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Micropropagation of *Albuca bracteata* and *A. nelsonii* — Indigenous ornamentals with medicinal value

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Abstract

In this study, two species from the genus *Albuca* (Hyacinthaceae) with ornamental and medicinal properties were micropropagated. Adventitious bulblets of *Albuca bracteata* were cut into quarters and used as explants to examine the effect of temperature (10, 15, 20, 25, 30 or 35 °C), carbohydrates (glucose, fructose or sucrose at 0, 87.5, 175, 262.5 or 350 mM) and hormones (BA, *m*TR, NAA, IAA, GA_3, ABA or methyl jasmonate each at 0, 0.1, 1.0 or 5.0 mg/L) on the induction and growth of bulblets. Temperatures above 35 °C completely inhibited bulb formation, while induction at all other temperatures was high. Heaviest and largest bulbs formed at 20 °C. Low concentrations (87.5 mM) of all tested carbohydrates increased bulb induction compared to media without a carbohydrate source, while higher levels decreased bulblet induction. The cytokinins *m*TR and BA inhibited bulb induction, diameter and mass at moderate (1.0 mg/L) and high (5.0 mg/L) concentrations. GA_3, NAA and particularly IAA promoted bulblet induction, while ABA and methyl jasmonate had no significant effect on the induction or bulblet growth. Leaf material and young inflorescences of *A. nelsonii* were removed, decontaminated, and dissected into seven explant types: leaves, peduncles, pedicels, whole flowers, tepals, ovaries and anthers. These were placed on MS media without hormones, or containing 0.5 mg/L *m*TR, 0.5 mg/L NAA or 0.5 mg/L *m*TR + 0.5 mg/L NAA to establish which explant type and hormone combination promoted shoot formation. Some tepal and pedicel explants were capable of shoot production on media with both *m*TR and NAA, but peduncle explants produced the most shoots when *m*TR and NAA were both present in the culture medium. Flowers, leaves, ovaries and anthers were completely unresponsive, irrespective of medium composition. These techniques will aid the further horticultural development of these plants, and can be easily adjusted for other species within the genus to promote conservation.

Keywords: Auxins; Carbohydrates; Cytokinins; Bulb formation; Shoot induction

1. Introduction

There are approximately 80 species of *Albuca*, of which about 60 are endemic to southern Africa (Manning et al., 2004). The Red Data List for South Africa lists three species as endangered (*A. clanwilliamigloria*), vulnerable (*A. crudenii*), and critically endangered (*A. thermarum*). Plants display a variety of flower size and colour (Fig. 1A and D), and have considerable potential for development as ornamental subjects.

Albuca species have been used for traditional medicine with bulb infusions taken as emetics to protect against sorcery and as general protective charms (Pooley, 1998). Several species are known to produce steroidal saponins, polyhydroxyalkaloids and homoisofavonoids (Dahlgren et al., 1985; Koorbanally et al., 2005), making them potential candidates for ethnomedicinal studies. Should unsustainable harvesting of these plants continue and escalate, more species could become threatened. This fact, combined with their ornamental potential, are significant motivating factors for exploration of micropropagation in this genus.

Our group has conducted much research into this particular area of micropropagation of plants with medicinal and ornamental value for conservation purposes. We have been successful in several genera, including *Crinum* (Fennell et al., 2001), *Tulbaghia*

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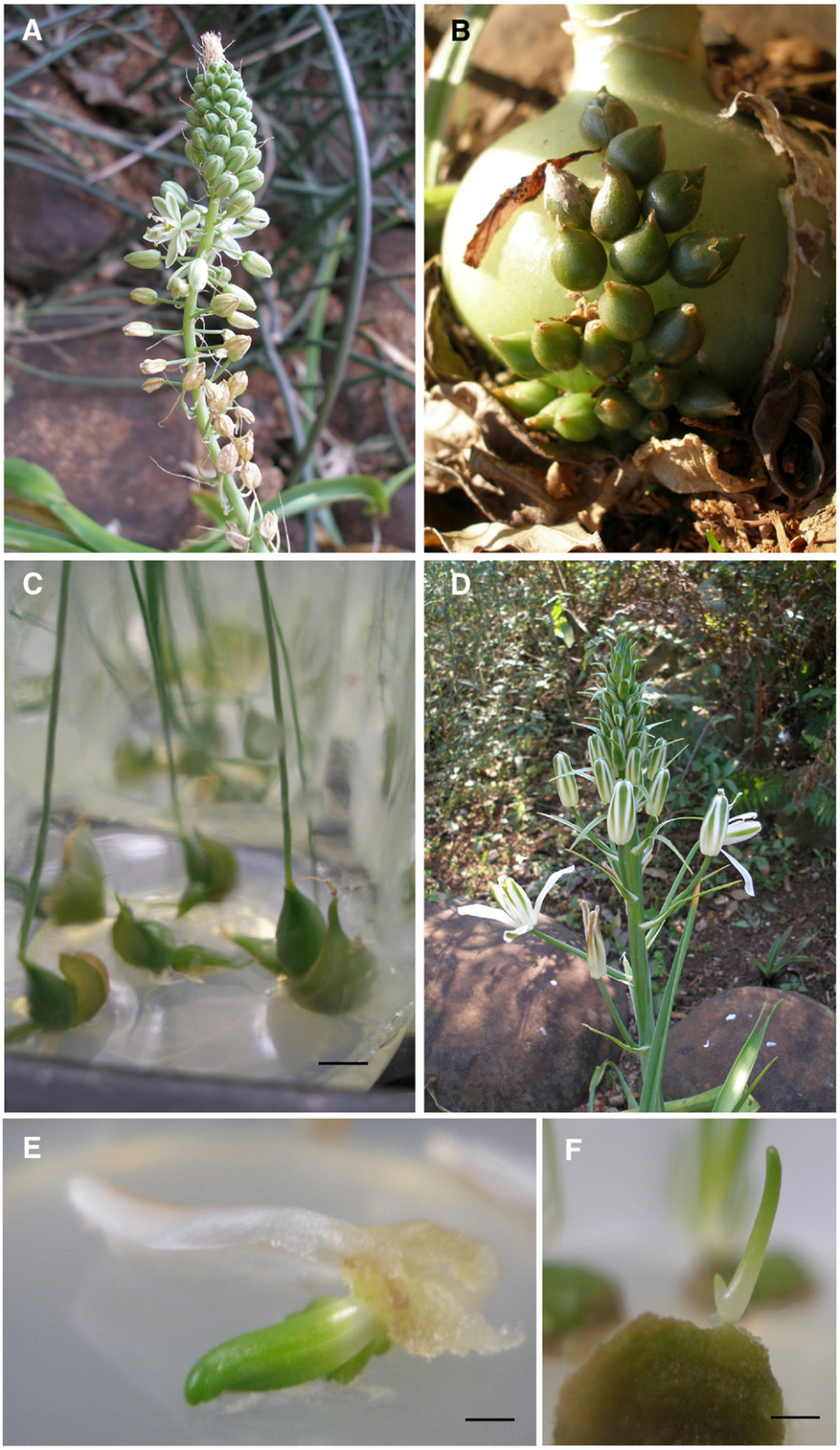

Fig. 1. Flowers and inflorescence of *Albuca bracteata* (A); adventitious daughter bulblets forming on outer scale of *A. bracteata* were used as explants (B); bulblets forming from quarter-bulb explants of *A. bracteata* (C, bar represents 5 mm); flowers and inflorescence of *A. nelsonii* (D); *in vitro* shoot formation from the base of a tepal (E, bar represents 2.5 mm); *in vitro* shoot production from peduncle explants after eight weeks on media containing 0.5 mg/L *m*TR+0.5 mg/L NAA (F, bar represents 2.5 mm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Zschocke and Van Staden, 2000), *Scilla* (McCartan and Van Staden, 1998), *Cyrtanthus* (McAlister et al., 1998), *Eulophia* (McAlister and Van Staden, 1998), *Eucomis* (McCartan and Van Staden, 1996), *Hypoxis* (Appleton and Van Staden 1995), *Bowiea* (Finnie et al., 1994), *Sandersonia* (Finnie and Van Staden, 1989), *Gloriosa* (Finnie and Van Staden, 1989), *Podocarpus* (Kowalski and Van Staden, 2001), *Cussonia* (Tetyana and Van Staden, 2001) and *Salvia* (Huang and Van Staden, 2002). To the best of our knowledge, there are no reports on the tissue culture of *Albuca* species, thus our goal was to propagate two members of this genus by investigating the effect of explant type on culture initiation of *A. nelsonii*, and the control of bulb formation in *A. bracteata*.

2. Materials and methods

2.1. Plant material and general micropropagation conditions

All plant material was obtained from existing plants growing in the Botanic Gardens of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37′30″ S, 30°24′15″ E). 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.

2.1.1. Albuca bracteata

Albuca bracteata plants can be propagated asexually since many bulbs produce adventitious bulblets on the outermost scale throughout the year (Fig. 1B). These bulblets were harvested, transferred to the laboratory and decontaminated. Bulblets were rinsed under running tap water for 2 min, followed by a 2 min submersion in 70% (v/v) ethanol. After transferring to sterile (autoclaved) beakers in a laminar flow hood, bulblets were surface decontaminated by immersion in a 50% (v/v) commercial bleach solution (3.5% w/v sodium hypochlorite) for 10 min, followed by 10 min in a 1% (w/v) mercuric chloride solution. Bulblets were washed three times in sterile distilled water before culture initiation. Individual bulblets were placed in 33 mL culture tubes containing 10 mL MS medium, capped and sealed with Parafilm®, and incubated in a growth room. Bulblets that exhibited signs of contamination were discarded. Bulblets that were successfully decontaminated after two weeks were cut longitudinally into halves and then again into quarters, and placed individually in 33 mL culture tubes containing 10 mL of MS media for subsequent experiments.

The effect of temperature, carbohydrates and hormones on bulb induction and growth of quarter-bulb explants were investigated. Explants were placed in growth chambers with a 16 h photoperiod at 10, 15, 20, 25, 30 or 35 °C. Alternatively, explants were placed singly in 33 mL culture tubes with 10 mL MS media, modified by addition of glucose, fructose or sucrose at 0, 87.5, 175, 262.5 or 350 mM and placed at 25 °C. Additionally, five explants were placed in 200 mL culture jars with 40 mL MS media modified by adding 6-benzyladenine (BA), *meta*-topolin riboside (*m*TR), α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), gibberellic acid (GA_3), methyl jasmonate or abscisic (ABA) at 0, 0.1, 1.0, or 5.0 mg/L and placed at 25 °C. Five to seven replicates were used for each treatment, and the experiment was repeated four times. Cultures were observed weekly, and after three months, bulb induction, number of bulbs per explant, bulb diameter and bulb mass were recorded.

Bulblets were transferred to a mist-house and laid on top of trays containing vermiculite. After two weeks roots and leaves had developed, and plantlets were transferred to trays containing soil, placed in a greenhouse, and watered three times a week.

2.1.2. Albuca nelsonii

As only a limited number of these plants were available in the Botanic Gardens, a non-destructive method of culture initiation was required. Leaf material and elongating inflorescences were cut from plants, placed in a beaker with distilled water and returned to the laboratory. Whole flowers with pedicels, leaf sections, and peduncle sections were decontaminated in separate beakers as follows: explants were washed under running tap water for 2 min, submerged in 70% (v/v) ethanol for 1 min and subsequently transferred to sterile (autoclaved) beakers in a laminar flow hood. Plant material was then surface decontaminated with a 50% (v/v) solution of commercial bleach (3.5% (w/v) sodium hypochlorite) for 10 min, and rinsed three times with sterile distilled water.

The effect of explant (leaf, peduncle, pedicel, whole flower, tepal, anther or ovary) and hormone type (no hormones, 0.5 mg/L *m*TR, 0.5 mg/L BA or 0.5 mg/L *m*TR+0.5 mg/L BA) on callus and shoot induction was investigated by placing four explants in a single jar of each media type. Three replicates of each media type were conducted. The experiment was not repeated since insufficient inflorescences were available, and the flowering season had come to an end. Explants were observed weekly for morphological changes, and after eight weeks, shoots were subcultured onto MS media containing 0.5 mg/L *m*TR for multiplication.

2.2. Data analysis

Once experiments had terminated, data were analysed for significant differences using ANOVA (Sigmaplat® 11) and means were separated by Fishers LSD ($\alpha=0.05$). Proportion bulb induction was arcsine transformed prior to ANOVA.

3. Results and discussion

3.1. Albuca bracteata

Bulbs were readily induced on quarter-bulb explants at all culture temperatures except 35 °C, where bulb induction was inhibited (Table 1). Bulbs formed at 20 °C had a significantly

Table 1
Effect of culture temperature on bulb induction and growth of quarter-bulb explants from *Albuca bracteata* after three months. Values represent means±se; values within a column with common letters are not significantly different (Fishers' LSD, $\alpha \geq 0.05$).

Temperature (°C)	Induction (%)	Bulb diameter (mm)	Bulb mass (mg)
10	92±12.9 a	3.01±0.23 c	30.4±5.4 b
15	100±0 a	3.71±0.22 abc	54.5±4.2 a
20	96±10.2 a	4.23±0.27 a	72.9±10.7 a
25	96±9.4 a	3.39±0.14 bc	54.1±5.8 a
30	100±0 a	3.77±0.20 ab	55.7±8.2 a
35	0 b		

greater diameter ($p \leq 0.05$) than those formed at 10 or 25 °C, but were not significantly different ($p > 0.05$) from those formed at 15 and 30 °C (Table 1). Heaviest bulbs were formed at 20 °C, and these were significantly heavier than those produced at 10 °C, but were not significantly different from those formed at 15, 25 and 30 °C (Table 1). Temperature is an important factor that regulates plant growth and development in general, but more specifically, storage organ formation and growth in vivo. In many species, reduced culture temperature (below 20 °C) improves storage organ formation (Ascough et al., 2008). In *Nerine bowdenni*, bulbs formed readily at lower culture temperatures (12–22 °C), but induction was repressed at 27 °C (Jacobs et al., 1992). Temperatures from 4 to 15 °C promoted bulb formation in *Lachenalia*, but at 21 °C bulb formation was inhibited (Slabbert and Niederwieser, 1999). For *A. bracteata*, a much wider temperature range for bulblet production exists (10–30 °C), and only at very high temperatures (35 °C) was bulb formation inhibited.

Bulb induction from quarter-bulb explants was low (41%) when no carbohydrate was present in the culture medium (Table 2). Greatest bulb induction (100% of explants) was observed in cultures supplemented with 87.5 mM sucrose. As sucrose levels were increased, bulb induction decreased. Similarly, as glucose or fructose levels in the media were increased from 87.5 mM, bulb induction decreased (Table 2).

Table 2
Effect of carbohydrate type and concentration on bulb induction and growth of quarter-bulb explants from *Albuca bracteata* after three months. Values represent means±se; values within a column with common letters are not significantly different (Fishers' LSD, $\alpha \geq 0.05$).

Treatment (mM)	Induction (%)	Bulb diameter (mm)	Bulb mass (mg)
Control 0	41±16.7 de	4.61±0.52 a	61.3±3.7 a
Sucrose 87.5	100±0 a	4.08±0.22 ab	51.1±6.6 ab
175	71 ± 10.2 bc	3.78±0.28 abc	35.3±4.3 bc
263.5	30±8.6 de	2.85±0.04 c	15.4±1.2 d
350	17±10.7 ef	2.84±0.64 c	22.5±8.6 cd
Glucose 87.5	86±9.9 b	4.28±0.31 ab	52.1±4.5 a
175	68±14.6 bc	4.04±0.19 ab	52.8±4.1 a
263.5	71±11.0 bc	3.61±0.28 bc	27.6±3.2 cd
350	40±11.4 de	3.00±0.25 c	25.6±5.3 cd
Fructose 87.5	76±8.1 b	4.23±0.50 ab	52.5±5.9 a
175	50±12.8 cd	4.18±0.05 ab	59.0±4.8 a
263.5	18±7.6 ef	4.06±0.53 ab	50.2±20.4 ab
350	0 f		

This suggests that increased carbohydrate supply, or increased medium osmolarity, inhibits bulb induction. Similar findings were reported for *Hyacinthus orientalis* where explants on media with 3% (w/v) sucrose (87.6 mM), fructose (166.5 mM) or glucose (166.5 mM) produced more bulblets than those cultured on 6% (Bach et al., 1992).

Bulb diameter was greatest in control cultures (no carbohydrates), and a significant decrease was only observed in cultures supplemented with sucrose and glucose at 263.5 and 350 mM (Table 2). In other treatments, bulb diameter was smaller, but not significantly so ($p > 0.05$). Similarly, heaviest bulbs were produced in cultures without carbohydrates, and high concentrations of sucrose and glucose (263.5 and 350 mM) significantly decreased bulb mass. This implies that while carbohydrates improve bulb induction compared to cultures without carbohydrates, they are not necessary for bulb growth. In contrast, *Lachenalia* leaf explants produced bigger bulbs on media containing 175 mM sucrose compared to those cultured on 87.5 mM sucrose (Slabbert and Niederwieser, 1999). This discrepancy in response to increasing carbohydrates within the Hyacinthaceae may be a reflection of different explant types responding in a different manner.

Bulb induction was inhibited ($p \leq 0.05$) by all concentrations of *m*TR, and moderate (1.0 mg/L) and high concentrations (5.0 mg/L) of BA (Table 3). In addition, primary bulb diameter and mass was also significantly reduced, implying that cytokinins are inhibitors of bulb formation and growth in *A. bracteata*. However, in other Hyacinthaceae such as *Muscari botryoides* and *Urginea maritima*, BA promoted bulb induction from bulb sections (El Grari and Backhaus, 1987; Kromer and Kukułczanka, 1992), while in *H. orientalis* "Pink Pearl", BA and kinetin had no affect on bulb production or growth (Pierik and Steegmans, 1975).

In contrast, moderate concentrations (1.0 mg/L) of NAA and all tested concentrations of IAA increased bulb induction compared to control cultures (Table 3). Primary bulb diameter was similar to that of control bulbs, while primary bulblet mass was significantly increased with all tested concentrations of IAA. When applied at 0.1 mg/L, IAA also significantly increased the diameter and mass of secondary bulblets (Table 3). This beneficial effect of auxin is consistent with several other species from the Hyacinthaceae. Bulb induction from bulb-scale explants of *Charybdis numidica* was promoted by NAA, *H. orientalis* "Carnegie" by IBA, and *H. orientalis* "Pink Pearl" by IAA, NAA and IBA (Kongbangkerd et al., 2005; Pierik and Steegmans, 1975; Yi et al., 2002). This indicates a possible role for auxin in bulb induction and growth within this family.

Low (0.1 mg/L) and moderate concentrations (1.0 mg/L) of GA_3 significantly increased bulb induction compared to control cultures, but had no significant effect on other characteristics (Table 3). At 5.0 mg/L, bulb induction in GA_3-supplemeted cultures was similar to the control, but bulb mass of primary and secondary bulbs was significantly increased, while bulb diameter remained unchanged from control cultures. While GA_3 promoted storage organ formation in some Iridaceae (Ascough et al., 2009), it inhibited bulb formation in *H. orientalis* (Pierik and Steegmans, 1975).

Table 3
Hormonal control of bulb induction and growth from quarter-bulb explants of *Albuca bracteata* after three months. Values are means ± se; values followed by the same letter within a column are not significantly different (Fishers' LSD, $\alpha \geq 0.05$). Due to contamination, values in parentheses represent the mean of one set of experiments, and were not considered during statistical analysis.

Treatment (mg/L)	Induction (%)	Bulbs per explant	Primary bulb diameter (mm)	Primary bulb mass (mg)	Secondary bulb diameter (mm)	Secondary bulb mass (mg)
Control	93.3±11.5 b	1.6±0.5 ab	5.57±0.29 abc	115.21±12.6c	4.21±0.48 bc	57.0±6.6 cde
BA 0.1	91.7±14.4 b	1.5±0.4 ab	4.64±0.22 efg	64.9±7.5 de	3.74±0.11 c	40.5±0 ef
1.0	62.2±3.8 d	1.1 ± 0.2 b	3.93±0.32 gh	54.1±9.3 e	(3.33)	(35)
5.0	0 e	0 c				
*m*T 0.1	80±0 c	1.2±0.1 b	3.77±0.44 h	47.0±15.7 e	3.58±0.36 c	26.0±4.1 f
1.0	0 e	0 c				
5.0	0 e	0 c				
NAA 0.1	(0)	(0)				
1.0	100±0 a	2.0±0.2 a	5.57±0.09 abc	124.1±11.3 bc	4.38±0.08 bc	71.2±1.2 bc
5.0	80±20 c	1.3±0.3 b	5.4.0±0.49 abcde	126.8±18.2 bc	(3.31)	(33.5)
IAA 0.1	100±0 a	1.2±0 b	6.27±0.34 a	186.7±25.4 a	5.51±0.09 a	113.0±9.8 a
1.0	100±0 a	1.8±0 ab	6.28±0.36 a	161.9±15.7 ab	4.18±0.13 bc	63.3±4.4 bcd
5.0	100±0 a	1.4±0.6 ab	5.85±0.20 ab	163.7±23.7 ab	3.90±0.60 bc	41.0±7.2 def
GA_3 0.1	100±0 a	1.3±0.1 ab	5.55±0.53 abcd	130.3±38.1 bc	3.33±0.83 c	28.0±16.2 f
1.0	100±0 a	1.2±0.3 b	5.39±0.20 abcde	118.8±15.3 c	3.73±0.81 c	43.3±20.9 def
5.0	91.7±14.4 b	1.2±0.1 b	6.18±0.12 a	178.9±7.2 a	4.95±0.07 ab	83±5.1 b
MeJa 0.1	(100)	(1.6)	4.98±0.95 bcdefg	83.2±33.6 cde	4.5±0.40 abc	70±11.1 bcd
1.0	100±0 a	1.1±0.1 b	4.73±0.51 cdefg	83.8±21.6 cde	(3.20)	(28)
5.0	(100)	(1)	5.36±0.40 abcdef	99±14 cde		
ABA 0.1	100±0 a	1.3±0.1 ab	5.29±0.69 defg	103.2±31.5 cde	4.20±0.57 bc	59.5±16.7 cde
1.0	100±0 a	1.3±0.3 ab	4.70±0.08 defg	71.3±5.7 de	4.22±0.10 bc	54.9±1.6 cde
5.0	100±0 a	1.5±0.1 ab	4.39±0.07 fgh	55.0±5.3 e	3.73±0.33 c	37.1±10.5 ef

Bulb induction in cultures treated with methyl jasmonate or ABA was marginally increased compared to control cultures, but all other parameters were similar to those observed in control cultures (Table 3), suggesting that these hormones are not involved in the regulation of bulb induction and growth.

3.2. *Albuca nelsonii*

The effect of explant and hormone type on shoot induction during culture initiation of *A. nelsonii* was tested by placing several different explants on media with various combinations of hormones (Table 4). After eight weeks, leaf explants had died on media without hormones and about half had remained green (but unresponsive) on media with *m*TR (Table 4). Approximately 50% of explants on NAA-containing media produced a small amount of translucent crystalline callus along the cut edges. No response was observed when this callus was subcultured onto media with both *m*TR and NAA, and within four weeks had turned brown and died (data not shown). Peduncle explants did not respond to control or cytokinin-containing media. Approximately 80% of explants produced roots in response to NAA, and when both NAA and *m*TR were present, shoots were produced (Table 4 and Fig. 1F). Pedicel explants responded in a similar fashion. Whole flowers simply senesced and died after eight weeks, although the ovaries of a few explants on media with both NAA and *m*TR remained green. A small number of tepals were capable of producing shoots on media with both NAA and *m*TR (Fig. 1E). In contrast, excised anthers and ovaries did not respond to any treatment and simply senesced and died after eight weeks (Table 4).

Table 4
In vitro response of various *Albuca nelsonii* explants to media containing no hormones, a cytokinin (0.5 mg/L *m*TR), an auxin (0.5 mg/L NAA) or both (*m*TR and NAA at 0.5 mg/L). C — crystalline callus.

Explant	Control	0.5 mg L^{-1} *m*TR	0.5 mg L^{-1} NAA	*m*TR + NAA
Leaf	Dead	50% survival	50% survival, C	70% survival, C
Peduncle	Dead	Dead	80% roots	80% shoots
Pedicel	Dead	Dead	60% roots	10% shoots
Whole flower	Dead	Dead	Dead	Ovaries green
Sepal	Dead	Dead	Dead	8% shoots
Anther	Dead	Dead	Dead	Dead
Ovary	Dead	Dead	Dead	Dead

It appears critical therefore, that both NAA and *m*TR be present in the medium for successful (i.e. shoot induction) culture initiation. Peduncle explants produced more shoots than other explants, most of which were unimpressively unresponsive. This indicates that cells in these tissues are competent to register hormonal stimuli and sufficiently developmentally plastic to respond by de-differentiation and re-differentiation. Unlike *A. bracteata*, explants contained no meristematic tissue, and this could explain why the response was substantially lower.

4. Conclusions

Temperatures below 35 °C did not affect bulb induction in *A. bracteata*, but bulb diameter and mass were greatest at 20 °C. Sucrose, glucose and fructose at low concentrations (87.5 mM) improved bulb induction compared to explants grown without a carbohydrate, and further increases in carbohydrate concentration did not improve bulb diameter or mass. Bulb induction was

promoted by low and moderate levels of IAA, NAA and GA_3, but inhibited by *m*TR and BA, suggesting that hormonal interactions may be the key regulatory switch for bulb formation in *A. bracteata*. Culture initiation was successful for *A. nelsonii*, with most consistent shoot induction occurring on peduncle explants in media supplemented with *m*TR and NAA. This will enable production of microplants without destructive harvesting of bulbs, and micropropagation of this species could be an effective way to prevent decline in natural populations due to unsustainable harvesting practices.

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