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Full Length Research Paper

Seed germination and *in vitro* regeneration of the African medicinal and pesticidal plant, *Bobgunnia madagascariensis*

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Propagation of the medicinal and pesticidal tree, *Bobgunnia madagascarensis* is difficult due to poor and erratic germination of its seeds and slow growth of its seedlings. This study involved two separate experiments. The first evaluated the effect of pre-sowing treatments and growing medium on *ex vitro* seed germination and early seedling development. The second experiment involved *in vitro* germination, shoot initiation and rooting of shoots. Pre-sowing seed treatments involved soaking seeds in cold and hot water for 12 and 24 h and soaking in different concentrations (0, 100, 200, 400 and 800 mg/l) of gibberellic acid for 24 h. Soaking of seeds in cold or hot water for up to 24 h did not achieve more than 45% germination, while seeds treated with gibberellic acid achieved <20% germination rates. On the other hand, *in vitro* procedures achieved 30 to 70% germination of seeds. Seedling survival of *ex-vitro* germinated seeds was higher (>76%) when seeds were sown in a growing medium without compost compared with a medium with compost (<43%). All shoot-tips isolated from the *in vitro* germinated seedlings on B5 media without plant growth regulators continued to grow as a single shoot, while shoot-tips cultured on B5 supplemented with 0.1 mg/l of naphthaleneacetic acid (NAA) and thidiazuron (TDZ) produced two shoots each after four weeks. It was concluded that *B. madagascariensis* seeds had very low *ex vitro* germination percentages. Although, *in vitro* cultures improved seed germination, axillary shoot multiplication and rooting were not satisfactory. Therefore, further studies are needed to develop an optimal *in vitro* multiplication protocol for *B. madagascariensis*.

Key words: Axillary shoot multiplication, gibberellic acid, *in vitro* regeneration, seed germination, *Swartzia.*

INTRODUCTION

Bobgunnia madagascariensis, formerly *Swartzia madagascariensis* (Kirkbride and Wiersema, 1997), is a leguminous tree widely distributed throughout the savannahs and dry forests across Africa (Kirkbride and Wiersema, 1997; Hostettmann et al*.,* 2000; Schaller et al*.,* 2001; Smith and Allen, 2004). Different parts of the tree are used in traditional medicine to treat diseases such as malaria, fever, syphilis and leprosy

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(Cunningham, 1993; Schaller et al., 2001; Kirkbride and Wiersema, 1997; Ouattaraa et al., 2006). In the laboratory, extracts of the root bark had very high activity against a chloroquine-resistant strain of the malaria parasite *Plasmodium falciparum* (Ouattaraa et al., 2006). The fruit pulp was shown to exhibit high trypanocidal activity in Nigeria (Atawodi, 2005). Antifungal products have also been isolated from the root bark (Hostettmann and Marston, 2002; Hostettmann et al., 2000; Schaller et al., 2001). The toxic properties of *B. madagascariensis* have also been exploited in the control of insect pests of medical, veterinary and agricultural importance (Schaller et al., 2001; Sileshi et al., 2008). The powdered pods are a potent insecticide against mosquito larva (Kirkbride and Wiersema, 1997). The molluscicidal activity of saponins from the aqueous extract of fruits (pods) has been demonstrated (Borel and Hostettmann, 1987; Lwambo and Moyo, 1991; Suter et al., 1986). In addition to its medicinal and insecticidal properties, this tree has a very strong, durable and termite resistant wood used in building, making of local musical instruments, veneer, curios and makes good firewood and charcoal (Kirkbride and Wiersema, 1997; Vermeulen et al., 1996).

The commercial harvesting and sale of roots of this species is widespread in Africa (Cunningham, 1993). This may involve uprooting of the whole plant or removal of the root, which may seriously damage the root system. Unsustainable harvesting of the pods could also reduce regeneration and thus, threaten local populations. This calls for cultivation on the farm to increase availability locally. Unfortunately, *B. madagascariensis* is a difficult tree to propagate because it has low seed viability and poor germination. Plants propagated from seed also exhibit high degree of genetic variability (Berger and Schaffner, 1995).

In vitro culture offers advantages over conventional methods in the propagation of some crops and species of conservation importance. Micropropagation can significantly improve regeneration rates in rare and threatened species with very small quantities of seeds and/or very poor seed germination (Sarasan et al., 2006). However, development of good quality cultures for large scale propagation for both commercial propagation and conservation can be limited by the recalcitrant nature of the species in many cases. Some leguminous tree species are recalcitrant in culture (Jordan et al., 2001). Berger and Schaffner (1995) made the first attempt to develop micropropagation protocols for *B. madagascariensis* and found that, the species show very high genotypic variability in rooting ability and low shoot induction on Gelerite-based media. Since then, no effort has been made to propagate *B. madagascariensis* either through *in vitro* or *ex vitro* methods. Therefore, the specific objectives of this study were (1) to identify pre-sowing seed treatments which can improve *ex vitro* germination rates; (2) assess the effect of growing media on seedling survival and growth; and (3) to undertake a feasibility study using seeds to develop protocols for *in vitro* propagation of *B. madagascariensis*.

MATERIALS AND METHODS

The study involved two separate experiments. The first experiment involved assessment of seed pre-treatment and growing medium on germination, seedling survival and growth at Chitedze Agricultural Research Station in Malawi. The second experiment involved *in vitro* culturing in the Conservation Biotechnology Unit (CBU) at the Royal Botanic Gardens Kew, United Kingdom. The seed used in both studies were from the same seed lots collected from the natural stands of scattered trees of various age in eastern Zambia.

Ex vitro germination and seedling survival

This experiment was conducted in January-April 2009 in a greenhouse with average temperatures varying between 13 and 40°C. The pre-sowing seed treatments involved soaking seeds in cold and hot water for 12 and 24 h and soaking in different concentrations (0, 100, 200, 400 and 800 mg/l) of gibberellic acid for 24 h. For each concentration, 200 seeds were immersed in 500 ml flasks containing 200 ml solution of a given gibberellic acid concentration and shaken on a reciprocating shaker. After 24 h of soaking, the solutions were drained off and seeds rinsed with distilled water for one minute. Seeds soaked in 0 mg/l gibberellic acid (soaking in distilled water) served as the control. The hypothesis tested was that, there is a direct relationship between gibberellic acid concentration and germination of *B. madagascariensis* seeds.

Each treated seed was sown in black polythene tube filled with either of the two growing medium: (1) compost manure + sand + forest soil in a 1:1:1 mixture by volume, which is the recommended potting mixture for growing tree seedlings in medium to light soils (Jaenicke, 1999) and (2) sand + forest soil in a 1:1 mixture. The compost manure was procured from the Four Seasons Nursery, a commercial producer of ornamentals. The manure was produced from a well composted mixture of maize stover, kitchen waste, chicken and cattle manure. The forest soil was collected from a mature miombo woodland (a deciduous forest type in southern Africa where *B. madagascarensis* grows naturally) and was sieved to remove unwanted materials.

The seed pre-treatment and growing media were combined in a factorial experiment in a completely randomized design with four replications (25 seeds per replicate). Watering was done when the surface of the soil seemed to have started drying out. Data were collected on germination, survival and height growth of the seedlings. Germination was defined as emergence of the cotyledons above the medium surface. The first germinating seeds acted as the baseline for recording data. A value of "1" was recorded if the seed had germinated and the value of "0" for otherwise. Germinating seeds were counted and recorded daily until no further germination was recorded for three consecutive days. The germination test lasted for seven weeks. The total numbers of the germinated seeds for each treatment were summed up to determine the cumulative germination. 11 weeks after sowing, the total number of seedlings that survived and their mean heights were recorded for each treatment.

Table 1. Significance (P value) effect of pre-sowing seed treatment and growing medium on germination of seeds, survival and height growth of *B. madagascariensis* seedlings.

Treatment	Germination	Survival	Height growth
Hot water	<0.001	0.876	0.524
Growing medium	<0.001	<0.001	0.003
Interaction	0.105	0.606	0.330
Cold water	<0.001	0.042	0.381
Growing medium	0.551	0.167	0.005
Interaction	0.009	0.002	0.081
Gibberellic acid concentration	0.144	0.218	0.259
Growing medium	0.824	0.019	0.135
Interaction	0.186	0.327	0.220

In vitro culture

The various preliminary *in vitro* culture tests were carried out between March and August, 2008 in Conservation Biotechnology Unit (CBU). Seeds were either scored along the middle with a scalpel blade or left intact before being soaked in deionised water with a drop of Tween 20 and placed on a shaker at 110 rpm for 24 h. Imbibed seeds were washed in deionised water to remove the seed coat and were sterilized by soaking in 0.5% (w/v) of sodium dichloroisocyanurate (SDICN) for an hour and 30 min. After sterilisation, seeds were washed in deionised water again and surfaces were dried on sterile filter paper. The sterilised seeds were cultured on Gamborg's B5 (Gamborg et al., 1968) and Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). All culture media for seed germination and multiplication contained 3% sucrose, while rooting media contained 2% sucrose unless otherwise stated. Activated charcoal was added to the media at a concentration of 1.5 g/l. All solid media were solidified with 0.8% tissue culture agar (A-7002, Sigma Chemicals, UK) and pH was adjusted to 5.8 before they were autoclaved at 121°C for 15 min. For seed germination and multiplication, about 100 ml of media were dispensed into honey jars (355 ml) with plastic screw-cap lids. All aseptic culture procedures were done in a sterile laminar airflow cabinet. The cultures were grown in a tissue culture growth room at 23±2°C and photosynthetic photon flux density (PFD: 50 µmol m^{-2} s^{-1}) under 18/6 h (day/night) photoperiod.

For shoot multiplication, nine shoot-tips were isolated, while the seedlings were still inside the jar by making a cut just below the second node of the germinated seedlings. The rest of the seedling (with the first node) was left in the medium for axillary shoot proliferation. The shoot-tips were cultured on MS medium without plant growth regulators (PGRs) (control) and MS medium containing 1 mg/l of naphthaleneacetic acid (NAA) and 1 mg/l of thidiazuron (TDZ). The *in vitro* germinated seedlings were monitored for their ability to produce axillary shoots after providing explants for sub-culturing. Six fresh explants harvested from the *in vitro* germinated seedlings were cultured on B5 liquid media supplemented with 0.1 mg/l of NAA and 0.1 mg/l of TDZ for axillary multiplication.

To induce rooting, shoot-tips were cultured on Florialite® cubes (Nisshinbo Industries Inc. Japan) in B5 liquid media supplemented with 1 mg/l of NAA and 1 mg/l of indole butyric acid (IBA). These were subjected to an improved air circulation with vented Magenta® B-cap (Sigma Chemicals, UK). The liquid medium used for this trial was devoid of sucrose.

Data analysis

Angular transformations of the seed germination and seedling survival data were subjected to analysis of variance (α = 0.05). Since seed germination was recorded as binary process (1 = germinated and 0 = not germinated) at a particular time, it was possible to model the probability distribution of germination in each treatment over the entire period of observation. The probability of germination was estimated using categorical models and the cumulative probabilities were plotted against time.

RESULTS

Ex-vitro seed germination, seedling survival and growth

Seed germination percentages significantly ($P < 0.01$) varied with the length of soaking in hot water and growing medium, but not with their interaction effect (Table 1). Germination percentages also significantly ($P < 0.01$) varied with the length of soaking in cold water and not with the growing media and the interaction effect of the length of soaking in cold water and growing medium (Table 1). On the other hand, the concentration of gibberellic acid, growing medium and their interactions did not significantly ($P > 0.05$) influence germination percentage (Table 1). Over the 50 day period, the highest (45%) cumulative germination was recorded in the seeds soaked in hot water for 24 h (Figure 1a). Higher and more uniform germination was recorded in the seeds soaked in hot water for 24 h and sown in the growing medium without compost manure (Figure 1a). On the other hand, lower and erratic germination was recorded in the seeds soaked in cold water for 24 h and sown in either growing

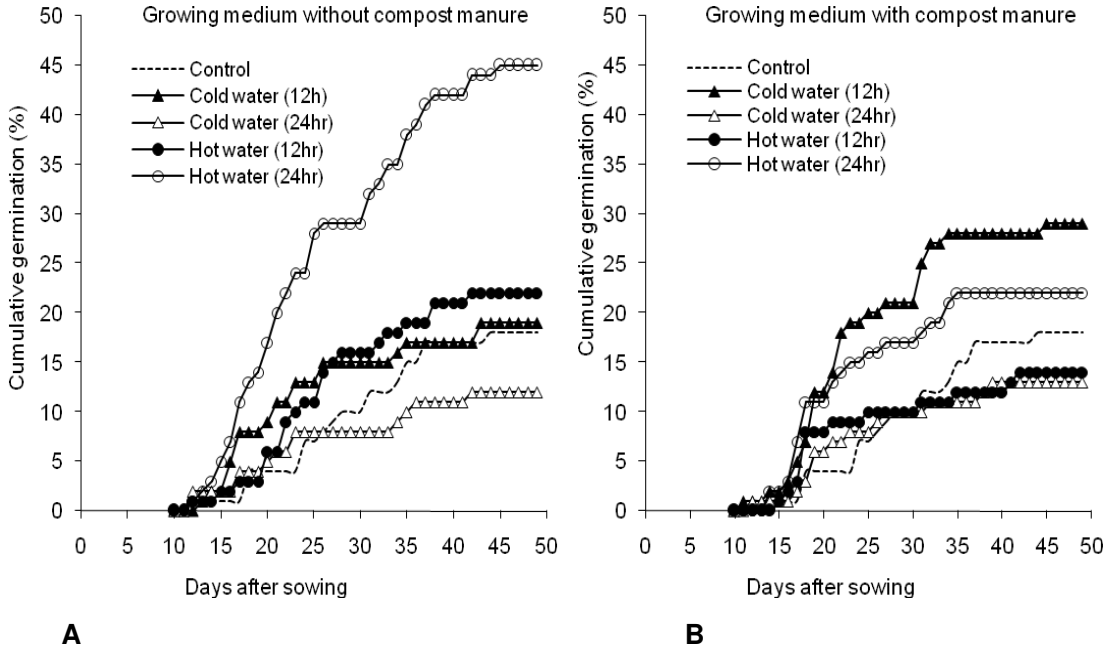

Figure 1. Cumulative distribution of germination of *B. madagascariensis* seeds as affected by soaking in water and growing medium.

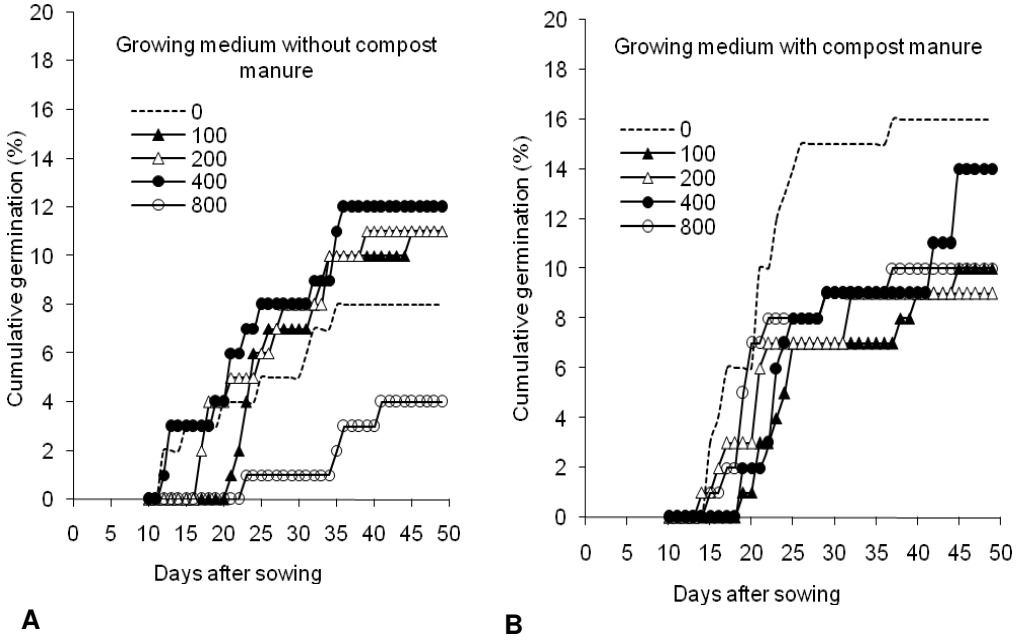

Figure 2. Cumulative distribution of germination of *B. madagascariensis* seeds as affected by soaking in various concentrations of gibberellic acid and growing medium.

medium (Figure 1a, b). Seeds soaked in 400 mg/l of gibberellic acid for 24 h had higher cumulative germination compared with those soaked in 400 mg/l of gibberellic acid when sown in growing medium without compost manure (Figure 2a). When sown in a growing medium with compost manure, cumulative germination

Table 2. The effect of pre-sowing seed treatment and growing medium on the survival rate (%) of *B. madagascariensis* seedlings.

Pre-treatment	Level	Germination (%)		Survival (%)		Height (cm)	
		Without compost	With compost	Without compost	With compost	Without compost	With compost
Control	0	18	18	88.9	40.1	79.2±2.5	57.3±17.8
Cold water	12	19	29	52.6	55.2	74.8±4.7	61.1±3.5
	24	12	13	58.3	84.6	66.9±6.1	58.1±6.6
Hot water	12	22	14	76.2	35.7	69.5±4.1	56.4±4.5
	24	45	22	80.0	31.7	73.8±3.0	60.0±6.9
Gibberellic acid	100	11	10	54.5	50.0	62.3±6.8	43.±7.5
	200	11	9	90.9	55.6	61.2±6.9	42.2±9.7
	400	12	14	66.7	35.4	66.1±6.9	45.8±9.0
	800	4	10	50.0	20.8	55.0±7.5	55.0±1.0

was lower in seeds soaked in gibberellic acid compared with the control (Figure 2b).

Seedling survival ranged from 21 to 92%, the highest (91%) being in seeds soaked in 200 mg/l of gibberellic acid and sown in media without compost (Table 2). Seedling survival did not significantly vary ($P > 0.05$) with the duration of soaking in hot water or various concentration of gibberellic acid. However, it significantly varied ($P < 0.01$) with the growing medium in the case of hot water treatment (Table 1). The growing medium without compost manure after hot water treatment had higher survival (76 to 80%) compared with the one with compost manure (32 to 36%) irrespective of the duration of soaking (Table 2).

Seedling height significantly differed ($P < 0.01$) only with growing medium following either cold or hot water treatment (Table 1). Untreated seeds (control) sown in a medium without compost manure on average grew taller (79.2 cm) than seeds sown in medium with compost (57.3 cm). Similarly, seeds soaked in hot water and sown in the medium without compost grew taller than those sown in medium with compost (Table 2).

In vitro culture

Seeds pre-treated by scoring and soaking gave high germination percentage (70%) compared with those soaked without scoring and cultured on Gamborg's B5 (35% germination) and MS media (30% germination). Explants tested for shoot multiplication on both MS media without PGRs and MS media supplemented with 1 mg/l of both NAA and TDZ neither elongated nor multiplied (Figure 3a). All six *in vitro* germinated seedlings left in B5 media without PGRs for axillary shoot proliferation produced a single shoot, after removing the shoot-tip in seven weeks (Figure 3c). All the six explants tested on B5 media supplemented with 0.1 mg/l of NAA and TDZ produced two shoots each after four weeks (Figure 3d). Explants cultured on Florialite® cubes in B5 liquid media supplemented with 1 mg/l of NAA and IBA with an improved air circulation failed to produce roots after four weeks.

DISCUSSION

This study and the literature (Mbuya et al., 1994) indicated that, *B. madagascariensis* seeds had very low *ex vitro* germination percentages. Efforts in the nursery at Kasisi Agricultural Training Centre (KATC) in Zambia to germinate it achieved less than 30% germination (Lesseps, pers. comm.). Seeds of *B. madagascariensis* could not be uniformly germinated by soaking in water. Seed dormancy caused by the hard seed coat or due to a deficit of endogenous gibberellins may cause low or erratic germination at times taking up to six months, while seeds are still germinating (Masamba, 1994). Treating seeds with gibberellic acid has been reported to overcome dormancy and ensure uniform germination (Al-Absi, 2010; Çetinbaş and Koyuncu, 2006). However, the various gibberellic acid concentrations did not signify-cantly improve germination percentage over the control, which also achieved less than 20% germination. On the other hand, *in vitro* procedures achieved over 70% germination. This is probably because of the controlled culture conditions provided under aseptic conditions for seed germination.

Amendment of the growing medium with compost

A

B

C

D

Figure 3. (A), Shoot-tips cultured on MS medium supplemented with 1 mg/l of NAA and TDZ; (B), MS medium without PGRs showing no multiplication after 4 weeks; (C), axillary shoot proliferation from seedling without shoot tips on B5 without PGRs after 7 weeks; (D) those cultured on B5 supplemented with 0.1 mg/l of NAA and TDZ after 4 weeks.

manure appeared to depress germination, seedling survival and height growth, contrary to earlier reports that compost suppresses disease incidence and increase survival of plants (Hoitink and Boeh, 1999; Mazzola, 2007; Raaijmakers et al., 2008; Termorshuizen et al., 2006). In traditional tree nurseries, growing media are amended with compost manure to improve soil organic matter content, increase soil water holding capacity and suppress diseases (Hoitink and Boehm, 1999; Mazzola, 2007; Raaijmakers et al., 2008; Termorshuizen et al., 2006). The lower germination percentage in the growing medium with compost manure could be attributed to factors such as poor water drainage and nitrogen supply. Brady and Weil (2002) noted that, addition of manure to growing medium increases nitrogen content.

Seeds and vegetative parts of some recalcitrant species may pose challenges at all stages of *in vitro* culture. The seed coat was proved to be the limiting factor in this case to achieve good germination. By scoring the seeds with a sharp blade germination percentage were improved. The poor shoot induction on B5 media is consistent with the findings of Berger and Schaffner (1995). However, the species multiplied well on MS and WP media (Berger and Schaffner, 1995). A half strength MS media containing 26.8 µM (5 mg/l) of NAA was used to effectively induce rooting (Berger and Schaffner, 1995). Further studies are needed to find the optimal culture medium and other growing conditions required to obtain maximum shoot multiplication from juvenile material. Achieving high percentage of rooting from these shoots and the successful transplantation of plantlets to the *ex vitro* conditions are further challenges when considering developing an efficient system of *in vitro* propagation. Initial trials done at CBU showed that, combining rooting and weaning using a photoautotrophic system of growing can be achieved (data not included). Growing cultures in a sucrose-free medium with either forced or diffusive ventilations systems simple photoautotrophic systems can be developed. Plants produced this way in culture will have functional stomata and well developed cuticle once they leave the culture environment to the greenhouse (Sarasan et al., 2006).

Large scale propagation of desired genotypes of *B. madagascariensis* is required for the sustainable utilisation of this important medicinal and pesticidal species. This approach can overcome the reported genetic variability exhibited especially by plants propagated from seed (Berger and Schaffer, 1995). Based on the trials conducted in this study, it is clear that *B. madagascariensis* seeds have very low *ex vitro* germination percentage. However, just developing *in vitro* raised seedlings may not solve the propagation problem to meet the demands in the market place. While *in vitro* cultures can improve germination, axillary multiplication and rooting efforts for rapid multiplication of this species were not satisfactory in this study. By using genetically diverse seed stocks, media and culture conditions can be improved. Therefore, further studies are needed to develop an efficient micropropagation system to produce quality propagules.

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REFERENCES

Atawodi SE (2005). Comparative *in vitro* trypanocidal activities of petroleum ether, chloroform, methanol and aqueous extracts of some Nigerian savannah plants. Afr. J. Biotechnol., 4: 177-182.

Al-Absi KM (2010) The effects of different pre-sowing seed treatments on breaking the dormancy of mahaleb cherries, *Prunus mahaleb* L. seeds. Seed Sci. Tech., 38: 332-340.

Berger K, Schaffner W (1995). *In vitro* propagation of the leguminous tree *Swartzia madagascariensis*. Plant Cell Tissue Organ., 40: 289-291.

Borel C, Hostettmann K (1987). Molluscicidal saponins from *Swartzia madagascariensis* Desvaux. Helv. Chim. Acta, 70: 571-576.

Brady NC, Weil RR (2002). The nature and properties of soils, 13th edn. Pearson Education, New Jersey.

Çetinbaş M, Koyuncu F (2006). Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. Hortscience, 33: 119–123.

Cunningham AB (1993). African medicinal plants: setting priorities at the interface between conservation and primary health care. People and Plants working paper 1, Paris, UNESCO.

Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50: 151-158.

Hoitink HAJ, Boehm MJ (1999). Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Ann. Rev. Phytopathol., 37: 427–446.

Hostettmann K, Schaller F (2000). United States patent No 5,929,124.

Hostettmann K, Marston A (2002). Twenty years of research into medicinal plants: Results and perspectives. Phytochem. Rev., 1: 275–285.

Hostettmann K, Marston A, Ndjoko K, Wolfender J-L (2000). The potential of African plants as a source of drugs. Curr. Org. Chem., 4: 973-1010.

Jaenicke H (1999). Good tree nursery practices. Practical guidelines for research nurseries. World Agroforestry Centre (ICRAF), Nairobi, Kenya. 90 pp.

Jordan M, Larrain M, Tapia A, Roveraro C (2001). *In vitro* regeneration of from seedling explants. Plant Cell Tissue Organ., 66: 89-95.

Kirkbride JH Jr., Wiersema JH (1997). *Bobgunnia*, a new African genus of tribe Swartzieae (Fabaceae, Faboideae). Brittonia, 49: 1-23.

Lwambo NJ, Moyo HG (1991). The molluscicidal activity of seed pods of

Swartzia madagascariensis on Marisa cornuarietis. East Afr. Med. J., 68: 827-30.
Masamba C (1994). Presowing seed treatments on four African Acacia species: appropriate technology for use in forestry for rural development. Forest Ecol. Manage., 64: 105-109.
Mazzola M (2007). Manipulation of rhizosphere microbial communities to induce suppressive soils. J. Nematol., 39: 213–220.
Mbuya LP, Msanga CK, Ruff, CK, Birnie A, Tengas B (1994). Useful trees and shrubs for Tanzania: identification, propagation and management for agricultural and pastoral communities. Regional Soil Conservation Unit (RSCU), Swedish International Development Authority (SIDA).
Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.
Ouattaraa Y, Sanonb S, Traoréc Y, Mahioud V, Azase N, Sawadogoa L *(*2006). Antimalarial activity of *Bobgunnia madagascariensis* Desv. (leguminosae), *Combretum glutinosum* Guill. & Perr. (Combretaceae) and *Tinospora bakis* Miers. (Menispermaceae), Burkina Faso medicinal plants. Afr. J. Trad. Compl. Alt. Med., 3: 75-81
Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2008). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil DOI 10.1007/s11104-008-9568-6
Sarasan V, Cripps R, Ramsey MM, Atherton C, McMichen M, Prendergast G, Rowntree JK (2006). Conservation *in vitro* of threatened plants; progress in the past decade. In Vitro Cell Dev. Biol. Plant, 42: 2006-214.
Schaller F, Rahalison L, Islam N, Potterat O, Hostettman K, Stoeckli-Evans H, Mavi S (2001). A new potent antifungal 'Quinone methide' diterpene with a cassane skeleton from *Bobgunnia madagascariensis*. Helv. Chim. Acta, 84: 222-229.
Sileshi G, Kuntashula E, Matakala P, Nkunika PO (2008). Farmers' perceptions of pests and pest management practices in agroforestry in Malawi, Mozambique and Zambia. Agrofor. Syst., 72: 87-101.
Smith P, Allen Q (2004). Field guide to the trees and shrubs of the Miombo woodlands. Royal Botanic Gardens, Kew, pp. 176.
Suter R, Tanner M, Borel C, Hostettmann K, Freyvogel TA (1986). Laboratory and field trials at Ifakara (Kilombero District, Tanzania) on the plant molluscicide *Swartzia madagascariensis*. Acta Trop., 43: 69-83.
Termorshuizen AJ, van Rijn E, van der Gaag DJ, Alabouvette C, Chen Y, Lagerlöf J, Malandrakis AA, Paplomatas EJ, Rämert B, Ryckeboer J, Steinberg C, Zmora-Nahum S (2006). Suppressiveness of 18 composts against 7 soilborne plant pathogens. Soil Biol. Biochem., 38: 2461–2477.
Vermeulen SJ, Campbell BM, Matzke GE (1996). The consumption of wood by rural households in Gokwe communal area, Zimbabwe. Hum. Ecol., 24: 479-491.