2.7 or 5.4 μM, IAA at 0, 0.6, 2.9 or 5.9 μM, or IBA at 0, 0.5, 2.5 or 4.9 μM) did not produce more shoots, but rooted easily. Longest roots were produced by control cultures (no hormones; Table 4). NAA at all concentrations inhibited root elongation significantly, with the resultant roots being short and thick. Roots produced by shoots grown in IBA had the most 'normal' appearance; long thin fibrous roots that did not differ significantly from control cultures (Table 4). Acclimatization was satisfactory (> 70%) in most cases, except for the highest concentration of NAA where all plantlets died on transferral to the mist-house (Table 4). Although root length varied significantly between auxins, acclimatisation was relatively unaffected. In other plants such as *Watsonia*, however, both auxin type and concentration affected rooting and acclimatisation (Ascough et al., 2007). Plants continued to grow in the greenhouse, and after one month, flowered (Fig. 1B).

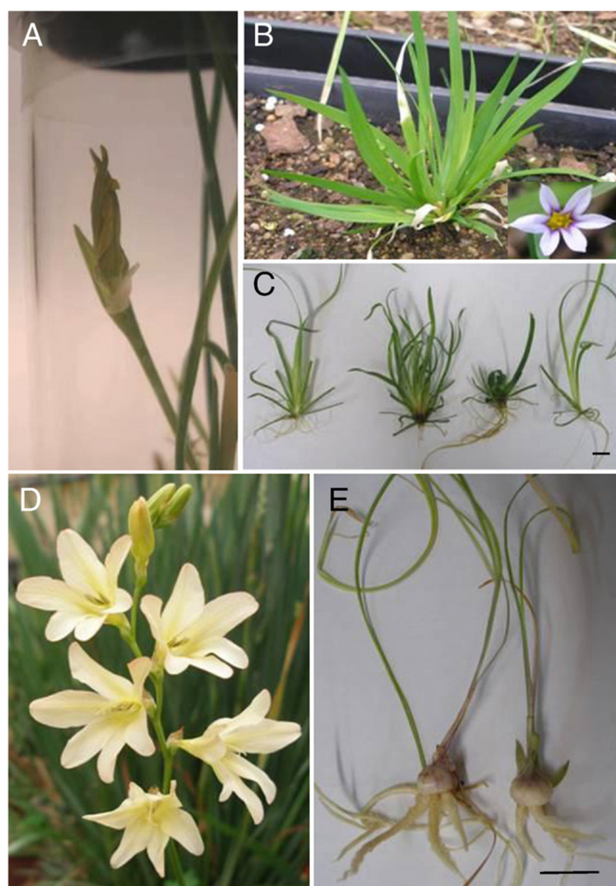

Fig. 1. (A) Unopened *in vitro* formed flower of *Romulea minutiflora* observed in a 33 ml culture tube with MS media supplemented with 175 mM sucrose and grown at 20 °C. This was only observed in one explant and the flower remained unopened; (B) Acclimatized plant of *Sisyrinchium laxum* and flower (inset); (C) *S. laxum* shoot explants grown in liquid-shake culture after three weeks without hormones (left), with 3.3 μM *m*T (second from left), with 3.4 μM paclobutrazol (third from left), and with 2.9 μM GA_3 (right); (D) Inflorescence of *Tritonia gladiolaris*; (E) Corm induction on *T. gladiolaris* shoot explants cultured at 15 °C for three months. Scale bar represents 10 mm.

Shoots were grown in a liquid medium on a rotary shaker (120 rpm) supplemented with *m*T, GA_3 or paclobutrazol. Shoot production increased in response to 3.3 μM *m*T, but this was not significantly higher than other treatments or the control cultures (Table 5; Fig. 1C). Root number increased significantly compared to control cultures in response to 2.9 μM GA_3. Unlike other Iridaceae grown in liquid-shake culture, no meristemoid (bud clusters) were observed in any treatment (Ascough et al., 2007; Madubanya et al., 2006; Ziv, 1991). Meristemoid formation allows even more rapid production of shoots and the possibility of scaling-up and mechanizing production techniques.

3.3. *Tritonia gladiolaris*

Seed germination in *T. gladiolaris* was followed by successful induction of shoot multiplication using 3.3 μM *m*T. Once sufficient clonal material had been generated, corm induction and growth was investigated by subculturing shoots onto MS media and incubating cultures at various temperatures (10, 15, 20, 25, 30 or 35 °C) or with hormones (GA_3, NAA,

Table 3
Shoot multiplication of *Sisyrinchium laxum* with 6-benzyladenine (BA) or *meta*-topolin (*m*T) after one month. Means in the same column with common letters are not significantly different (Fischer's LSD, p ≥ 0.05).

Treatment		Number of shoots per explant (mean ± s.e.)
Control		1 ± 0 e
BA	4.4 μM	4.1 ± 2.3 cd
	8.9 μM	5.5 ± 1.2 bc
	22.2 μM	8.3 ± 3.2 c
*m*T	4.1 μM	2.5 ± 1.1 d
	8.3 μM	10.2 ± 9.0 abcd
	20.7 μM	14.9 ± 6.2 ab

Table 4
Effect of α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) on *in vitro* rooting and *ex vitro* acclimatization of *Sisyrinchium laxum* shoot explants. Means in the same column with common letters are not significantly different (Fischer's LSD, p ≥ 0.05).

Treatment		Mean root length per explant (mm ± s.e.)	Acclimatization (%)
Control (no hormones)		118.65 ± 7.4 a	85.7 a
NAA	0.5 μM	21.45 ± 6.8 cd	100 a
	2.7 μM	9.83 ± 0.8 c	100 a
	5.4 μM	5.00 ± 0 d	0 c
IAA	0.6 μM	72.32 ± 2.4 b	100 a
	2.9 μM	69.80 ± 28.3 abcd	100 a
	5.9 μM	21.59 ± 3.4 c	75 a
IBA	0.5 μM	97.02 ± 6.4 ab	75 a
	2.5 μM	78.45 ± 23.8 abcd	100 a
	4.9 μM	94.36 ± 6.9 ab	67 ab

paclobutrazol or methyl jasmonate all at 1 mg/L) at either 15 or 25 °C. Corm induction was best at 10 and 15 °C, while corms formed at a significantly lower frequency at 20 °C (Table 6; Fig. 1E). Temperatures above 20 °C completely inhibited corm induction. Heaviest corms were produced at 20 °C. Corms produced at 15 °C were significantly lighter than those at 20 °C, but significantly heavier than those formed at 10 °C (Table 6). A two-stage system could therefore be developed whereby shoots are incubated at 15 °C for a period to induce corm formation, followed by transfer to 20 °C to increase corm growth. Reduced temperature is a known promoter of storage organ induction, and is probably perceived as a trigger of unfavourable approaching environmental conditions that can be survived by formation of a dormant storage organ (Ascough et al., 2008). Producing larger corms *in vitro* could be an advantage since in many species, a minimum corm size is needed before plants will flower. Producing a large corm *in vitro* could reduce the time to flowering by reducing the number of seasons required to obtain a corm of sufficient size for flowering.

The effect of various hormones (GA_3, NAA, paclobutrazol and methyl jasmonate) on corm induction and growth of *T. gladiolaris* was tested at both 15 and 25 °C to determine if there was a temperature–hormone interaction, and to establish if a hormonal treatment could promote corm induction at a non-inducing temperature. Disappointingly, no corm induction occurred at 25 °C (Table 7). Methyl jasmonate and NAA inhibited corm formation significantly, while paclobutrazol decreased corm formation compared to control cultures, although this was not significant. Corm mass and corm diameter were similar in all treatments (Table 7). This suggests that hormonal treatments lowering corm induction do not impinge on subsequent growth of the corm at this single temperature (15 °C). Temperature therefore, appears to be a greater factor influencing corm growth than hormones.

Table 5
Effect of *meta*-topolin (*m*T), gibberellic acid (GA_3) and paclobutrazol on shoot and root formation in *Sisyrinchioum laxum* shoot explants grown in a liquid-shake culture (120 rpm) for three weeks. All explants in all treatments produced roots (rooting = 100%). Means in the same column with common letters are not significantly different (Fischer's LSD, p ≥ 0.05).

Treatment	Number of roots per explant (mean ± s.e.)	Number of shoots per explant (mean ± s.e.)
Control	4.1 ± 0.5 b	1.0 ± 0.2 b
*m*T 3.3 μM	4.6 ± 0.7 ab	3.0 ± 1.2 a
GA_3 2.9 μM	6.5 ± 1.0 a	1.3 ± 0.1 b
Paclobutrazol 3.4 μM	6.4 ± 1.4 ab	1.6 ± 0.6 ab

Table 6
Corm induction and mass of *Tritonia gladiolaris* shoot explants cultured in MS media for three months at various temperatures. Means in the same column with common letters are not significantly different (Fischer's LSD, p ≥ 0.05).

Temperature (°C)	Corm induction (%)	Corm mass (mg)
10	81.3 ± 12.0 a	80.4 ± 14.5 c
15	95.0 ± 5.0 a	248.5 ± 19.7 b
20	37.5 ± 23.9 b	456.0 ± 92.3 a
25	0 c	
30	0 c	
35	0 c	

Table 7
Corm induction, mass and diameter of *Tritonia gladiolaris* shoot explants cultured in MS media containing either gibberellic acid (GA_3), α-naphthaleneacetic acid (NAA), paclobutrazol or methyl jasmonate at 15 °C. Means in the same column with common letters are not significantly different (Fischer's LSD, p ≥ 0.05).

Treatment	Corm induction (percentage ± s.e.)	Corm mass (mg ± s.e.)	Corm diameter (mm ± s.e.)
Control	65.9 ± 12.0 a	131.7 ± 0.2 a	5.85 ± 0.11 a
GA_3 2.9 μM	64.3 ± 7.1 a	126.6 ± 3.1 a	5.92 ± 0.10 a
NAA 5.4 μM	0 c		
Paclobutrazol 3.4 μM	33.3 ± 19.1 ab	125.1 ± 25.7 a	5.89 ± 0.44 a
Methyl jasmonate 4.5 μM	14.3 ± 0 b	147.0 ± 20.8 a	6.31 ± 0.29 a

4. Conclusions

The current research has shown the ease of developing rapid micropropagation protocols from Iridaceae having ornamental potential. In all cases, seeds were used as the initial explants for culture initiation, thereafter shoots or hypocotyl sections were used for multiplication. Where species produce a dormant storage organ such as a corm (*Tritonia* and *Romulea*), experiments optimizing factors controlling corm formation and growth were performed to allow rapid production of propagules that do not require acclimatization and are easily shipped. For *Sisyrinchium* that produce rhizomes, rooting and acclimatization optimization were successfully performed.

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References

Appleton, M.R., Van Staden, J., 1995. Micropropagation of some South African *Hypoxis* species with medicinal and horticultural potential. Acta Horticulturae 420, 75–77.

Ascough, G.D., Van Staden, J., 2010. Micropropagation of *Albuca bracteata* and *A. nelsonii* — indigenous ornamentals with medicinal value. South African Journal of Botany 76, 579–584.

Ascough, G.D., Erwin, J.E., Van Staden, J., 2007. *In vitro* propagation of four *Watsonia* species. Plant Cell, Tissue and Organ Culture 88, 135–145.

Ascough, G.D., Erwin, J.E., Van Staden, J., 2008. *In vitro* storage organ formation on ornamental geophytes. Horticultural Reviews 34, 417–446.

Ascough, G.D., Erwin, J.E., Van Staden, J., 2009a. Micropropagation of Iridaceae — a review. Plant Cell, Tissue and Organ Culture 97, 1–19.

Ascough, G.D., Novák, O., Pěnčík, A., Rolčík, J., Strnad, M., Erwin, J.E., Van Staden, J., 2009b. Hormonal and cell division analyses in *Watsonia lepida* seedlings. Journal of Plant Physiology 166, 1497–1507.

Baker, J.G., 1972. Handbook of the Irideae. Strauss and Crater, GmbH, Germany.

Cheesman, L., Finnie, J.F., Van Staden, J., 2010. *Eucomis zambesiaca* Baker: factors affecting *in vitro* bulblet induction. South African Journal of Botany 76, 543–549.

De Vos, M.P., 1972. The genus *Romulea* in South Africa. Journal of South African Botany Supplementary volume 9.

Dickens, C.W.S., Kelly, K.M., Manning, J.C., Van Staden, J., 1986. *In vitro* propagation of *Gladiolus flanaganii*. South African Journal of Botany 52, 485–487.

Drewes, F.E., Van Staden, J., 1994. *In vitro* propagation of *Gethyllis linearis* L. Bak., a rare indigenous bulb. South African Journal of Botany 60, 295–296.

Fennell, C.W., Crouch, N.R., Van Staden, J., 2001. Micropropagation of the river lily, *Crinum variabile* (Amaryllidaceae). South African Journal of Botany 67, 74–77.

Finnie, J.F., Van Staden, J., 1989. *In vitro* propagation of *Sandersonia* and *Gloriosa*. Plant Cell, Tissue and Organ Culture 19, 151–158.

Goldblatt, P., Rudall, P., Henrich, J.E., 1990. The genera of the *Sisyrinchium* alliance (Iridaceae: Iridoideae): phlogeny and relationships. Systematic Botany 15, 497–510.

Jäger, A.K., McAlister, M.G., Van Staden, J., 1998. *In vitro* culture of *Gladiolus carneus*. South African Journal of Botany 64, 146–147.

Madubanya, L.A., Makunga, N.P., Fennell, C.W., 2006. *Dierama luteoalbidum*: liquid culture provides an efficient system for the *ex situ* conservation of an endangered and horticulturally valuable plant. South African Journal of Botany 72, 584–588.

Manning, J.C., Goldblatt, P., 1996. West coast. South African Wild Flower Guide, volume 7. Botanical Society of South Africa, Kirstenbosch.

Manning, J.C., Goldblatt, P., 2001. A synoptic review of *Romulea* (Iridaceae: Crocoideae) in sub-Saharan Africa, the Arabian Peninsula and Socotra including new species, biological notes, and a new infrageneric classification. Adansonia 23, 59–108.

Manning, J.C., Goldblatt, P., Snijman, D., 2002. The color encyclopedia of Cape Bulbs. Timbers Press, Portland, USA.

McAlister, B.G., Jäger, A.K., Van Staden, J., 1998. Micropropagation of *Babiana* spp. South African Journal of Botany 64, 88–90.

McCartan, S.A., Van Staden, J., 1998. Micropropagation of the medicinal plant, *Scilla natalensis* Planch. Plant Growth Regulation 25, 177–180.

Morán, G.P., Colque, R., Viladomat, F., Bastida, J., Codina, C., 2003. Mass propagation of *Cyrtanthus clavatus* and *Cyrtanthus spiralis* using liquid medium culture. Scientia Horticulturae 98, 49–60.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15, 472–497.

Ngugi, C.W., Jäger, A.K., Van Staden, J., 1998. *In vitro* propagation of *Drimia robusta* Bak. South African Journal of Botany 64, 266–268.

Nhut, D.T., 1998. Micropropagation of lily (*Lilium longiflorum*) via *in vitro* stem node and pseudo-bulblet culture. Plant Cell Reports 17, 913–916.

Page, Y.M., Van Staden, J., 1985. *In vitro* propagation of *Dierama latifolium*. HortScience 20, 1049–1050.

Rabe, T., Van Staden, J., 1999. *In vitro* propagation of three *Haemanthus* species. South African Journal of Botany 65, 438–440.

Taylor, J.L.S., Van Staden, J., 1997. *In vitro* propagation of *Veltheimia bracteata* and *V. bracteata* "Lemon Flame". South African Journal of Botany 63, 159–161.

Ziv, M., 1989. Enhanced shoot and cormlet proliferation in liquid cultured *Gladiolus* by growth retardants. Plant Cell, Tissue and Organ Culture 17, 101–110.

Ziv, M., 1991. Morphogenic patterns of plants micropropagated in liquid medium in shaken flasks or large-scale bioreactor cultures. Israel Journal of Botany 40, 145–153.

Zschocke, S., Van Staden, J., 2000. *In vitro* propagation of *Tulbaghia simmleri*. South African Journal of Botany 66, 86–89.

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