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Full Length Research Paper

Propagation of the African medicinal and pesticidal plant, *Securidaca longepedunculata*

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Propagation methods do not exist for *Securidaca longepedunculata*. In this study, *ex vitro* and *in vitro* experiments were conducted to identify simple propagation methods for this species. *Ex vitro* germination rates did not exceed 43%, whereas *in vitro* procedures achieved 67 to 90% germination rates. *In vitro* germinated seedlings produced at least two re-sprouts and consistently showed vigorous growth. Explants cultured on B5 medium supplemented with indole-3-butyric acid (IBA) and 1-naphthalene-3-acetic acid (NAA) successfully produced roots within 4 weeks. It is concluded that germination of seeds, shoot multiplication and rooting of *S. longepedunculata* can be improved through appropriate in *vitro* procedures.

Key words: Agroforestry, axillary shoot multiplication, *ex vitro* germination, gibberellic acid, *in vitro* propagation.

INTRODUCTION

Securidaca longepedunculata is widely used in African traditional medicine (Ajali and Chukwurah, 2004; Dapar et al., 2007; Lino and Deogracious, 2006; Maiga et al., 2005; Meyer et al., 2008; Ojewole et al., 2001; Okoli et al., 2005; Pallant and Steenkamp, 2008; Rakuambo et al., 2004). Recent studies have also reported antimicrobial activity against protozoa, bacteria and fungi (Ajali and Chukwurah, 2004; Lino and Deogracious, 2006; Maiga et al., 2005; Pallant and Steenkamp, 2008). The alkaloid securinine confers activity against *Plasmodium falciparum*, the causative agent of malaria (Maiga et al., 2005), while the root extract was trypanocidal against both *Trypanosoma brucei* and *Trypanosoma congolense* (Atawodi et al., 2003). The xanthones from its root bark have also shown promise in the treatment of erectile dysfunction (Meyer et al., 2008; Rakuambo et al., 2004). In addition, the root bark is used by farmers as a pesticide in stored grain (Belmain and Stevenson, 2001; Boeke et al., 2004; Stevenson et al., 2009). The efficacy of this has been validated in laboratory studies (Jayasekera et al., 2005; Stevenson et al., 2009). Molluscicidal properties of the root extract has also been recently demonstrated (Olofintoye, 2010).

Natural stands are under considerable pressure from harvesting of roots for its numerous uses (Ouedraogo et al., 2003). Natural regeneration is limited because this plant has low seed germination rates and slow seedling

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Abbreviations: B5, Gamborg's B5 medium; **GA,** gibberelic acid; **IBA,** indole-3-butyric acid; **2ip,** isopentenyladenine; **MS,** Murashige and Skoog's medium; **NAA,** 1-naphthalene-3-acetic acid; **SDICN,** sodium dichloroisocyanurate; **TDZ,** thidiazuron.

growth (Mbuya et al., 1994). Planting on farm land is hoped to reduce pressure on the natural stands. However, little effort has also been made to develop propagation methods to facilitate planting of *S. longepedunculata* in agroforestry systems. Therefore, the specific objectives of the present work were to: (1) determine the effect of seed pre-treatment on germination rates, seedling growth and survival; and (2) explore the feasibility of *in vitro* micropropagation as a tool for propagation of *S. longepedunculata*.

MATERIALS AND METHODS

An *ex vitro* experiment was conducted in a glasshouse with average temperatures varying between 13 and 40°C at Chitedze Agricultural Research Station during January to April 2009. The seeds used in all studies were collection from Chitedze in Malawi. Seed treatment involved soaking for 24 h in different concentrations (0, 100, 200, 400 and 800 mg/L) of gibberellic acid (GA). Seeds soaked in 0 mg/L GA (that is, no soaking in water or GA) served as the control. Each treated seed was sown in a black polythene tube filled with either of two growing media: 1 = compost manure + sand + forest soil in a 1:1:1 mixture by volume or 2 = sand + forest soil in a 1:1 mixture. The seed pre-treatment and growing media were combined in a factorial experiment in a completely randomized design with four replicates of 25 seeds per replicate. Data were collected on seed germination, seedling survival and height growth. Germinated seeds were counted and recorded daily until no further germination was recorded for three consecutive days. Eleven weeks after sowing, the total number of seedlings that survived and their mean heights were recorded for each treatment. Angular transformations of the seed germination and seedling survival data were subjected to analysis of variance (ANOVA).

In addition, preliminary *in vitro* tests were carried out between March and August 2008 in the Conservation Biotechnology Unit of the Royal Botanic Gardens, Kew in the UK. The seeds were soaked in water, peeled and washed in deionised water and sterilised in 0.5 % (w/v) bleach, sodium dichloroisocyanurate (SDICN) with a drop of Tween 20 for 30, 45 and 60 min. A total of 30 seeds were used for each length of sterilization. All the seeds were later washed to remove the excess bleach in deionised sterile water and surface dried on sterile filter paper. The pH of the media was set at 5.8 and solidified with 0.8% agar before autoclaving at 121°C for 15 min. Activated charcoal was added to the media at a concentration of 1.5 g/L. The seeds were cultured on MS media in 9 cm Petri dishes. Three lengths of sterilisation (30, 45 and 60 min) were applied as treatments, which were laid out in a completely randomized design with three replicates. A total of 90 seeds were cultured with each replicate having 10 seeds. All the aseptic culture procedures were done in a sterile laminar airflow cabinet and cultured seeds were incubated at 26°C in the dark in an incubator. After germination, they were moved to the growth room (PPFD: 25 µmol/m/s) under a 12-h day/12-h /night photoperiod at 23 ± 2°C.

Epicotyl segments from seven weeks old *in vitro* germinated seedlings were subcultured on MS media. Nine explants were cultured on MS media without plant growth regulators (PGRs) as a control and a further nine explants were cultured on MS media supplemented with 1 mg/L of 1-naphthalene-3-acetic acid (NAA) and 1 mg/L of thidiazuron (TDZ). These treatments were laid out in a completely randomised design with three replicates. Each replicate had three explants in 355 ml culture vessel.

Six *in vitro* germinated seedlings were maintained in the growth room and observed for axillary shoot multiplication. These were maintained in MS medium without PGRs. These original seedlings further provided 15 explants tested on B5 media. Five of these explants were cultured on B5 media supplemented with 3 mg/L of isopentenyladenine (2iP), a further five were cultured on B5 media supplemented with 0.1 mg/L of TDZ and a control comprised of five explants cultured on B5 without PGRs. Semi-solid sucrose-free rooting medium containing B5 nutrients with 0.1 mg/L of indole-3-butyric acid (IBA) and 0.1 mg/L of NAA in honey jars and Florialite® plugs made of vermiculite and paper pulp were used for rooting under photoautotrophic conditions. Kruskal-Wallis H test was applied to the data on explants response to hormones sterilization lengths on the *in vitro* germination. The Mann-Whitney U test was used for the data on explants response to hormones.

RESULTS AND DISCUSSION

Germination rates of *S. longepedunculata* seed were very low (<45%) in all treatments. This is in agreement with the findings of Ouedraogo et al. (2003). However, germination rates differed significantly with GA concentrations ($F = 10.1$; $P < 0.001$), growing medium ($F = 4.4$; $P = 0.044$) and their interaction effects ($F = 6.5$; $P < 0.001$). In the growing medium with compost manure, cumulative germination was highest (42.6%) in the control and lowest (10%) in seeds treated with 200 mg/L of GA (Figure 1a). The lowest germination rate (2%) was recorded in seeds treated with 800 mg/L of GA and sown in the growing medium with compost manure (Figure 1b). While germination commenced 15 days after planting, it was extended over a period of 50 days or more showing lack of uniformity.

Seeds treated with 800 mg/L GA and sown in a growing medium with compost manure germi-nated only after 45 days. Germination rates generally declined with increasing concentrations of GA in the growing medium with compost manure (Figure 1c).

Seedling height did not significantly differ with GA concentration ($P > 0.05$). However, it differed significantly with growing media ($F = 11.7$; $P = 0.002$) and its interaction with GA concentration ($F = 3.5$; $P = 0.018$). Eleven weeks after sowing, seedlings sown in the growing medium without compost manure were signify-cantly taller (306 mm) than those in growing medium with compost manure (175 mm). The shortest seedlings were recorded in seeds treated with 800 mg/L GA and sown in a growing medium with compost manure (Figure 1d). Seedling survival did not significantly differ with GA concentration ($P > 0.05$). However, it differed with growing medium ($F = 8.3$; $P = 0.007$). Survival was higher in the growing medium without compost manure at all GA concentrations, except in 100 mg/L (Figure 1e). Seedling survival rates generally declined with increasing concen-trations of GA in growing medium with compost manure (Figure 1e).

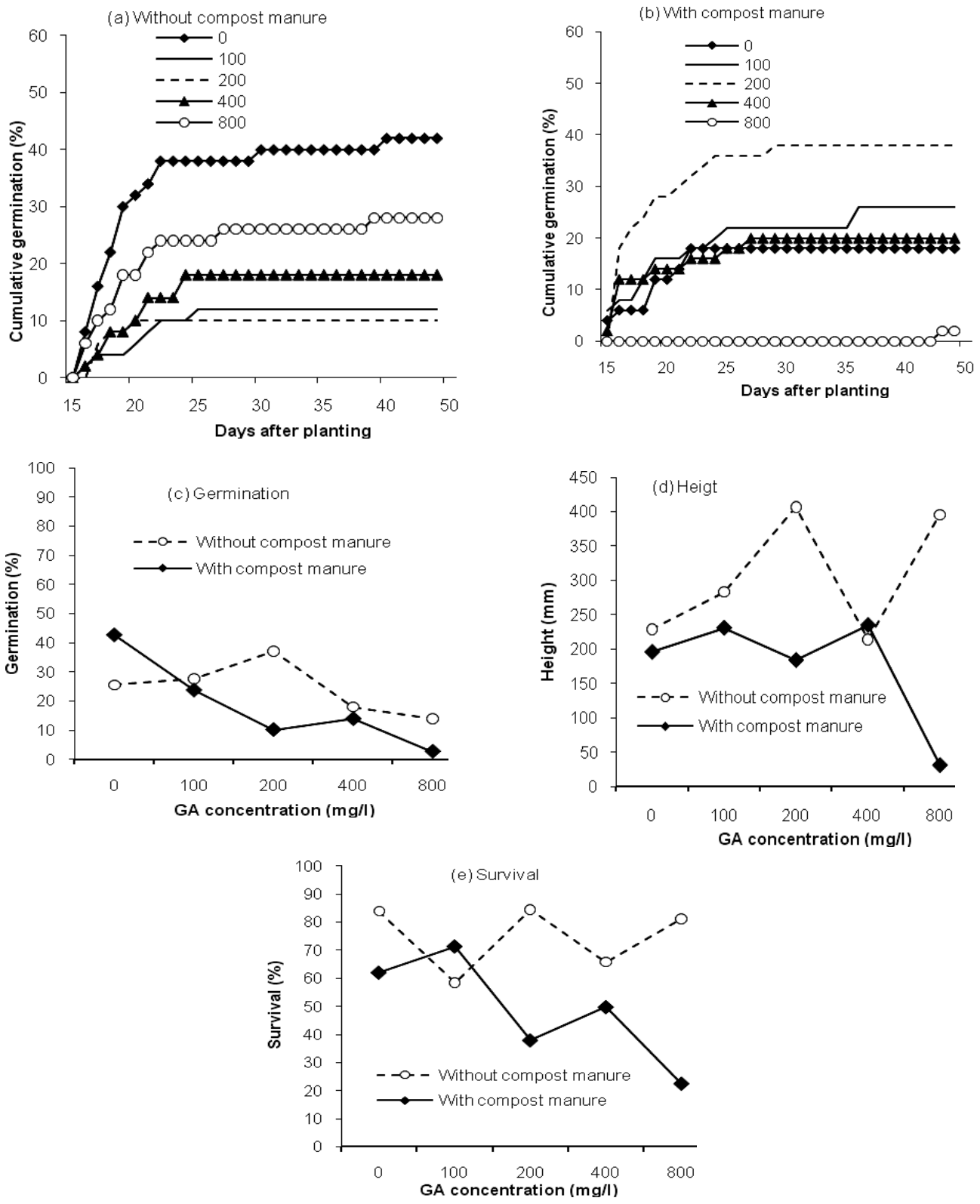

Figure 1. Effect of different concentrations (0, 100, 200, 400 and 800 mg/L) of GA and growing media on germination of *S. longipedunculata* seed (a to c), seedling growth (d) and survival (e).

(a) Axillary shoot multiplication

(b) Explants cultured on vermiculite and paper pulp

(c) Rooting on B5 liquid media + 0.1 mg/l of IBA and NAA

Figure 2. a: Axillary shoot multiplication and rooting in *S. longipedunculata*; b: Explants cultured on vermiculite and paper pulp (B5 liquid media with 0.1 mg/L of NAA and TDZ) never produced roots; c: Explants cultured on B5 liquid media supplemented with 0.1 mg/L of IBA and NAA rooted after 4 weeks.

In the *in vitro* germination test, the 30, 45 and 60 min sterilization gave 90, 73 and 66.7% germination, respectively. However, the differences between the treatments were no statistically significant ($P > 0.05$). The improvement in germination success is probably because of the aseptic conditions in the *in vitro* cultures. Removing the seed coat could have reduced contamination sources while improving the imbibition of water and subsequent germination.

Production of new nodes also did not differ significantly ($U = 0.164$, $P > 0.05$) between MS supplementation with PGRs and the control. Explants cultured on MS media supplemented with 1 mg/L of NAA and 1 mg/L of TDZ showed vigorous growth and greater overall size than those cultured on MS media without PGRs (control). The *in vitro* germinated seedlings produced at least two re-sprouts in seven weeks as potential explants sources for subsequent sub culturing. Explants supplemented with 0.1 mg/L of TDZ significantly ($H = 10.05$, $P = 0.007$) improved shoot multiplication when compared with the control. After the shoot tips were cut back, the five explants cultured on B5 supplemented with 3 mg/L of 2iP produced a total of ten shoots in four weeks and all these cultures re-sprouted with two shoots each. The five explants cultured on B5 supplemented with 0.1 mg/L of TDZ produced a total of 13 shoots. The five explants cultured on B5 without PGRs re-sprouted and produced a total of six shoots after their tips were cut back. Plantlets developed in Florialite® plugs were healthier when compared with the ones produced in agar (Figure 2b). Shoot-tips cultured on B5 medium supplemented with 0.1 mg/L of IBA and NAA successfully produced roots within 4 weeks (Figure 2c). The results indicate that germination of seeds, axillary shoot multiplication and rooting ability of

S. longepedunculata explants can be improved. However, further detailed research is needed to identify the best media and culture conditions to achieve optimal multiplication rate. This can facilitate mass-propagation of elite genotypes for wider planting in agroforestry systems or in its natural habitat where restoration and conservation is envisaged.

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