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Micropropagation and bioreactor studies of the medicinally important plant *Lessertia* (*Sutherlandia*) *frutescens* L.

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Abstract

This study describes a protocol for rapid and efficient in vitro propagation of *Lessertia frutescens* (cancer bush), a medicinally important plant species native to southern Africa. Single node explants were grown in various culture regimes of MS medium containing 30 g/l sucrose supplemented with various concentrations of cytokinins and auxins and solidified with 8 g/l agar. These were (a) 2.22, 4.44, 13.32 and 22.19 μM BA; 2.32, 4.65, 13.95 and 23.23 μM K and 0.45, 2.27, 4.54 and 13.62 μM TDZ (b) a combination of 2.22 μM BA with 0.57, 2.85, 5.71 and 11.42 μM IAA, 0.49, 2.46, 4.9 and 9.8 μM IBA or 0.54, 2.69, 5.37 and 10.74 μM NAA and (c) different media types viz. MS, SH basal salt medium and WPM at 1, ½ and ¼ salt strength which were each supplemented with 2.22 μM BA and 0.54 μM NAA. Single node explants were also grown in MS liquid medium supplemented with 2.22 μM BA and 0.54 μM NAA in temporary and continuous immersion bioreactors. Maximum number of shoots (12.9) per single node explant was obtained in the temporary immersion bioreactor but 50% of these shoots showed symptoms of hyperhydricity. In solid culture the best shoot multiplication response (10 shoots) was obtained in full strength MS. Roots were induced using shoot tips cultured in ½ MS solid medium supplemented with various concentrations of IBA or NAA. The highest rooting percentage (78%) was achieved in 19.6 μM IBA. Rooted plantlets were cultured in a mixture of perlite and vermiculite (1:1; v/v) and successfully acclimatized in a growth chamber with an 85% survival rate.

Keywords: Auxin; Bioreactor; Cancer bush; Cytokinin; Rooting; Shoot multiplication

1. Introduction

The vast majority of Africans utilize traditional medicines for their primary health care requirements. Their conviction lies in the indigenous knowledge of traditional healers, *sangomas* (soothsayers) and *inyangas* (herbalists) (vernacular names used by the Nguni people of South Africa), who derive their medicinal preparations from a rich diversity of flora which provide secondary metabolites shown to possess antimicrobial and antiviral properties (Cowan, 1999). One such medicinally reputed plant species, *Lessertia frutescens* L. (syn. *Sutherlandia frutescens* L.; Fabacaeae), is commonly known as cancer bush (or *unwele*—Zulu, *insiswa*—Koi San, *mukakana*—Tswana or *kankerbos*—Afrikaans). The plant is a perennial woody shrub indigenous to hot dry habitats in southern Africa. Striking scarlet flowers have made it a popular ornamental especially grown *en masse*. It is reported that British botanists first encountered this species when it was used by Zulu traditional healers against the influenza pandemic which claimed nearly 20 million lives in the early 1900s (Haffajee, 2002). Traditionally, the aerial parts are boiled in water to yield a bitter tea used in folk remedies (Van Wyk et al., 1997). Extracts are regarded as multipurpose, adaptogenic, efficacious stimulants when consumed under the supervision of a doctor or healthcare professional in addition to an optimal diet. Its safety originates from extensive use in folklore remedies (including the treatment of gynaecological, gastrointestinal, urogenital and

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musculoskeletal system disorders) with no adverse results recorded (Xaba and Notten, 2003; Scott and Springfield, 2004).

Scientifically validated reports show that extracts of *L. frutescens* play a significant role in the management of HIV/AIDS (Hartnett et al., 2005), cancer (Tai et al., 2004; Chinkwo, 2005; Steenkamp and Gouws, 2006; Stander et al., 2007) and diabetes (Ojewole, 2004; Chadwick et al., 2006). The plant's active ingredients (L-canavanine, D-pinitol, γ-amino-butyric acid and a novel triterpenoid glucoside known as "SU1") are reported to possess anti-inflammatory (Na et al., 2004; Ojewole, 2004; Kundu et al., 2005), antioxidant (Fernandes et al., 2004; Katerere and Eloff, 2005) and anticonvulsant properties (Ojewole, 2007).

Natural plant resources are usually inadequate or difficult to obtain, hence, alternate methods of desired resources are deemed necessary. In the case of *L. frutescens* an estimated 10 to 20 t of dried leaves per month are considered necessary for the production of herbal dietary supplements sufficient for one million people in South Africa alone (Haffajee, 2002). This enormous demand on plant resources to manufacture low-cost phytopharmaceutical drugs could possibly result in the attrition of natural populations to the point of extinction as well as a decrease in the genetic diversity of this species. Consequentially, the potential economy and efficiency of tissue culture production is important. Plant tissue culture is a valuable tool to produce medically useful metabolites by turning plant tissues into factories for sought-after phytocompounds speedily, economically and consistently. However, this technique has not been studied in *L. frutescens*. Furthermore, the conventional propagation method through seeds is unsatisfactory resulting in poor germination not exceeding 10% (Shaik et al., 2008). In addition, herbal preparations from local South African herbal shops demonstrated the presence of bacterial and fungal contaminants (Govender et al., 2006; Street et al., 2008). Our objective therefore was to develop an efficient in vitro protocol for the micropropagation of *L. frutescens* using nodal explants followed by successful acclimatization.

2. Materials and methods

2.1. Plant material

Potted plants of *L. frutescens* were purchased from Silverglen Nature Reserve, Durban in July 2006 and were grown in the greenhouse at the University of KwaZulu-Natal, Durban, South Africa. The plants were manually watered every week and maintained in the greenhouse at 25 °C.

2.2. Establishment of aseptic culture

In December 2006, nodal explants (segments containing 3–4 buds) were decontaminated by washing several times with sterile distilled water followed by a wash with 70% (v/v) ethanol for 30 s, then hand agitated for 5 min in a solution of 1.75% (v/v) sodium hypochlorite containing 1 drop of Tween 20. Final rinsing consisted of three rinses of 5 min each in sterile distilled water, with hand agitation. Thereafter, single node explants (1.5–2 cm) were cultured in 300 ml Magenta jars (Sigma) containing 50 ml MS (Murashige and Skoog, 1962) basal salt medium supplemented with 30 g/l sucrose and solidified with 8 g/l agar (Sigma-Aldrich). A large number of in vitro plantlets were generated through the regular sub-culture (every 4 weeks) of nodal explants and shoot tips onto fresh MS solid medium. The pH of all media was adjusted to 5.8 with NaOH before autoclaving at 121 °C and 1.2 kg cm^{-2} for 20 min. Cultures were incubated under diffuse white light at 55 μmol m^{-2}/s, with an 18 h photoperiod provided by cool white fluorescent tubes (Phillips, Holland) at 25 °C in a growth room. These cultures were used as plant material for shoot multiplication and rooting experiments.

2.3. Shoot multiplication in solid culture

Single node explants (4 per culture vessel) of about 2 cm in length were cultured in 300 ml Magenta jars containing 50 ml MS medium supplemented with 30 g/l sucrose and 8 g/l agar containing varying concentrations of different cytokinins and auxins. These were (a) benzyladenine (BA) at 2.22, 4.44, 13.32 and 22.19 μM, kinetin (K) at 2.32, 4.65, 13.95 and 23.23 μM and thidiazuron (TDZ) at 0.45, 2.27, 4.54 and 13.62 μM (b) a combination of 2.22 μM BA with indole-3-acetic acid (IAA) at 0.57, 2.85, 5.71 and 11.42 μM, indole-3-butyric acid (IBA) at 0.49, 2.46, 4.9 and 9.8 μM or 1-naphthaleneacetic acid (NAA) at 0.54, 2.69, 5.37 and 10.74 μM and (c) different media salt strength of MS, SH (Schenk and Hilderbrandt, 1972) and WPM (Lloyd and McCown, 1980) at 1, ½ and ¼ strength which were each supplemented with 2.22 μM BA and 0.54 μM NAA. MS solid medium without plant growth regulators was used as a control. The pH of all media was adjusted to 5.8 with NaOH before autoclaving at 121 °C and 1.2 kg cm^{-2} for 20 min. All cultures were maintained under diffuse white light of 55 μmol m^{-2}/s, with an 18 h photoperiod at 25 °C for 6 weeks.

2.4. Shoot multiplication in bioreactor cultures

Continuous and temporary immersion bioreactor systems using 5 l glass balloon-type bubble bioreactors (BTBB) were used to further investigate shoot multiplication of *L. frutescens*. Bioreactor systems were autoclaved at 121 °C and 1.2 kg cm^{-2} for 50 min. Forty single node explants of about 2 cm in length were transferred into each bioreactor containing a working volume of 1500 ml MS liquid medium supplemented with 30 g/l sucrose, 2.22 μM BA and 0.54 μM NAA. The pH of the medium was adjusted to 5.8 with NaOH before autoclaving at 121 °C and 1.2 kg cm^{-2} for 20 min. Ultra-filtered air was supplied at 1.0 vvm to the bioreactors and the immersion cycle was set at 30 min every 4 h. Bioreactor cultures were maintained under diffuse white light of 55 μmol m^{-2}/s, with an 18 h photoperiod at 25 °C for the culture period of 6 weeks.

2.5. In vitro rooting

Shoot tips of about 3–5 cm in length, following 6 previous sub-culture cycles, were cultured in 300 ml Magenta jars

containing 50 ml MS medium at half salt strength supplemented with 30 g/l sucrose, 8 g/l agar and varying concentrations of IBA and NAA at 4.9, 9.8, 19.6, 29.4 μM and 5.37, 10.74, 21.48, 32.22 μM respectively. Basal and half salt strength media without plant growth regulators were used as a control. All cultures were maintained for 4 weeks under diffuse white light of 55 μmol m^{-2}/s with an 18 h photoperiod at 25 °C.

2.6. Acclimatization

Following 4 weeks of growth post rooting, in vitro plantlets measuring 3–4 cm in height and consisting of about 6–8 roots and 4–5 leaves were washed free of agar before their transfer to polystyrene cups filled with an autoclaved and cooled mixture of perlite and vermiculite (1:1; v/v). All pots were maintained under controlled growth chamber conditions at 25±2 °C, 40–50% relative humidity and 100 μmol m^{-2}/s photosynthetic photon flux with a 16 h photoperiod using halide lamps. The plants were watered weekly with a nutrient solution of half strength of MS basal salts. The polystyrene cups were covered for 10 days with transparent plastic bags to maintain high humidity. These bags were gradually perforated after 10 days and completely removed after 4 weeks. Uncovered plants were housed in the growth room thereafter.

2.7. Experimental design

Experiments were set up in a completely randomized design. Each treatment had three replications and there were 4 explants per replicate. Several growth parameters were assessed including shoot length, shoot number, root length, root number, rooting percentage, explant height, number of leaves per explant, fresh weight and dry weight. Dry weight was recorded after drying the material for 48 h at 60 °C. Data were subjected to ANOVA and Duncan's multiple range test using the SAS program (Version 6.12, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Effects of cytokinins and auxins on shoot multiplication

Shoot multiplication of *L. frutescens* (expressed as number of axillary shoots on each single node explant) was most effective at 2.22 μM BA, followed by 0.45 μM TDZ and then 13.95 μM K where the number of shoots produced was 8.8, 7.4 and 7.1 respectively, compared to the control at 5.8 (Table 1). The data also revealed that shoot multiplication was significantly influenced by cytokinin concentration. BA was shown to be effective in promoting shoot multiplication in other woody species such as *Feronia limonia* (Hiregoudar et al., 2003) and in other plants (Lu, 2005). The results also showed that shoot length responses were significantly influenced by the type of cytokinin used. In the K-treated explants, shoot length decreased with increasing K-concentrations. The mechanism of action of different cytokinins varies as a result of their differential uptake rate in different genomes, varied transportation rates to growing regions and degradation of the cytokinins through metabolic processes (Blakesey, 1991).

Table 1
Effect of cytokinins on shoot multiplication and growth of *L. frutescens* after 6 weeks of culture.

Cytokinin (μM)	No. of shoots per explant	Shoot length (mm)	Fresh weight per explant (mg)	Dry weight per explant (mg)
MS without PGR	5.8 bc [b]	42.1 a	89.4 abcd	13.3 a
Kin 2.32	3.3 de	24.4 b	61.4 bcd	8.1 bc
4.65	4.2 cde	21.9 bc	86.9 abcd	9.8 b
13.95	7.1 ab	20.7 bcd	110.2 a	10.6 ab
23.23	5.9 bc	16.2 cde	78.0 abcd	8.2 bc
BA 2.22	8.8 a	15.8 cde	90.9 abc	7.7 bcd
4.44	5.1 cde	12.6 de	52.4 bcd	4.5 cde
13.32	4.3 cde	11.8 e	57.4 bcd	4.6 cde
22.19	2.9 e	10.8 e	41.8 d	4.2 de
TDZ 0.45	7.4 ab	13.6 de	95.3 ab	8.4 b
2.27	5.2 bcde	15.2 cde	63.4 abcd	4.7 cde
4.54	3.2 e	15.3 cde	46.3 cd	3.8 e
13.62	3.3 e	12.3 e	53.5 bcd	3.7 e
Significance [a]				
Cytokinin type (CT)	***	***	NS	***
Cytokinin conc. (CC)	NS	NS	NS	NS
CT × CC	***	NS	*	*

[a] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.
[b] Mean separation within columns by Duncan's multiple range test at 5% level.

Further evaluation of shoot multiplication in *L. frutescens* revealed that the best cytokinin and auxin combination for enhancing shoot multiplication was 2.22 μM BA and 0.54 μM

Table 2
Effect of BA (2.22 μm), IAA, IBA, and NAA on shoot multiplication and growth of *L. frutescens* after 6 weeks of culture.

Auxins [a] (μM)	No. of shoots per explant	Shoot length (mm)	Fresh weight per explant (mg)	Dry weight per explant (mg)
Control [b] (BA 2.22)	7.8 bc [d]	24.7 a	104.2 c	10.3 c
IAA 0.57	5.5 bcd	17.9 b	447.1 bc	39.8 bc
2.85	5.3 de	19.4 b	1041.7 a	86.5 a
5.71	4.6 de	19.6 b	1095.3 a	83.4a
11.42	4.4 e	22.1 ab	1183.0 a	97.7 a
IBA 0.49	7.4 bc	19.8 b	679.2 ab	59.9 ab
2.46	7.6 bc	21 ab	767.0 ab	68.6 ab
4.90	7.7 bc	19.2 b	737.3 ab	65.6 ab
9.80	8.3 ab	21.3 ab	905.6 ab	77.0 ab
NAA 0.54	10.0 a	19.6 b	655.6 ab	58.6 ab
2.69	7.4 bc	20.2 ab	689.6 ab	61.8 ab
5.37	7.4 bc	20.7 ab	1000.4 ab	79.5 ab
10.74	6.1 cde	20.3 ab	993.3 ab	81.9 a
Significance [c]				
Auxin type (AT)	***	NS	NS	NS
Auxin conc. (AC)	NS	NS	NS	NS
AT × AC	**	NS	*	*

[a] All auxin treatments supplemented with 2.22 μM BA.
[b] Medium supplemented with 2.22 μM BA alone.
[c] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.
[d] Mean separation within columns by Duncan's multiple range test at 5% level.

NAA (Table 2; Fig. 1a). Using this combination, the number of shoots increased from 8.8 (with 2.22 μM BA only) to 10. Shoot induction using BA in the presence of NAA or other auxins has also been recorded for other legumes (Shekhawat et al., 1993; Al-Wasel, 2000). The results also showed that the shoot number in all other treatments was lower than that of the control. In all combinations of IBA used, the shoot number increased with a corresponding increase in auxin concentration. However, the

Fig. 1. In vitro shoot multiplication, rooting and acclimatization of *Lessertia frutescens*: (a) shoot multiplication on MS supplemented with BA (2.22 μM) and NAA (0.54 μM) after 6 weeks in culture; (b) shoot multiplication in different media after 6 weeks in culture; (c) shoot multiplication in temporary immersion bioreactor showing symptoms of hyperhydricity after 6 weeks in culture; (d) in vitro rooting after 4 weeks in culture; (e) plantlets at day zero of acclimatization; (f) acclimatized plant.

Table 3
Effect of media type and salt strength on shoot multiplication and growth of *L. frutescens* after 6 weeks of culture.

Media type	Salt strength[a]	No. of shoots per explant	Shoot length (mm)	Fresh weight per explant (mg)	Dry weight per explant (mg)
MS	¼	3.3 cd[c]	8.7 c	310.9 bcd	49.4 bc
	½	4.3 bc	9.8 bc	330.8 bcd	50.4 bc
	Full	10.4 a	18.3 a	845.1 a	78.6 a
SH	¼	0.0 f	0.0 d	185.0 cde	29.5 cd
	½	0.8 ef	1.4 d	406.0 bc	58.5 ab
	Full	2.0 de	7.4 c	503.5 b	63.1 ab
WPM	¼	0.6 ef	2.3 d	68.5 e	13.9 d
	½	2.9 cd	6.1 c	155.7 de	25.1 cd
	Full	5.8 b	12.7 b	259.2 cde	40.6 bc
Significance[b]					
Media type (mt)		***	***	***	***
Salt strength (ss)		***	***	***	***
mt × ss		NS	NS	*	NS

[a] All salt strengths supplemented with BA 2.22 μM and NAA 0.54 μM.
[b] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.
[c] Mean separation within columns by Duncan's multiple range test at 5% level.

opposite was true for all IAA and NAA concentrations. In general, the shoot length increased as auxin concentration in the medium increased as was reflected in the fresh and dry weights. High concentration of cytokinins (>4.44 μM) and/or auxins (>4.9 μM) resulted in callus formation at the shoot base (data not shown).

3.2. Effect of media salt strength on shoot multiplication

Since the best shoot multiplication response (10 shoots) was obtained in the 2.22 μM BA and 0.54 μM NAA combination, this treatment was used in combination with different media salt strengths of WPM, MS and SH at ¼, ½ and full strength to further assess shoot multiplication in *L. frutescens* (Table 3; Fig. 1b). By comparing the three media, it was evident that full strength MS was superior to SH or WPM media with regards to shoot number (10.4 shoots per explant). Poor shoot multiplication (0.6–5.8) was obtained in all WPM strengths tested, while very few (0.8–2.0) or no shoots were produced in the SH media (Fig. 1b). The results revealed similar trends for shoot length, fresh and dry weights. The nitrogen content in MS is higher than that in WPM or SH, and it is therefore likely that this important macronutrient was probably required in larger quantities for optimal shoot growth and multiplication of *L. frutescens*.

Table 4
Effect of culture type on shoot multiplication and growth of *L. frutescens* after 6 weeks of culture.

Culture type[a]	No. of shoots per explant	Shoot length (mm)	Fresh weight per explant (mg)	Dry weight per explant (mg)	Hyperhydricity
Solid culture	7.8 b[b]	24.7 b	104.2 c	10.3 c	0 c
Bioreactor culture					
Temporary immersion	12.9 a	36.2 a	3109.0 a	351.8 a	50 b
Continuous immersion	9.0 b	20.0 b	1648.8 b	146.2 b	80 a

[a] All cultures supplemented with BA 2.22 μM and NAA 0.54 μM.
[b] Mean separation within columns by Duncan's multiple range test at 5% level.

3.3. Shoot multiplication in bioreactors

The bioreactor studies revealed that shoot multiplication was highest (12.9 shoots) in temporary immersion (Table 4; Fig. 1c). Temporary immersion yielded the best growth in terms of shoot number, shoot length and fresh weight when compared continuous immersion and/or solid culture. A probable reason for this may lie in the occasional contact between the explants and the liquid medium (30 min every 4 h) which periodically supplies the necessary nutrients and oxygen crucial for growth. The temporary immersion system has been successfully applied for propagation of many species of medicinal plants such as *Artemisia judaica* and *Hydrastis canadensis* (Liu et al., 2004; He et al., 2007). However, in the present study a large number of micropropagated *L. frutescens* shoots from the temporary immersion bioreactor (50%) and from the continuous immersion bioreactor (80%) showed thick broad leaves that were wrinkled and/or curled and brittle, symptoms of hyperhydricity. The environment inside bioreactors normally used in plant micropropagation is characterised by high relative humidity, poor gaseous exchange between the internal atmosphere of the bioreactor and its surrounding environment, and the accumulation of ethylene, conditions that may induce physiological disorders (Dewir et al., 2006). Considering the natural habitat of *L. frutescens*, which is a typical plant of arid environments, it is clear that these conditions hinder large-scale micropropagation in bioreactors.

3.4. In vitro rooting and acclimatization

There was considerable rooting activity in *L. frutescens* which differed significantly amongst the auxin type and concentration (Table 5; Fig. 1d). Clearly, the best rooting medium was ½ MS supplemented with 19.6 μM IBA which yielded 78% rooted shoots with an average of 8.1 roots per shoot and an average root length of 11 mm. Rooting percentage was reduced with increasing IBA concentration. However, IBA proved more efficient than NAA at all concentrations tested. Inducing of roots has previously been a limiting factor in the micropropagation of woody species (Nemeth, 1986). IBA has successfully been used in root induction for other species (Al-Wasel, 2000; Mereti et al., 2002; He et al., 2007). From the above results, it is clear that an exogenous supplement of auxins is necessary to improve rooting in *L. frutescens* since endogenous auxin levels are not adequate. Primary roots, which were thin but mostly branched and white in colour, became visible after 10 days of culture. Root nodules, which are characteristic of leguminous plants, were observed at root tips

Table 5
Effect of IBA, NAA on rooting and growth of *L. frutescens* after 4 weeks of culture.

Auxin (μM)	Rooting %	No. of roots per plantlet	Root length (mm)	Plant height (mm)	Number of leaves per plantlet	Fresh weight per plantlet (mg)	Dry weight per plantlet (mg)
½ MS without auxins	33 d [b]	5.7 e	15.3 a	37.9 ab	4.0 ab	155.5 e	25.1 d
IBA 4.90	22 e	7.0 d	8.0 h	29.4 b	4.5 ab	187.1 de	26.6 d
9.80	56 b	7.8 c	10.4 d	30.4 b	4.6 ab	238.5 cde	29.9 d
19.60	78 a	8.1 b	11.0 c	44.8 a	5.9 a	304.7 cd	39.8 cd
29.40	44 c	9.0 a	12.5 b	36.4 ab	4.0 ab	365.7bc	48.4 bc
NAA 5.37	44 c	4.5 g	9.5 f	29.9 b	4.8 ab	454.1 b	59.5 ab
10.74	56 b	4.6 f	8.4 g	30.0 b	4.5 ab	466.0 b	53.5 abc
21.48	33 d	7.0 d	10.0 e	30.6 b	3.5 b	636.3 a	67.1 a
32.22	0 f	0.0 h	0.0 i	40.1 a	1.8 c	641.6 a	57.9 ab
Significance [a]							
Auxin type (AT)	***	**	**	***	***	***	***
Auxin conc. (AC)	***	**	*	NS	*	NS	NS
AT × AC	***	NS	NS	*	NS	NS	NS

[a] NS, *, **, *** not significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively.
[b] Mean separation within columns by Duncan's multiple range test at 5% level.

of explants treated with 4.9 or 9.8 μM IBA and at most concentrations of NAA. In general, all other growth parameters i.e. root length, explant height and number of leaves per explant, increased with increasing concentration of auxins. However, NAA treatments resulted in callus formation at the base of explants.

In vitro rooted plantlets were planted for 4 weeks in a mixture of perlite and vermiculite (1:1; v/v) (Fig. 1 e,f). The plants were successfully acclimatized in the growth room with a survival rate of 85% and were morphologically similar to the mother plant.

4. Conclusion

The present study provides a simple in vitro protocol for micropropagation of *L. frutescens* using nodal explants. The most effective cytokinin for shoot multiplication was 2.22 μM BA. The best cytokinin and auxin combination for enhancing shoot multiplication was 2.22 μM BA and 0.54 μM NAA. Full strength MS was the most effective medium salt strength to increase the shoot number. In the liquid media tested using bioreactor systems, temporary immersion yielded the highest number of shoots, however, 50% of these shoots were hyperhydric. Further investigations into this culture system are required to optimize the environmental conditions for development of elite plant tissues of *L. frutescens*. The best rooting medium was ½ MS supplemented with 19.6 μM IBA. Acclimatization of the plants was successful with a survival rate of 85%. Chemical analysis of in vitro propagated tissues of this species is currently being undertaken to validate and quantify the phytochemicals that are known to occur in naturally growing plants. Characterization and quantification will be carried out using RP-HPLC, LC-MS, GC-MS and/or NMR.

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References

Al-Wasel, A.S., 2000. Micropropagation of *Acacia seyal* Del. in vitro. Journal of Arid Environments 46, 425–431.
Blakesey, D., 1991. Uptake and metabolism of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. Plant Cell, Tissue and Organ Culture 66, 25–29.
Chadwick, W.A., Roux, S., Van de Venter, M., Louw, J., Oelofsen, W., 2006. Antidiabetic effects of *Sutherlandia frutescens* in Wistar rats fed a diabetogenic diet. Journal of Ethnopharmacology 109, 121–127.
Chinkwo, K.A., 2005. *Sutherlandia frutescens* extracts can induce apoptosis in cultured carcinoma cells. Journal of Ethnopharmacology 98, 163–170.
Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564–582.
Dewir, Y.H., Chakrabarty, D., Ali, M.B., Hahn, E.J., Paek, K.Y., 2006. Lipid peroxidation and antioxidant enzyme activities of *Euphorbia millii* hyperhydric shoots. Environmental and Experimental Botany 58, 93–99.
Fernandes, A.C., Cromarty, A.D., Albrecht, C., Jansen van Rensburg, C.E., 2004. The antioxidant potential of *Sutherlandia frutescens*. Journal of Ethnopharmacology 95, 1–5.
Govender, S., Du Plessis-Stoman, D., Downing, T.G., Van de Venter, M., 2006. Traditional herbal medicines: microbial contamination, consumer safety and the need for standards. South African of Journal Science 102, 253–255.
Haffajee, F., 2002. Home-grown healing. New Internationalist 349, 20–21.
Hartnett, S.M., Oosthuizen, V., Van de Venter, M., 2005. Anti-HIV activities of organic and aqueous extracts of *Sutherlandia frutescens* and *Lobostemon trigonus*. Journal of Ethnopharmacology 96, 113–119.
He, S.S., Liu, C.Z., Saxena, P.K., 2007. Plant regeneration of an endangered medicinal plant *Hydrastis canadensis* L. Scientia Horticulturae Amsterdam 113, 82–86.
Hiregoudar, L.V., Murthy, H.N., Hema, B.P., Hahn, E.J., Paek, K.Y., 2003. Multiple shoot induction and plant regeneration of *Feronia limonia* (L.) Swingle. Scientia Horticulturae Amsterdam 98, 357–364.
Katerere, D.R., Eloff, J.N., 2005. Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed anti-HIV/AIDS phytomedicine. Phytotherapy Research 19, 779–781.
Kundu, J.K., Mosanda, K.S., Na, H., Surh, Y.J., 2005. Inhibitory effects of the extracts of *Sutherlandia frutescens* (L.) R. Br. and *Harpagophytum procumbens* DC. on phorbol ester-induced COX-2 expression in mouse skin: AP-1 and CREB as potential upstream targets. Cancer Letters 218, 21–31.
Liu, C.Z., Murch, S.J., El-Demerdash, M., Saxena, P.K., 2004. *Artemisia judaica* L.: micropropagation and antioxidant activity. Journal of Biotechnology 110, 63–71.
Lloyd, G.B., McCown, B.H., 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proceedings, International Plant Propagator's Society 30, 421–427.
Lu, M.C., 2005. Micropropagation of *Vitis thunbergii* Sieb. et Zucc., a medicinal herb, through high-frequency shoot tip culture. Scientia Horticulturae Amsterdam 107, 64–69.
Mereti, M., Grigoriadou, K., Nanos, G.D., 2002. Micropropagation of the strawberry tree, *Arbutus unedo* L. Scientia Horticulturae Amsterdam 93, 143–148.
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiologia Plantarum 15, 473–497.

Na, H.K., Mossanda, K.S., Lee, J.Y., Surh, Y.J., 2004. Inhibition of phorbol ester-induced COX-2 expression by some edible African plants. Biofactors 21, 149–153.

Nemeth, G., 1986. Induction of rooting. In: Bajaj, Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry-Trees, vol. 1. Springer-Verlag, New York, pp. 49–64.

Ojewole, J.A., 2004. Analgesic, anti-inflammatory and hypoglycemic effects of *Sutherlandia frutescens* R. Br. (variety *Incana* E. MEY.) [Fabaceae] shoot aqueous extract. Methods and Findings in Experimental and Clinical Pharmacology 26, 409–416.

Ojewole, J.A.O., 2007. Anticonvulsant property of Sutherlandia frutescens R. BR. (variety Incana E. MEY.) [Fabaceae] shoot aqueous extract. Brain Research Bulletin 75, 126–132.

Schenk, R.U., Hilderbrandt, A.C., 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canadian Journal of Botany 50, 199–204.

Scott, G., Springfield, E.P., 2004. Pharmaceutical Monographs for 60 South African Plant Species Used as Traditional Medicines. South African National Biodiversity Institute (SANBI). (online) Available http://www.plantzafrica.com/medmonographs (Accessed 10 March 2006.

Shaik, S., Dewir, Y.H., Singh, N., Nicholas, A., 2008. Influences of pre-sowing seed treatments on germination of the cancer bush (*Sutherlandia frutescens*), a reputed medicinal plant in arid environments. Seed Science and Technology 36, 795–801.

Shekhawat, N.S., Rathore, T.S., Singh, R.P., Deora, N.S., Rao, S.R., 1993. Factors affecting in vitro clonal propagation of *Prosopis cineraria*. Plant Growth Regulators 12, 273–280.

Stander, B.A., Marais, S., Steynberg, T.J., Theron, D., Joubert, F., Albrecht, C., Joubert, A.M., 2007. Influence of *Sutherlandia frutescens* extracts on cell numbers, morphology and gene expression in MCF-7 cells. Journal of Ethnopharmacology 112, 312–318.

Steenkamp, V., Gouws, M.C., 2006. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer. South African Journal of Botany 72, 630–633.

Street, R.A., Stirk, W.A., Van Staden, J., 2008. South African traditional medicinal plant trade—challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology 119, 705–710.

Tai, J., Cheung, S., Chan, E., Hasman, D., 2004. In vitro culture studies of *Sutherlandia frutescens* on human tumor cell lines. Journal of Ethnopharmacology 93, 9–19.

Van Wyk, B.B., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, pp. 246–247.

Xaba, P.M., Notten, A., 2003. *Sutherlandia frutescens*. South African National Biodiversity Institute (SANBI). (online) Available http://www.plantzafrica.com/plantqrs/sutherfrut.htm (Accessed 12 February 2007.

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