

Full Length Research Paper

Effect of temperature, light intensity and growth regulators on propagation of *Ansellia Africana* from cuttings

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Accepted 18 June, 2010

Ansellia africana (Orchidaceae) is an important endangered medicinal plant species of South Africa which has been heavily exploited in recent years. Experiments were conducted in growth rooms at different temperatures (16, 26, 36°C) and in a nursery at different light intensities induced by shade cloth densities (200, 400, 600, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light) at the University of Zululand, South Africa. Mature *A. africana* plants were cut into two lengths, the top leafy shoot and the bottom part with roots (10 – 15 cm in length, 3 - 8 mm diameter). The top leafy or leafless cuttings were used in all the experiments. Bud formation and rooting competence of cuttings were compared by growing cuttings treated with solutions of varying naphthaleneacetic acid (NAA) and kinetin concentrations in river sand. Three types of cuttings were used: a) mature cuttings with both green leaves and an inflorescence, b) mature cuttings with yellow leaves (or leafless) and an inflorescence, and c) young cuttings with green leaves but no inflorescence. The lowest percentage death was recorded in cuttings with both green leaves and an inflorescence in the growth room (10%) and nursery (5%), respectively. The same type of cuttings gave significantly higher ($P < 0.05$) percentage bud formation in the incubator (18%) and nursery (37%), respectively. NAA was effective in root initiation when applied after bud break. The best results for root number, root length and root dry weight were achieved at NAA concentration of 1 or 2 mg l^{-1} . Optimum conditions for bud formation were temperature of 36°C, light intensity of 200 - 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and kinetin concentration of 1 or 2 mg l^{-1} . A maximum value of 57% was recorded for bud formation in this study.

Key words: *Ansellia Africana*, cuttings, kinetin, naphthaleneacetic acid, propagation.

INTRODUCTION

South Africa has the richest temperate flora in the world, many of which are medicinally useful (Van Wyk and Smith, 2001). The increased rate of harvesting and the number of plant gatherers has made exploitation of this biodiversity unsustainable (Afolayan and Adebola, 2004). A study in the Eastern Cape province documented 166 plant taxa in trade, providing 525 tonnes of plant material valued at R27 million annually (Dold and Cocks, 2002).

Of the 60 most frequently traded species, 93% were

being harvested unsustainably and 34 species were prioritized for conservation and management attention.

Survival of many species of Orchidaceae in the wild is currently threatened by over-collection and reduction in their natural habitats, and *Ansellia africana* is classified as endangered. The genus *A. africana* is vulnerable in terms of conservation status and the whole plant is used for medicinal purposes (Ndawonde et al., 2007). *A. africana* is used as love charm, antidote to bad dreams, and to ward off lightning (Pooley, 1998). Traditional healers, in collaboration with conservation organizations, have useful strategies of using medicinal plants sustainably, such as the establishment of medicinal plant gardens. Plant traders, characterized by unsustainable harvesting, have not been exposed to environmental

Abbreviations: **CLI**, Mature cuttings with green leaves and an inflorescence; **CL**, young cuttings with leaves and no inflorescence; **C**, mature leafless cuttings with an inflorescence.

education. Reliable methods of mass propagation of endangered species are highly desirable in order to meet the demand of botanical gardens and plant traders and eventually restore the plants to their natural environment (Giusti et al., 2002).

Plant traders harvest whole plants from the wild for medicinal purposes whereas traditional healers use the top leafy shoots in a mixture with other plants to treat their patients. Traditional healers harvest in a sustainable way of leaving the bottom rooted part in the soil for new shoot formation. Because *A. africana* resembles sugarcane which is propagated from leafy shoots, it was hypothesized that this genus could be propagated from cuttings. There was no evidence from the literature that this species could be propagated from cuttings. The genus *A. africana* is currently propagated from seed and by separation of shoots (Hew, 2001).

The relationship between nitrogen (N), carbohydrates and rooting in Pelargonium cuttings depend on current photosynthesis, which is influenced by ambient light conditions (Druege et al., 2004; Rapaka et al., 2005). Growth of adventitious roots in *Euphorbia pulcherrima* is highly dependent on the available light for current photosynthesis (Lopez and Runkle, 2008). Light requirements for successful propagation in *E. pulcherrima* was 630 - 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the green house (Zerche and Druege, 2009). Exogenous auxins are commonly used to improve rooting efficiency and quality of stem cuttings. NAA (1-naphthaleneacetic acid) stimulates adventitious rooting while kinetin promotes bud formation (Copes and Mandel, 2000; Zobolo et al., 2009).

The aim of the present study is to investigate the factors affecting rooting ability and bud formation of mature stem cuttings of *A. africana* using non-mist propagators. The factors investigated were: 1) temperature, 2) light intensity, 3) growth regulators and their interactions.

MATERIALS AND METHODS

Plant materials

Plants were obtained from plant traders at Nongoma Muthi market. Mature *A. africana* plants were cut into two lengths, top leafy shoot (10 – 15 cm) without roots, bottom (6 – 10 cm) with two nodes and roots. The bottom part of the shoot with roots was germinated in pots for another experiment which was not reported in this study. The top leafy or leafless cuttings were used in all the experiments for the present study. Cuttings were sterilized using 1% sodium hypochlorite for 1 min prior to incubation. The procedure was repeated once a week for four weeks. The basal ends of cuttings were dipped in one of the six concentrations of growth regulators (0.5, 1.0 and 2.0 mg l^{-1} NAA or kinetin) for 10 min, thereafter planted in 20 l plastic pots filled with river sand (particle size <2 mm diameter). The choice of sand as a medium for propagation was based on the recommendation by Atangana et al. (2006) as used in the species *Allanblackia floribunda*. The control plants were dipped in distilled water. Cuttings were inserted in the rooting media using a wooden dipper to save the base of the cutting from injury. The pots were placed in a random design at a spacing of 20 x 20 cm.

Growth parameters investigated were: Shoot death (%), root formation (%), bud formation (%), root dry weight, root length and shoot length. Bud formation was defined as released buds longer than 0.5 cm (Hansen, 1989). Three types of shoots were used, namely mature cuttings with green leaves and an inflorescence (CLI), young cuttings with leaves and no inflorescence (CL), mature leafless cuttings with an inflorescence (C). Each treatment consisted of 20 cuttings and was repeated thrice. Humidity conditions within the propagators were enhanced by spraying the cuttings with water at 07.30 and 17.30 h each day using a hand-held sprayer. The relative humidity ranged from 80 to 90%. Cuttings were assessed weekly for callus development, the presence of roots (≥ 2 mm in length), leaf shedding, shoot formation and cutting mortality. The experiments were terminated between 60 and 90 days after treatment.

Temperature and light intensity

Experiments were conducted in the growth rooms (16, 26 and 36°C) and in the nursery with varying light (200, 400, 600, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$) at the University of Zululand, South Africa. In the growth room, light was provided with white fluorescent lamps at a photosynthetic photon flux of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Air temperature of the nursery ranged from 25 to 38°C depending on the light intensity.

Plant growth regulators

Solutions of NAA and kinetin were prepared from chemicals purchased from Sigma (St. Louis, MO, USA) by dissolving the hormone in a mixture of absolute ethanol and methanol in a 1:1 ratio.

Statistics

Data were analyzed by two ways ANOVA in a randomized complete design. Means were separated according to Duncan's test ($p < 0.05$).

RESULTS

Effect of temperature on percentage death and bud formation of cuttings

The death of the cuttings incubated at a temperature of 36°C was significantly lower ($p < 0.05$) than at 16 and 26°C. The CLI and CL types of cuttings showed a significantly lower ($p < 0.05$) percentage of death than the C type of cuttings at 16, 26 and 36°C, suggesting that the leaves played a role in the survival of these cuttings (Figure 1A). In all the cuttings, the decomposition started from the cut end inside the culture medium and progressed towards the shoot tip.

There were no buds formed at 16°C in all the cuttings during the experimental period. The percentage of bud formation recorded at a temperature of 36°C showed a significantly higher ($p < 0.05$) value in the CLI type of cuttings compared with CL and C types (Figure 1B). The CL type of cuttings also showed a significantly higher percentage of bud formation than the C type of cuttings at 36°C. The percentage of bud formation at 36°C for CLI type of cuttings was also significantly higher than the one

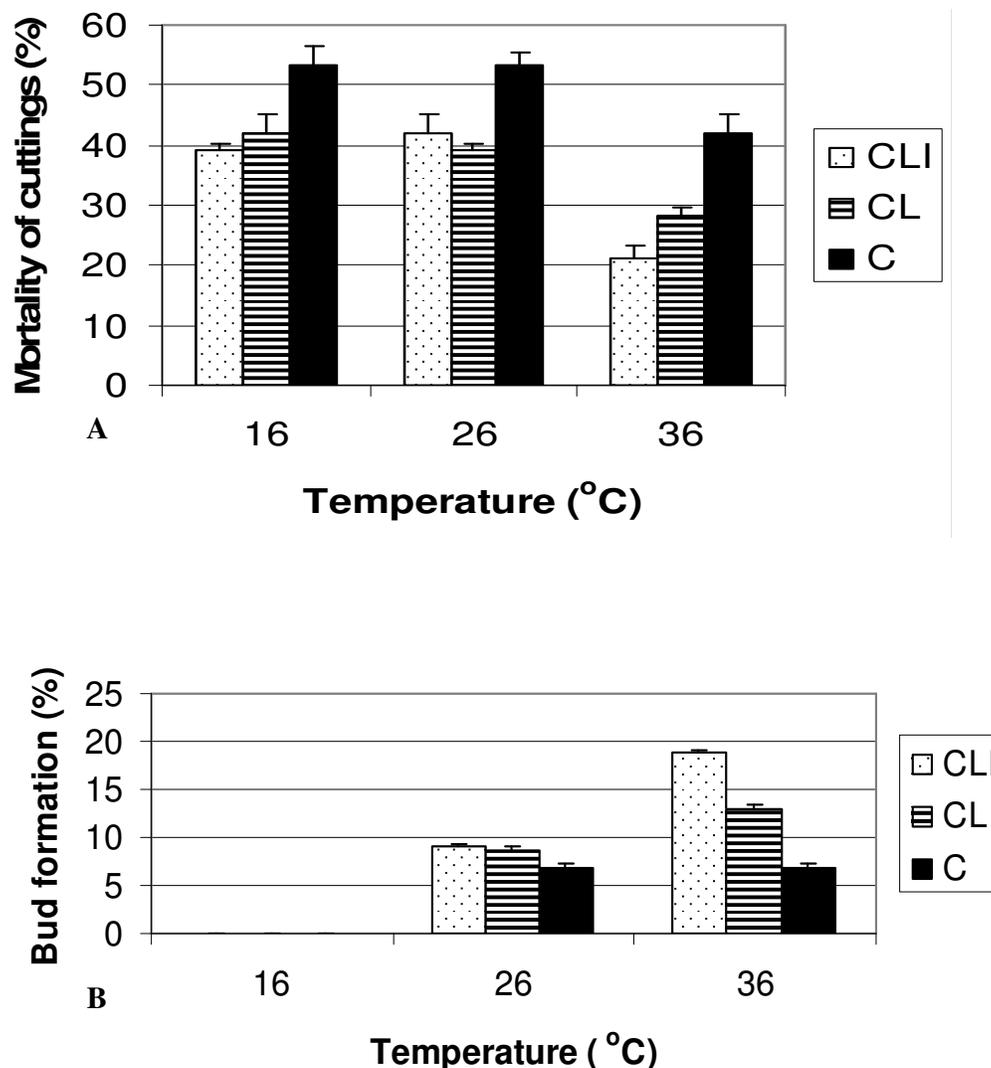


Figure 1. Effect of temperature on mortality of cuttings (A) and on bud formation (B) in three types of *Ansellia africana* cuttings grown in pots in the incubator at 90 days after treatment. Definitions of cuttings are: CLI = mature cuttings with both green leaves and an inflorescence, CL= young cuttings with green leaves and no inflorescence, C = mature leafless cuttings with an inflorescence.

recorded at 26 °C.

Effect of light intensity on percentage death and bud formation of cuttings

The death of CLI, CL and C cuttings grown in the nursery at light intensities of 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were significantly lower ($p < 0.05$) than the ones grown at light intensities of 600 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The death of CLI type of cuttings was significantly lower ($p < 0.05$) than that of CL at light intensities of both 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At a light intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CLI type of cuttings showed the lowest percentage of death, out performing both CL and C types of cuttings (Figure 2A).

The percentage of bud formation from CLI and C type

of cuttings was significantly higher ($p < 0.05$) than that of CL type at all light intensities. These cuttings performed best at a light intensity of 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2B). There were no significant differences between CLI and C type of cuttings at all light intensities.

Effect of NAA on bud formation, root and shoot growth

i. NAA applied before bud formation

Bud formation

An increase in NAA concentration applied to cuttings

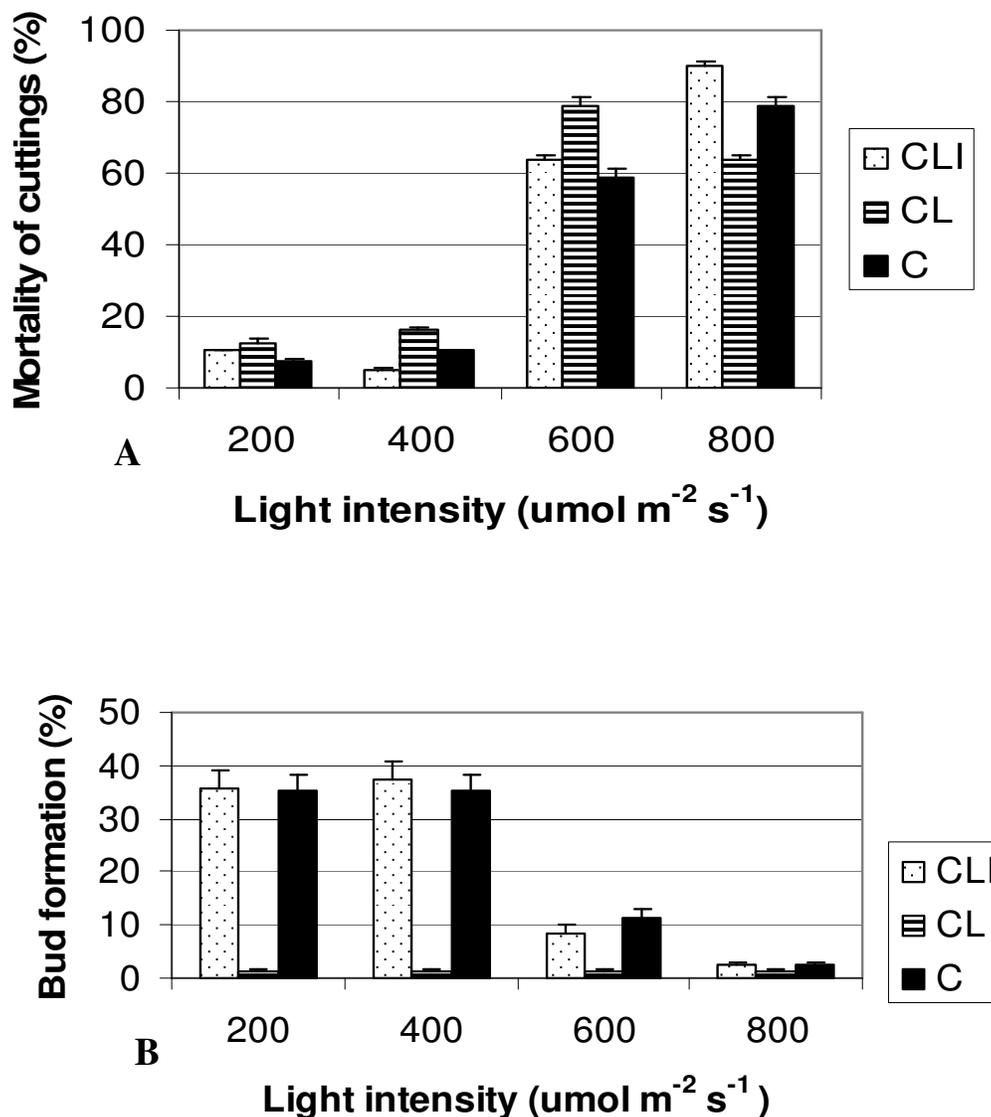


Figure 2. Effect of light intensity on mortality of cuttings (A) and on bud formation (B) of three types of *Ansellia africana* cuttings grown in pots in a nursery at 90 days after treatment. Definitions of cuttings are: CLI = mature cuttings with both green leaves and an inflorescence, CL= young cuttings with green leaves and no inflorescence, C = mature leafless cuttings with an inflorescence.

without buds resulted in a significant decrease in the percentage of bud formation. The control plants produced a significantly higher ($p < 0.05$) percentage of buds than those treated with 1.0 and 2.0 mg l^{-1} NAA under incubator and nursery conditions (Table 1). The percentage bud formation under nursery conditions was significantly higher than the one from growth room at 0 and 0.5 mg l^{-1} NAA.

Number of buds per plant

Under growth room conditions, there were no significant differences in the number of buds per plant at all NAA

concentrations (Table 1). The control plants and those treated with 0.5 mg l^{-1} produced a significantly higher number of buds than the ones treated with either 1.0 or 2.0 mg l^{-1} NAA under nursery conditions. Under nursery conditions, the control plants produced significantly more buds per plant compared to growth room conditions. There were no significant differences between nursery and growth room at 0.5, 1.0 and 2.0 mg l^{-1} NAA.

Shoot length

The control plants and those treated with 0.5 mg l^{-1} NAA produced significantly longer shoots than those treated

Table 1. Effect of NAA concentration applied before (top) and after (bottom) bud formation on growth of *Ansellia africana* cuttings with both green leaves and an inflorescence at 90 and 60 days after treatment respectively grown in a nursery.

Growth parameter	Experimental condition	NAA concentration (mg l ⁻¹) applied before bud formation (90 days after treatment)			
		0	0.5	1.0	2.0
Bud formation (%)	Growth room	19.00 ± 0.71c	15.40 ± 1.33b	6.20 ± 0.66a	7.00 ± 0.71a
	Nursery	35.40 ± 3.01b*	37.60 ± 3.33b*	11.20 ± 1.77a	10.60 ± 1.50a
Number of buds/plant	Growth room	1.20 ± 0.20a	1.20 ± 0.20a	0.80 ± 0.20a	0.60 ± 0.24a
	Nursery	2.40 ± 0.60b*	2.00 ± 0.31b	1.40 ± 0.25a	1.00 ± 0.00a
Shoot length (mm)	Growth room	20.36 ± 1.29c	17.96 ± 1.39c	5.18 ± 0.33b	1.88 ± 0.22a
	Nursery	40.40 ± 2.50c*	37.80 ± 2.99c*	12.80 ± 0.86b*	4.60 ± 0.51a
Root length (mm)	Growth room	36.40 ± 1.86b	34.40 ± 2.29b	10.40 ± 0.40a	6.60 ± 0.81a*
	Nursery	43.60 ± 0.93c*	43.80 ± 1.36c*	20.60 ± 0.93b*	2.40 ± 0.51a
Root dry weight g/plant	Growth room	0.28 ± 0.05d	0.21 ± 0.01c	0.17 ± 0.01b	0.08 ± 0.01a
	Nursery	0.56 ± 0.02d*	0.42 ± 0.01c*	0.33 ± 0.01b*	0.15 ± 0.02a*
Growth parameter	Experimental condition	NAA concentration (mg l ⁻¹) applied after bud formation (60 days after treatment)			
		0	0.5	1.0	2.0
Shoot length (mm)	Growth room	17.96 ± 1.39a	17.52 ± 1.25a	17.96 ± 1.26a	18.56 ± 1.55a
	Nursery	37.80 ± 2.99a*	36.80 ± 1.98a*	35.80 ± 1.83a*	36.80 ± 2.27a*
Root length (mm)	Growth room	10.80 ± 0.86a	11.00 ± 0.84a	30.20 ± 1.69b	32.00 ± 2.00b
	Nursery	20.60 ± 0.93a*	20.00 ± 0.71a*	44.00 ± 1.14b*	43.80 ± 1.36b*
Root dry weight g/plant	Growth room	0.16 ± 0.04a	0.17 ± 0.01a	0.21 ± 0.01b	0.22 ± 0.01b
	Nursery	0.33 ± 0.01a*	0.33 ± 0.01a*	0.42 ± 0.01b*	0.41 ± 0.01b*

Means that are followed by different letters within a row differ significantly ($p \leq 0.05$). Means followed by asterisk (*) within a column differ significantly ($p \leq 0.05$), $n = 20$.

with 1.0 and 2.0 mg l⁻¹ in the growth room and nursery. There was a decline in shoot length as the NAA concentration increased from 0 to 2 mg l⁻¹. The nursery conditions produced significantly longer ($p < 0.05$) shoots than in the growth room at 0, 0.5 and 1.0 mg l⁻¹ (Table 1).

Root length

The control plants and those treated with 0.5 mg l⁻¹ NAA produced significantly longer roots than those treated with 1.0 and 2.0 mg l⁻¹ in the growth room and nursery. The roots produced under nursery conditions were significantly longer than the ones from growth room at 0, 0.5 and 1.0 mg l⁻¹ (Table 1).

Root dry weight

The highest root dry weight was recorded in the control plants from both growth room and nursery. An increase in NAA concentration resulted in a decrease in root dry weight in both growth room and nursery. The root dry weight under nursery conditions was significantly higher than the one from the growth room (Table 1).

ii. NAA applied after bud formation

Shoot length

The longest shoots were recorded at a concentration of 2 mg l⁻¹ NAA in plants grown in both growth room and nursery. The shoots produced under nursery conditions were significantly longer than those from growth room (Table 1).

Root length

The longest roots were recorded at a concentration of 2 mg l⁻¹ NAA in plants grown in both growth room and nursery. The roots produced under nursery conditions were significantly longer than those from growth room (Table 1).

Root dry weight

The highest root dry weight was recorded at 2 mg l⁻¹ NAA in both growth room and nursery. The root dry weight

Table 2. Effect of kinetin concentration on bud formation, shoot and leaf growth of *Ansellia africana* cuttings with both green leaves and an inflorescence at 60 days after treatment grown in a nursery.

Growth parameter	Experimental condition	Kinetin concentration (mg l ⁻¹)			
		0	0.5	1.0	2.0
Bud formation (%)	Growth room	17.60 ± 0.510a	17.6 ± 0.678a	22.20 ± 0.374b	22.8 ± 0.735b
	Nursery	30.60 ± 1.503a*	31.2 ± 1.772a*	57.60 ± 3.326b*	35.4 ± 3.009b*
Number of buds/plant	Growth room	0.60 ± 0.244a	1.2 ± 0.200a	2.00 ± 0.316b	2.4 ± 0.245b
	Nursery	1.20 ± 0.200a	2.2 ± 0.374b*	8.40 ± 0.510c*	9.6 ± 0.510d*
Shoot length (mm)	Growth room	19.80 ± 1.241a	20.0 ± 0.707a	32.80 ± 1.463b	32.8 ± 1.594b
	Nursery	36.40 ± 1.860a*	36.6 ± 1.568a*	68.20 ± 1.114b*	70.0 ± 1.581b*
Leaf length (mm)	Growth room	1.60 ± 0.511a	2.4 ± 1.249ab	4.60 ± 0.927b	4.6 ± 0.678b
	Nursery	3.24 ± 0.847a	4.0 ± 1.981a	8.74 ± 1.746b*	8.4 ± 1.288b*
Leaf width (mm)	Growth room	3.00 ± 0.707ab	2.0 ± 0.894a	4.20 ± 0.583ab	4.8 ± 0.735b
	Nursery	5.76 ± 1.668ab	3.9 ± 1.880a	9.42 ± 1.047b*	9.6 ± 1.503b*

Means that are followed by different letters within a row differ significantly ($p \leq 0.05$). Means followed by asterisk (*) within a column differ significantly ($p \leq 0.05$). $n=20$.

produced under nursery conditions was significantly higher than the one from growth room (Table 1).

Effect of kinetin on bud formation, shoot and leaf growth

Bud formation

The highest percentage of bud formation was recorded at a concentration of 2.0 mg l⁻¹ kinetin in both growth room and nursery. The percentage bud formation was significantly higher in the nursery than in the growth room (Table 2).

Number of buds per plant

The number of buds increased with an increase in kinetin concentration. The number of buds produced under nursery conditions was significantly higher than the one from growth room (Table 2 and Figures 3 and 4).

Shoot length

The longest shoots were recorded at a concentration of 1.0 or 2.0 mg l⁻¹ kinetin under both nursery and growth room conditions. Nursery conditions produced significantly longer shoots than the growth room (Table 2).

Leaf length and width

Leaf length and width were highest at a concentration of 1.0 or 2.0 mg l⁻¹ kinetin in both growth room and nursery.

Plants growth under nursery conditions produced significantly longer and wider leaves than the ones from growth room at a concentration of 1.0 or 2.0 mg l⁻¹ kinetin (Table 2).

DISCUSSION

The death of the cuttings incubated at a temperature of 36°C was significantly lower ($p < 0.05$) than at 16 and 26°C (Figure 1). Hvoslef-Eide (1991) also reported the death of *Nephrolepis exaltata* cuttings after 90 days of exposure to 15°C. In their experiments, survival of cuttings was achieved above 27°C. The CLI and CL types of cuttings showed a significantly lower ($p < 0.05$) percentage of death than the C type of cuttings at 36°C, suggesting that the leaves played a role in the survival of cuttings (Figure 1A). These results were consistent with the findings of Leakey et al. (1982) who reported the death of leafless cuttings within three weeks at a temperature below 23°C.

The high percentage of bud formation at 36°C for CLI type of cuttings was also significantly higher than the one recorded at 26°C, suggesting that temperature played a role in bud formation (Figure 1B). The results are in agreement with those reported by Hansen (1989) for *Stephanotis floribunda* which showed that rooting and bud formation were delayed at the temperature of 17°C.

The death of CLI, CL and C cuttings grown in the nursery at light intensities of 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were significantly lower ($p < 0.05$) than the ones grown at light intensities of 600 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At a light intensity of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, CLI type of cuttings showed the lowest percentage of death, outperforming both CL and C types of cuttings (Figure 2A). Since the CL type of cutting was immature, its death was probably due to



Figure 3. Rooting and bud formation of *Ansellia africana* cuttings with both green leaves and an inflorescence treated with 0.5 mg l^{-1} kinetin concentration in a nursery at 120 days after treatment.



Figure 4. Propagation of *Ansellia africana* from cuttings with both green leaves and an inflorescence treated with various concentrations of kinetin in a nursery at 120 days after treatment.

tissue dehydration (Grange and Loach, 1983) while the C type of cutting died due to inability to photosynthesize (Del Rio et al., 1991) as it had yellow or no leaves.

The percentage of bud formation under nursery conditions (light intensity $200\text{--}400 \mu\text{mol m}^{-2} \text{s}^{-1}$) and the growth room (light intensity $40 \mu\text{mol m}^{-2} \text{s}^{-1}$) was 40 and 20% respectively (Figures 1B and 2B). These results were consistent with the findings of other workers where shoot length was significantly lower at $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Morus latifolia* (mulberry). Furthermore, a high light intensity ($140 \mu\text{mol m}^{-2} \text{s}^{-1}$) during mulberry culture increased FW and accelerated shoot multiplication to about six fold per month (MeiChun,

2002). This might be due to photosynthesis rate increase as shown in grapevines (Amancio et al., 1999).

When NAA was applied before bud formation, the control plants and those treated with 0.5 mg l^{-1} NAA produced significantly more buds, longer shoots and roots than those treated with 1.0 or 2.0 mg l^{-1} in both growth room and nursery (Table 1). Applied auxins have been shown to inhibit bud growth (Ofori et al., 1996), as injection of *Nauclea diderrichii* with auxin delayed sprouting of cuttings taken from the same plant (Leakey, 1990). It was observed in the present study that bud formation was initiated when the shoot tip had already formed an inflorescence. This might be due to the strong apical

dominance exerted by shoot tip meristem with the consequent inhibition of axillary buds as have been previously reported by Geetha and Shetty (2000). In other studies, incorporation of NAA in the medium inhibited shoot bud formation and encouraged callus formation in the genus *Garcinia indica* (Malik et al., 2005).

The application of NAA to cuttings with buds increased root length and root dry weight significantly in all the concentrations compared to control cuttings (Table 1). Auxins are well known to play a significant role in stimulating root initiation in stem cuttings of most plants (Tchoundjeu and Leakey, 1996, 2000; Tchoundjeu et al., 2002, 2004). The stimulatory effect of auxins has been attributed to enhanced transport of carbohydrates to the base of the cutting (Mesen et al., 1997). In the present study, rooting was preceded by shoot bud formation, thus carbohydrates were initially needed for bud development and later for root growth.

The application of 1 or 2 mg l⁻¹ kinetin to cuttings improved bud formation, shoot length and leaf growth significantly under incubator and nursery conditions (Table 2). Maximum bud development and elongation were achieved when kinetin in the range 1-2mg l⁻¹ were used in the genus *Rosa hybrida* (Ibrahim and Debergh, 2001). In another experiment, a concentration of 1.5 mg l⁻¹ kinetin promoted bud formation in shoots of *Pappea capensis* significantly (Mng'omba et al., 2007). The use of kinetin reduced the time needed for bud formation from 90 to 60 days after treatment. The duration of 90 days prior to formation of a new bud of shoot in the absence of kinetin is in agreement with the findings of Hansen (1989) in the genus *Stephanotis floribunda*.

Conclusions

Plants are endangered by a combination of factors: over-collecting, unsustainable agriculture and forestry practices, urbanization, pollution, habitat destruction, fragmentation and degradation, spread of invasive alien species and climate change (Pitman and Jorgensen, 2002). In the present study, the problem was over-collecting for commercial purposes. The main objective of this study was to save the genus *A. Africana* from extinction. The present study investigated the importance of temperature, light intensity and growth regulators in the propagation of *A. africana* from leafy and leafless cuttings. Optimum conditions for bud formation were temperature (36°C), light intensity (200 - 400 μmol m⁻² s⁻¹) and kinetin (1 or 2 mg l⁻¹) in cuttings with both green leaves and an inflorescence. It is concluded that, with an overall budding success of 57%, the production of planting material from *A. Africana* through vegetative means, and through use of non-mist propagators, offers a cheaper alternative means of propagation. Further experiments are needed to test other growth regulators with a

view to increase bud formation from 57% to at least 80%.

ACKNOWLEDGEMENTS

This work was financially supported by the National Research Foundation and the University of Zululand, Kwa Dlangezwa, South Africa.

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