

# **Repeated exposure of jacket plum (*Pappea capensis*) micro-cuttings to indole-3-butyric acid (IBA) improved *in vitro* rooting capacity**

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## **Abstract**

The objective of the trial was to determine an effective propagation protocol for jacket plum (*Pappea capensis*) tree species. Experiments on *in vitro* propagation and rooting of stem cuttings were carried out. Dipping stem cuttings in half strength Murashige and Skoog (MS) media for 12 h prior to application of rooting hormones improved bud break and prolonged survival of stem cuttings on a mist bed. Early leaf loss was observed for stem cuttings planted without MS treatment. However, rooting was poor (11% for cuttings pre-treated in MS and 0% for those not pre-treated). For micro-propagation, significant differences ( $P < 0.05$ ) in shoot multiplication and root regeneration were found. MS media supplemented with 2.0 mg l<sup>-1</sup> benzylaminopurine was superior in the number of micro-shoots produced. Rooting capacity of micro-cuttings was improved from 42% to 64% when MS media supplemented with 0.5 mg l<sup>-1</sup> IBA were repeated for the micro-cuttings that initially failed to root. There was 70% survival rate of plantlets after hardened off.

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## 1. Introduction

Jacket plum (*Pappea capensis* L.) tree belongs to the Litchi family (Sapindaceae). It is a good fodder tree for livestock and produces edible fruits. The fleshy pulp is used to make vinegar, jelly and jam (Fivaz and Robbertse, 1993 and Venter and Venter, 1996). Seeds are rich in edible, non-drying and fairly viscous oil (about 74%) used for making soap and oiling guns (Venter and Venter, 1996 and van Wyk and Gericke, 2000). The oil can be exploited as a potential source of bio-fuel and such vegetable fuels are renewable and friendly to the environment (Ramadhas et al., 2005 and Canoira et al., 2006). Moreover,

vegetable oil yielding trees are produced locally and contribute low levels of the net greenhouse gasses to the atmosphere than fossil diesel (Bouaid et al., 2005). Recently, there is increasing interest to select superior germplasm for multiplication. Consequently, trials on the contributions of jacket plum trees to bio-diesel have been initiated at the University of Pretoria. However, wide cultivation of these trees or managing them in their natural habitats will largely depend on efficient propagation techniques that result in large quantities and good quality of planting materials. This tree is propagated by seeds but seedling growth is extremely slow (Palmer and Pitman, 1972). Vegetative propagation is preferred but poor rooting is a setback for many woody trees (Dick and Leakey, 2006). The challenge encountered in vegetative propagation of many tropical woody trees is root regeneration. This is unlike sexually propagated planting materials where seedlings easily develop normal and functional roots (Kwapata et al., 1999 and Dick and Leakey, 2006). There has been no scientific research done on vegetative propagation of *P. capensis* trees and the objective was to determine an effective propagation protocol for jacket plum tree species.

## 2. Materials and methods

### 2.1. Stem cuttings

Semi-hardwood cuttings (epicormic shoots) were collected from mature trees at the National Botanical Institute (1360 m above sea level, 25° 44'S, 28° 16'E), northeast of Pretoria in South Africa (Botha et al., 2000) from October 2004 to February 2005. These epicormic stem cuttings were selected because they are not completely juvenile, and hence they are expected to fruit earlier than seed-derived plants (George, 1993). Juvenile stem cuttings were not selected because of a long vegetative phase often exhibited by juvenile planting materials. A long vegetative phase is common to woody perennial trees and this is undesirable for fruit trees. The stem cuttings (10–15 cm long) were dipped in ½ Murashige and Skoog medium (Murashige and Skoog, 1962) for 12 h or planted without pre-treating in MS medium. Rooting hormones applied were (i) Seradix® No. 2 (0.3% indole-3-butyric acid, IBA in powder form) (Bayer, Pretoria, South Africa) and (ii) Seradix® No. 3, (iii) Dip 'n Grow (10 g l<sup>-1</sup> IBA and 5 g l<sup>-1</sup> NAA in liquid form) or (iv) IBA (0.1 g l<sup>-1</sup> in liquid form). These stem cuttings were planted in tray flats containing

sterile fine quartz sand growth media. The trays were placed on mist beds where there was a jet of mists for every 4 min. The relative humidity in the mist propagation chamber was at 70–95% and  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR.

The experiment was a randomised complete block design with a  $2 \times 4$  factorial arrangement and four replicates. There were eighty (80) stem cuttings per treatment combination and 640 stem cuttings were used for this experiment. Rooting of stem cuttings was assessed for a period of 16 weeks.

## **2.2. *In vitro* seed germination**

Jacket plum seeds were soaked in 98% sulphuric acid (3 min) to remove the tough seed coats and then rinsed in sterile water. The seeds were then decontaminated in 1.75% sodium hypochlorite (7 min) before rinsing in sterile water for four consecutive times. The seeds were germinated on hormone free and half strength MS media supplemented with 3% sucrose, solidified with 0.3% (w/v) gellan gum (Gelrite®). The medium was adjusted to  $\text{pH } 5.6 \pm 2$  with 1 N KOH or 1 N HCL before addition of Gelrite. The MS media were dispensed into  $25 \times 125$  mm test tubes and covered with caps before autoclaving at about  $100^\circ\text{C}$  under 121 psi pressure (15 min). Cultures were incubated under a 12 h photoperiod and  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR using two cool white fluorescent tubes per shelf. Temperatures in the incubation chamber were maintained at  $23 \pm 2^\circ\text{C}$ .

## **2.3. Pilot experiment**

Eleven different MS medium supplements were evaluated and the promising supplements were selected for further experimentation. Epicotyl shoots were excised from two-week old seedlings and explanted on MS media supplemented ( $\text{mg l}^{-1}$ ) with (i) 0.1 thidiazuron (TDZ), (ii) 0.1 TDZ and 0.1 indole-3-butyric acid (IBA), (iii) 2.0 benzylaminopurine (BAP), (iv) 1.0 BAP and 1.0 kinetin (KIN), (v) 0.1 BAP and 0.1 KIN, (vi) 0.2 BAP and 0.1 KIN, (vii) 1.5 KIN and 0.05  $\alpha$ -naphthaleneacetic acid (NAA), (viii) 3.0 BAP and 0.01 IBA, (ix) 5.0 BAP and 0.01 IBA, (x) 0.1 BAP and 0.05 NAA, or (xi) 3.0 BAP and 0.1 indole-3-acetic acid (IAA). The MS medium supplements were selected based on rate of bud break, growth of micro-shoots, presence of necrotic shoot tips and callusing. The MS media, with 3% sucrose and  $\text{pH } 5.6 \pm 2$  adjusted with 1 N KOH or 1 N HCL, were gelled

with 0.3% Gelrite. The MS media (10 ml aliquot) were dispensed into 25 × 125 mm test tubes and then covered with caps before autoclaving at about 100 °C under 121 psi pressure (15 min). Ten test tubes were used per treatment and then sealed with parafilm strips. Cultures were incubated under a 12 h photoperiod and 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR using two cool white fluorescent tubes per shelf. Temperatures were maintained at 23 ± 2 °C. This experiment was carried out for a period of 4 weeks, but not repeated.

#### **2.4. Shoot multiplication**

Six MS medium supplements selected were (i) 1.5 mg l<sup>-1</sup> kinetin, (ii) 5.0 mg l<sup>-1</sup> BAP, (iii) 0.1 mg l<sup>-1</sup> BAP and 0.05 mg l<sup>-1</sup> NAA, (iv) 2.0 mg l<sup>-1</sup> BAP, (v) 1.0 mg l<sup>-1</sup> BAP and 1.0 mg l<sup>-1</sup> kinetin or (vi) 1.0 mg l<sup>-1</sup> BAP and 5.0 mg l<sup>-1</sup> gibberellic acid (GA<sub>3</sub>). For the last medium treatment, 5.0 mg l<sup>-1</sup> GA<sub>3</sub> replaced kinetin since a combination of 1.0 mg l<sup>-1</sup> BAP and 1.0 mg l<sup>-1</sup> kinetin promoted a high level of callusing despite a good bud break. The MS media with 3% sucrose, pH 5.6 ± 2 and solidified with 0.3% Gelrite, were then dispensed into 25 × 125 mm test tubes. Each test tube contained 10 ml aliquot of MS media and then covered with caps. They were autoclaved at about 100 °C under 121 psi pressure (15 min). Excised shoots with two nodes were explanted and ensuing micro-shoots were subcultured three times on the same MS media. Test tubes were sealed with parafilm strips and incubated under a 12 h photoperiod and 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR using two cool white fluorescent tubes per shelf. Temperatures were maintained at 23 ± 2 °C. This experiment was a completely randomised design with six treatments and three replicates. There were twenty test tubes per treatment and this experiment was carried out for a period of 12 weeks. Data collected included the number of micro-shoots produced per responding explant, rate of callusing and shoot tip necrosis.

#### **2.5. *In vitro* root regeneration**

Micro-cuttings produced from axillary shoots with at least two leaves were excised and then explanted on ½ MS media supplemented with IAA, IBA, NAA or their different combinations. The experiment was a randomised complete block design with ten rooting treatments (mg l<sup>-1</sup>), namely (i) 0.1 IBA, (ii) 0.5 IBA and 0.5 NAA, (iii) 1.0 IBA, (iv) 1.0 IBA and 0.5 IAA, (v) 0.5 IBA, (vi) 0.1 NAA, (vii) 0.5 IBA and 0.5 IAA, (viii) 0.5 IAA,

(ix) 0.5 NAA or (x) 1.5 IBA. The  $\frac{1}{2}$  MS media also contained 3% sucrose and gelled with 0.3% Gelrite. The MS medium was adjusted to  $\text{pH } 5.6 \pm 2$  with 1 N KOH or 1 N HCL before Gelrite was added. The culture media were dispensed into  $25 \times 125$  mm test tubes. Test tubes containing 10 ml aliquot of MS media were covered with caps. They were autoclaved at about  $100^\circ\text{C}$  under 121 psi pressure (15 min) and then sealed with parafilm strips after culture initiation. All cultures were incubated under a 12 h photoperiod and  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR using two cool white fluorescent tubes per shelf. Temperatures were maintained at  $23 \pm 2^\circ\text{C}$ . Micro-cuttings were maintained on the same MS media for 4 weeks.

Plantlets were transferred onto  $\frac{1}{2}$  MS media without hormones soon after root induction. Micro-cuttings that failed to root were transferred onto  $\frac{1}{2}$  MS media without hormones after 4 weeks of exposure to the rooting hormones. Data collected included the number of rooted explants and the number of roots per responding explant. Plantlets were hardened off in a mist bed with 70–95% relative humidity and  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR.

## **2.6. Repeated exposure of micro-cuttings to IBA**

All micro-cuttings that failed to root were maintained on  $\frac{1}{2}$  MS media without hormones for 4 weeks. Terminal shoots (3–4 cm long) were excised from these micro-cuttings and explanted on  $\frac{1}{2}$  MS media supplemented with  $0.5 \text{ mg l}^{-1}$  IBA since this was the most effective rooting treatment established from the previous experiment. The medium pH was adjusted to  $5.6 \pm 2$  with 1 N KOH or 1 N HCL. The culture media contained 3% sucrose and solidified with 0.3% Gelrite. All test tubes ( $25 \times 125$  mm) containing 10 ml aliquot of MS media were covered with caps. The media were autoclaved at about  $100^\circ\text{C}$  under 121 psi pressure (15 min) and test tubes were sealed with parafilm strips after cultures were explanted. Cultures were incubated under a 12 h photoperiod and  $60 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR using two cool white fluorescent tubes per shelf. Temperatures were maintained at  $23 \pm 2^\circ\text{C}$ . There were fifteen test tubes used to assess the rooting capacity of these micro-cuttings and this was repeated three times. All the cultures were maintained on the same MS media for a period of 4 weeks. Plantlets were hardened off in a mist bed with 70–95% relative humidity and  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR.

## 2.7. Statistical analysis

Data were analysed using GenStat 4.24DE (Rothamsted Experimental Station) after angular transformation (Steel and Torrie, 1980).

# 3. Results and discussion

## 3.1. Stem cuttings

There was early leaf loss in all the stem cuttings followed by regeneration of new leaves and then bud break. Stem cuttings pre-treated in MS medium remained green and continued to sprout for 12 weeks. Many stem cuttings not pre-treated in MS medium wilted within 8 weeks and died. Although stem cuttings pre-treated in MS medium continued to sprout, rooting was very poor (11%). This is despite that the cuttings were from epicormic shoots. There were no significant differences ( $P < 0.05$ ) between the rooting treatment combinations. Dipping cuttings in MS media only extended the leaf regeneration period and bud break. This study shows rooting difficulties in jacket plum trees and with hardwood and mature stem cuttings rooting will be hard to achieve.

## 3.2. Pilot experiment

Six promising MS medium supplements were selected from the pilot experiment. Inclusion of auxins, especially IBA increased the rate of callusing at the bases of explants. MS media supplemented with TDZ and IBA highly favoured callus production (Fig. 1) at the bases and shoot tips of explants, and hence this treatment was not selected. MS media with  $5.0 \text{ mg l}^{-1}$  BAP and  $0.01 \text{ mg l}^{-1}$  IBA resulted in good bud break despite a high level of callusing and this was attributed to the presence of  $0.01 \text{ mg l}^{-1}$  IBA. Therefore, MS media were only supplemented with  $5.0 \text{ mg l}^{-1}$  BAP. The MS media with  $0.1 \text{ mg l}^{-1}$  BAP and  $0.05 \text{ mg l}^{-1}$  NAA also resulted in a high rate of bud break and rapid growth of micro-shoots despite a great amount of callusing at the bases of explants (Fig. 2A). This MS medium supplement was selected for further experimentation.

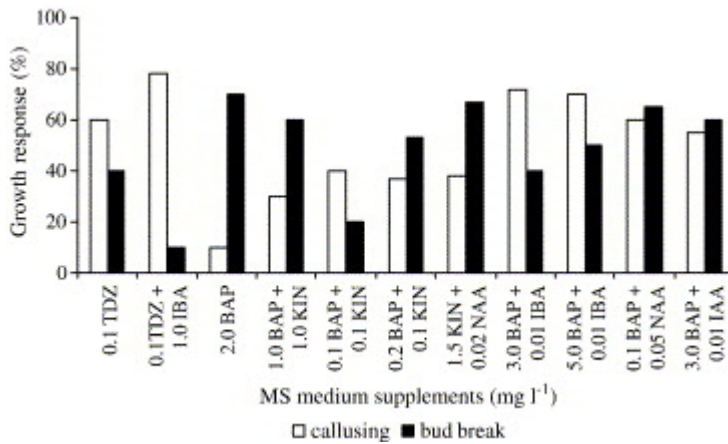


Fig. 1. Percentage callusing and bud break of jacket plum (*P. capensis*) micro-shoots on Murashige and Skoog (MS) media supplemented with (mg l<sup>-1</sup>) benzylaminopurine (BAP), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), kinetin (KIN) and  $\alpha$ -naphthaleneacetic acid (NAA) or thidiazuron (TDZ) after 4 weeks.

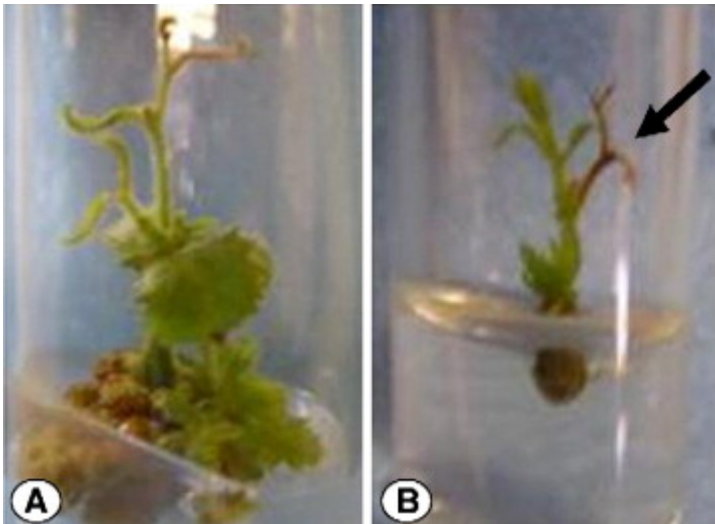


Fig. 2. Jacket plum (*P. capensis*) micro-shoots on Murashige and Skoog media (A) a high level of callusing at the bases of micro-shoots on 0.1 mg l<sup>-1</sup> benzylaminopurine and 0.05 mg l<sup>-1</sup>  $\alpha$ -naphthaleneacetic acid (B) micro-shoot with a necrotic shoot tip (arrow shows shoot tip death) and new micro-shoots being produced.



### 3.3. *In vitro* shoot multiplication

Data in Table 1 shows mean number and condition of micro-shoots produced on different MS media for a period of 12 weeks. There were significant differences ( $P < 0.05$ ) between treatments. MS media with 2.0 mg l<sup>-1</sup> BAP was superior (4.1) to 1.5 mg l<sup>-1</sup> kinetin (2.9), 5.0 mg l<sup>-1</sup> BAP (2.3), 1.0 mg l<sup>-1</sup> BAP and 1.0 mg l<sup>-1</sup> kinetic (2.5), and 1.0 mg l<sup>-1</sup> BAP and 5.0 mg l<sup>-1</sup> GA<sub>3</sub> (2.0). Matu et al. (2006) found that BAP was superior to kinetin for shoot multiplication in *Maytenus senegalensis* trees. Our results agree with their findings. Shoot tip necrosis was observed when micro-shoots were kept on the media for 4 weeks or longer before subculturing. This could be attributed to prolific micro-shoot growth (2–5 micro-shoots per explant per week). Once there was a shoot tip death, new micro-shoots were produced (Fig. 2B).

Table 1.

Mean number and condition of jacket plum (*P. capensis*) micro-shoots explanted on Murashige and Skoog (MS) media supplemented with benzylaminopurine (BAP), kinetin (KIN),  $\alpha$ -naphthaleneacetic acid (NAA) and gibberellic acid (GA<sub>3</sub>)

MS medium number	Supplements (mg l <sup>-1</sup> )	Mean number of shoots per explant	Condition of micro-cuttings
1.	1.5 KIN	2.9 <sup>b,c</sup>	No shoot tip necrosis
2.	5.0 BAP	2.3 <sup>b,c</sup>	No shoot tip necrosis
3.	0.1 BAP + 0.05 NAA	3.4 <sup>a,b</sup>	Base callusing
4.	2.0 BAP	4.1 <sup>a</sup>	Shoot tip necrosis
5.	1.0 BAP + 1.0 KIN	2.5 <sup>b,c</sup>	Little shoot tip necrosis
6.	1.0 BAP + 5.0 GA <sub>3</sub>	2.0 <sup>c</sup>	No shoot tip necrosis
	SE	0.36	
	CV (%)	21.7	

Numbers with the same letters within a column are not significantly different ( $P < 0.05$ ).

*In vitro* shoot tip necrosis has been reported in *Pistacia vera* explants on MS medium and this was attributed to slow calcium absorption (Barghchi and Alderson, 1985). Bhalla and

Mulwa (2003) also reported a high rate of necrotic shoot tips (80%) in macadamia shoots. In this trial, adding  $0.3 \text{ mg l}^{-1}$  casein hydrolysate slightly reduced shoot tip necrosis and frequent subculturing (short passages) was also effective in reducing shoot tip necrosis. A combination of cytokinin and a low auxin concentration or another cytokinin improved growth of micro-shoots. This agrees with the findings of Huetteman and Preece (1993). MS media with  $1.0 \text{ mg l}^{-1}$  BAP and  $1.0 \text{ mg l}^{-1}$  kinetin, and  $0.1 \text{ mg l}^{-1}$  BAP and  $0.05 \text{ mg l}^{-1}$  NAA resulted in a high rate of shoot multiplication. Jain et al. (1990) also found cytokinin and auxin combination to be effective for *Morus* species but Islam et al. (1993) found the combination to be less effective in *M. laevigata*.

Fig. 3A shows three micro-shoots produced on MS medium supplemented with  $2.0 \text{ mg l}^{-1}$  BAP which was an optimal formulation. Bhalla and Mulwa (2003) reported the highest percentage bud break and shoot multiplication on MS media containing  $2 \text{ mg l}^{-1}$  BAP. Subculturing ortet explants on MS media supplemented with  $2.0 \text{ mg l}^{-1}$  BAP after excising micro-shoots promoted production of prolific single micro-shoot (Fig. 3B). Prolific growth of a single micro-shoot makes it possible to get multiple micro-cuttings within 2 weeks.



Fig. 3. Jacket plum (*P. capensis*) shoot multiplication on Murashige and Skoog media supplemented with  $2.0 \text{ mg l}^{-1}$  benzylaminopurine (BAP) after 2 weeks (A) three healthy micro-cuttings (B) single shoot produced after subculturing the ortet explant.

### 3.4. *In vitro* root regeneration

Roots were observed after 10–15 days. Fig. 4 shows rooting percentage of jacket plum micro-cuttings explanted on different medium supplements. There were significant differences between rooting treatments ( $P < 0.05$ ). The MS media supplemented with  $0.5 \text{ mg l}^{-1}$  IBA were superior in rooting (42%) jacket plum micro-cuttings (Fig. 4). *In vitro* rooting of micro-shoots is affected by several factors (Rugini et al., 1993 and Kwapata et al., 1999). Le Roux and van Staden (1991) reported a wide range in rooting percentage within *Eucalyptus* species. In their trial, a range of 0%–67% rooting was obtained for *E. macarthurii*, 2%–30% for *E. smithii* and 21%–100% for *E. saligna*. This indicates that woody perennial tree species show variations in *in vitro* rooting capacity.

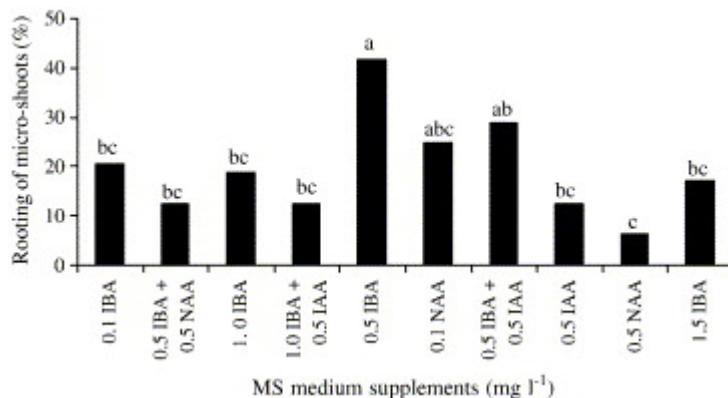


Fig. 4. Rooting percentage of jacket plum (*P. capensis*) micro-shoots explanted on Murashige and Skoog (MS) media supplemented with ( $\text{mg l}^{-1}$ ) indole-3-butyric acid (IBA),  $\alpha$ -naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA) after 3 weeks.

Fig. 5 shows mean number of roots regenerated and there were significant differences ( $P < 0.05$ ) between treatment means. A significant number of roots (3) per plantlet was obtained on MS media supplemented with  $0.5 \text{ mg l}^{-1}$  IBA (Fig. 5). The data shows that IBA was superior to NAA and IAA in root regeneration. Matu et al. (2006) also found that IBA was better than NAA and IAA in root regeneration.

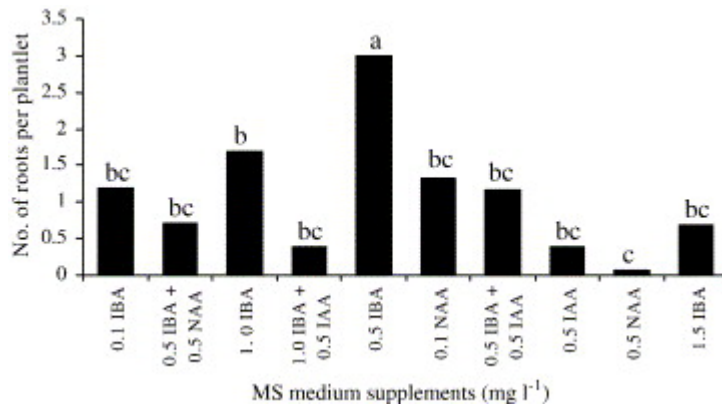


Fig. 5. Mean number of roots regenerated on jacket plum (*P. capensis*) micro-cuttings rooted on Murashige and Skoog (MS) with different concentrations ( $\text{mg l}^{-1}$ ) of indole-3-butyric acid (IBA),  $\alpha$  naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA). A combination of IBA and NAA or IBA alone induced callusing at the bases of explants (Fig. 6A). Johnston and Armstrong (2003) reported callus development at the bases, petioles and leaves of Christmas bush (*Ceratopetalum gummiferum* Sm.) explants on MS media supplemented with IBA or NAA. Despite the large quantity of callus, they reported a high rooting percentage (80–93%) and this suggests that callus development does not hinder rooting. However, *in vitro* rooting experiments for woody tree species have shown different responses (Williams et al., 1985). In this study, observations showed that MS media supplemented with a combination of  $0.5 \text{ mg l}^{-1}$  IBA and  $0.5 \text{ mg l}^{-1}$  IAA resulted in slender or weak roots. Some of the thread like roots curled away from the rooting media.

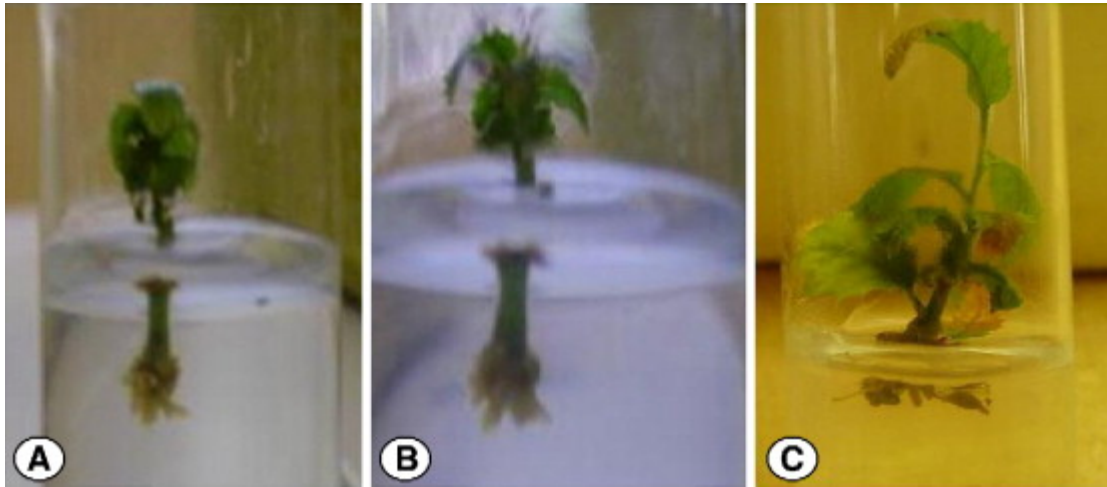


Fig. 6. Jacket plum (*P. capensis*) micro-cuttings rooted on Murashige and Skoog media supplemented with  $0.5 \text{ mg l}^{-1}$  indole-3-butyric acid and explanted for the second time on the same media (A) plantlet with root initials after 2 weeks (B) plantlet after 3 weeks (C) rooted micro-cuttings after 4 weeks.

It was further observed that micro-cuttings with a few or no leaves had good root regeneration and they were able to regenerate new shoots and leaves upon transferring to  $\frac{1}{2}$  MS media. Huettelman and Preece (1993) attributed rooting difficulties to 'carry over' effect from shoot multiplication media. Scott et al. (1961) and Maliro (1997) attributed a delay in root induction, and fewer and weak roots occurring in many woody tree species to inadequate light quality exposure.

In this trial, shoot tip necrosis was absent in all the jacket plum plantlets even those with weak and a few roots. However, shoot tip necrosis was pronounced during *in vitro* shoot multiplication. Therefore, this means poor absorption of plant nutrients by micro-shoots plays a major role in the development of shoot tip necrosis. There could be a seasonality effect on rooting of micro-cuttings, a common phenomenon in many woody tree species. Poor rooting has been reported in many tropical woody trees (Kwapata et al., 1999) and that there is less than 30% of explants regenerating normal and functional roots (Ahee and Duhoux, 1994). This is often attributed to inadequate light quality and intensity (Torrey, 1952 and Maliro, 1997). However, there are several factors that affect *in vitro* rooting of micro-shoots (Rugini et al., 1993).

### 3.5. Repeated exposure of micro-cuttings to IBA

In this trial, rooting was improved from 42% to 64% when micro-cuttings were maintained on ½ MS media without hormones for 4 weeks before exposing to MS media supplemented with 0.5 mg l<sup>-1</sup> IBA for the second time. Different stages of root regeneration are shown in Fig. 6A–C. We suspect that ‘carry over’ effect was broken by exposing jacket plum micro-cuttings to hormone free MS media for 4 weeks. Amin and Jaiswal (1993) improved rooting of jackfruit (*Artocarpus heterophyllus*) explants from 40% after the fourth subculture to 80% after the tenth subculture. In this trial, rooting was improved by exposing micro-cuttings to MS media supplemented with 0.5 mg l<sup>-1</sup> IBA for the second time.

### 3.6. Acclimatization

Plantlets were removed from the MS media and the roots were washed with distilled water to remove the MS medium. Plantlets were hardened off in a mist propagation bed and 70% survival rate of plantlets was achieved.

## 4. Conclusion

Jacket plum stem cuttings are difficult to root despite good sprouting ability, leaf retention, especially when pre-treated in ½ MS media. For micro-propagation, MS media supplemented with 2.0 mg l<sup>-1</sup> BAP was optimal for rapid multiplication of micro-shoots. This makes rapid production of jacket plum plantlets possible. Repeated exposure of micro-cuttings to MS media supplemented with 0.5 mg l<sup>-1</sup> IBA improved rooting to 64%. This is an efficient protocol for mass multiplication of jacket plum planting materials.

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