Journal of Horticulture and Forestry

Volume 7 Number 1 January 2015
ISSN 2006-9782

Academic Journals

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Vol. 7(1), pp. 1-7, January, 2015
DOI: 10.5897/JHF2014.0370
Article Number:13B4D5149545
ISSN 2006-9782 ©2014

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Journal of Horticulture and Forestry

Full Length Research Paper

Effect of *Aloe vera* gel coating on postharvest quality and shelf life of mango (*Mangifera indica* L.) fruits Var. 'Ngowe'

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Received 16 September, 2014; Accepted 6 November, 2014

Mango is a highly perishable fruit and high post-harvest losses occur in Africa. In order to address this problem, 4 concentrations of *Aloe vera* gel (AG) (0, 25, 50 and 75%) and chitosan (1%) were tested at two temperature levels (room temperature 15-22°C and 13°C) to determine their effect on the postharvest life of mango (var.'Ngowe'). The experimental design was a 5 by 2 factorial experiment embedded in a complete randomized design with three replications. Data were recorded on titratable acidity, fruit colour, ascorbic acid and anthracnose disease incidence. The results showed that at both temperatures 50 and 75% Aloe concentrations significantly increased the shelf life evidenced by reduced decrease in titratable acidity. Fruit colour and ascorbic acid were also maintained for longer periods in these treatments. Findings of this study demonstrate the potential of using *Aloe vera* gel at 50% as a coating for improved postharvest shelf life and maintaining quality of mango fruits hence reduced postharvest losses.

Key words: *Aloe vera* gel, postharvest shelf life, mango.

INTRODUCTION

Mango (*Mangifera indica* L.) is the most economically important fruit of the Anacardiaceae family (Tharanathan et al., 2006). World trade in mangoes has been increasing over the years, and both exports from Kenya and local consumption is growing. The world market continues to become more price-competitive in spite of postharvest challenges e.g. losses caused by diseases (HCDA, 2011). Mango is one of the most popular fruits all over the world as it has an attractive color, delicious taste and excellent nutritional properties. However, mango fruits are climacteric and ripen rapidly after harvest, this limits their storage, handling and transport potential (Lalel et al., 2003).

Mango is an easy access to post-harvest disease infection and production and consumption imbalances after harvesting lead to considerable losses (Zeng et al., 2006). Therefore, scientists are working towards prolonging the shelf life of the fruit by slowing down the ripening process while maintaining quality and flavor. Fruit coating after harvesting is becoming popular in this respect (Gill et al., 2005). However, possible health risks associated with the residue of the coating materials like fungicides are reducing the scope of coatings. Edible coatings have no residue associated risks and are

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possible alternative options (Ergun and Satici, 2012).

The use of *Aloe vera* gel has drawn interest in the food industry (Arowora et al., 2013). *A. vera* based edible coatings have been shown to prevent loss of moisture and firmness, control respiration rate and development and maturation, delay oxidative browning, and reduce microorganism proliferation in fruits such as sweet cherry, table grapes and nectarines (Valverde et al., 2005; Matinez-Romero et al., 2005; Ahmed et al., 2009). In addition to the traditional role of edible coatings as a barrier to water loss and delaying fruit senescence, the new generation coatings are being designed for incorporation and/or for controlled release of antioxidants, nutraceuticals, chemical additives and natural antimicrobial agents. It has also been reported that *A. vera* extracts possess antimicrobial activity against gram positive and gram negative bacterial pathogens (Adetunji, 2008).

The use of *A. vera* gel as an edible surface coating has been reported to prolong the shelf life and to delay changes in parameters related to deterioration of quality in sweet cherry and table grapes (Martinez- Romero et al., 2006; Serrano et al., 2006), yet no studies have demonstrated the use of *A. vera* natural plant extract based on its antifungal properties on enhancement of shelf life and quality of mango fruits. Therefore, this study was conducted with the objective of evaluating the effects of the different *A. vera* gel on postharvest life of mango fruits.

MATERIALS AND METHODS

Research site

The postharvest study was carried out in a laboratory at Egerton University, Njoro, Kenya. The laboratory lies at a latitude of 0° 23' South, longitude 35° 35' East, altitude of approximately 2,238 m a.s.l in the Lower Highland 3 (LH3) agroecological zone (Jaetzold and Schmidt, 1983). The laboratory records average maximum and minimum temperatures of 19 to 22°C and 5 to 8°C, respectively (Egerton Metrological Station, 2009).

Materials

Mango

The variety 'Ngowe' was used. 'Ngowe' is a popular mango variety in Kenya, which has little fibre and has excellent eating quality but it is susceptible to anthracnose. All the fruits that were used in this study were acquired from a grower in Masii in Machakos County, Kenya. The fruits were harvested at the mature green stage having no visible blemish. The fruits were transported to the laboratory on the same day.

Aloe vera

Leaves of *A. vera* were harvested from Lare in Nakuru County, Kenya. Only the fully extended mature leaves were harvested. The leaves were then stored in plastic papers and transported to the laboratory within same day.

Chitosan

Crushed chitosan powder food grade was purchased from Kobian Chemicals Company, Nairobi.

Preparation of coating solutions

Aloe gel was obtained from fresh aloe leaves, the matrix was separated from the outer cortex of the leaves and the colourless hydroparenchyma homogenized in a blender. The resulting mixture was filtered using Whatman filter paper number 100 to remove the fibres. The liquid constituted fresh *A. vera* gel. The gel matrix was pasteurized at 70°C for 45 min. For stabilizing, the gel was cooled immediately to an ambient temperature and 4.5 g of ascorbic acid was added; 4.5 g of citric acid was then added to adjust the pH to 4. To prepare chitosan coating, 1% Chitosan (Kobian Chemical Co.) was dissolved in a 0.5% glacial acetic acid and distilled water. The pH value of the chitosan solution was then adjusted to 5.6 using 0.1 M NaOH.

Application of treatments and experimental design

The coating solutions were: aloe gel (0%) as a negative control, aloe gel (25%), aloe gel (50%), aloe gel (75%), and chitosan (1%) as a positive control. Fresh fruits were dipped completely into the coatings solutions at room temperature for 25 min. The fruits were allowed to drain and then dried at room temperature to allow a thin film layer to be formed on the fruits. The fruits were then stored at room temperature and at 13°C. Mature, green fruits, without any visible blemish, were selected and the pedicels were removed. The fruits were then randomly divided into eight lots of twenty fruits each. The first lot constituted the positive control and was coated with chitosan. The second, third, fourth and fifth lots were coated by dipping completely in *A. vera* gel at concentrations of 0, 25, 50 and 75% respectively and stored at room temperature and at 13°C. The experiment was laid out as a 5 by 2 factorial experiment embedded in a completely randomized design with three replications. Various parameters were evaluated at 4 day intervals until the overall acceptability became unsatisfactory for each lot of samples (the fruit was considered as waste when it is infected by disease.

Titrable acidity (TA)

TA was determined by titrating 100 ml of juice against sodium hydroxide having concentration of 0.1 N (AOAC 2000) and expressed as the percentage of citric acid per 100 g fresh mass. Individual mango fruit from each treatment was ground in a blender to obtain freshly prepared juice which was then filtered using filter papers.

Colour

Peel color was measured at the equator on opposite cheeks of the fruit. Flesh color was measured in the center of one cut cheek. Both peel and flesh colours were measured using portable whiteness colourimeter (WSD-3 TYPE). Measurements were recorded using standard Hunter L a b chromatic system and were expressed as lightness (L), greenness (-a), redness (+a) yellowness (+b), blueness (-b) colour space coordinates. The instrument was calibrated with a standard white ceramic tile and black tile and set up for D65 as illuminate and a 10° observer angle.

Vitamin C content

This was determined by titrating 10 g of mixed pulp sample against

Table 1. Effect of *Aloe vera* gel on Anthracnose severity and anthracnose disease incidents.

Treatment	Severity index		Anthracnose incidence (%)
	% skin area	scores	
Chitosan	12.5[a]	2	45
0% Aloe	63.6[a]	4	93
25% Aloe	57.1[a]	4	80
50% Aloe	50.0[a]	3	73
75% Aloe	45.6[a]	3	60

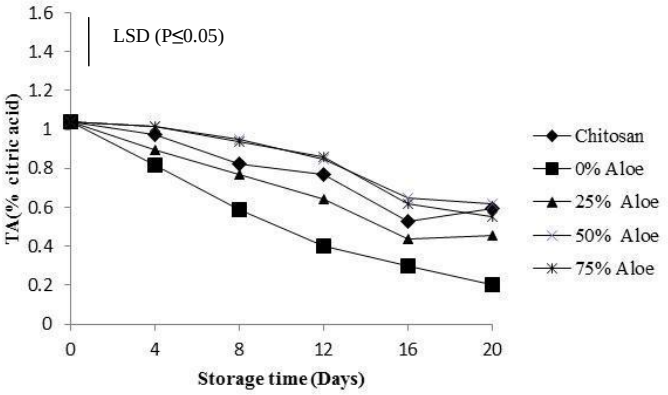

Figure 1. Titratable acidity of mango fruits var. 'Ngowe' as affected by *A. vera* gel coatings

the standard 2, 6 dichlorophenol dyes following the procedure outlined in AOAC (2000).

Degree and rate of anthracnose incidents

Anthracnose severity was assessed by measuring the diameter of anthracnose lesions on mango fruits and ranked by use of scale 1–5 where 1=0% of fruit surface rotten, 2=1–25%, 3=26–50%, 4=51–75% and 5=76–100%)

Data analysis

The data collected was subjected to Analysis of Variance (ANOVA) at P ≤ 0.05, using PROC GLM code of SAS (version 9, 2005) and means for significant treatments separated using the Tukey's Honestly Significant Different Test at P ≤ 0.05.

RESULTS AND DISCUSSION

Titratable acidity

Mango fruits with coating presented a statistically higher titratable acidity (TA) during storage in spite of the slight decrease observed (Figure 1). TA increased during storage in all treatments but the rate of increase in treated fruits was comparatively slower compared to the control. At day zero there was no significant difference (P≤0.05) between the treatments (Table 1). The initial TA was 1.04% citric acid. In day four, fruits coated with 50 and 75% *A. vera* gel concentration had a significantly lower TA value compared with those coated with 0% *A. vera* gel.

On day-8, fruits coated with 50% *A. vera* gel had a significantly lower TA values compared with those coated with other fruit coating treatments. On day-12, the highest TA was observed in fruits coated with 75% *A. vera* gel and the lowest readings were recorded for fruits coated with 0% *A. vera* gel. At day sixteen of the storage period, the highest TA was observed on fruits coated with 50% *A. vera* gel and the lowest readings were recorded for fruits coated with 0% *A. vera* gel. At day at the end of storage period (twenty days), fruits coated with 50% *A. vera* gel had the highest TA while the control had the lowest TA value.

Generally TA was maintained for those fruits coated with 50 and 75% *A. vera* gel. TA decreased gradually in all treatments but the rate was slower in treated fruits compared to negative control. There was a lower decrease in the titratable acidity for *A. vera* and chitosan coated mangoes. *A. vera* gel and chitosan coating must have modified internal atmosphere thus reducing ripening and maintenance of the TA (Nabigol and Asghari, 2013). Reduction in TA for uncoated fruits is due to conversion of acids into sugars and their further utilization in the metabolic processes of the fruit. Doreyappa and Huddar (2001) reported the similar pattern in different varieties of mango fruits stored at 18 to 34°C. They observed a series of physico-chemical changes during ripening and the major changes were decrease in acidity. The acidity of the fruit is an important character to determine its quality and acceptability. Very high or very low values of the acidity are not recommended for good fruit.

Fruit colour

The coatings were effective on peel colour change of the mangoes stored under room temperature conditions and 13°C (colour change during ripening for this variety of mango is from green to yellow). Color changes in peel are presented as L*, a*, b* and were expressed as lightness (L*), greenness (-a*), yellowness (+b*), colour space coordinates. Fruits from each treatment for both

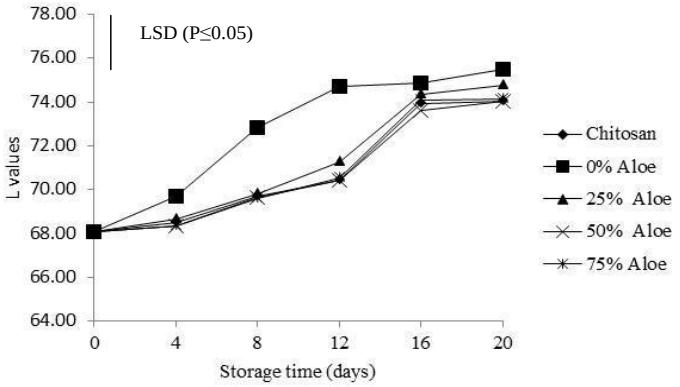

Figure 2. Effect of different *A. vera* gel concentrations on L* value of the peel colour of mango fruits var. 'Ngowe'.

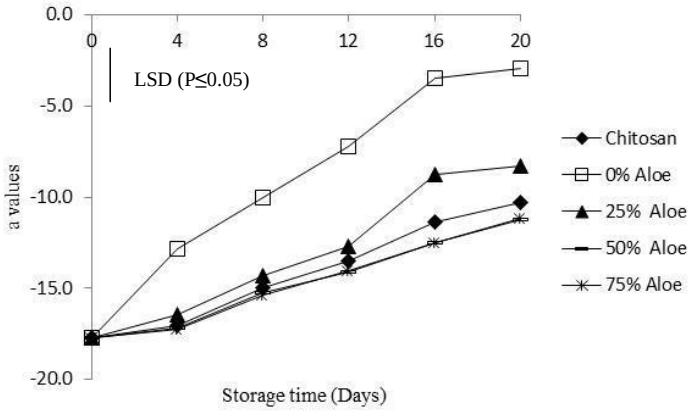

Figure 3. Effect of different *Aloe vera* gel concentrations on Chromatic a* of the peel color of mango fruits var. 'Ngowe'.

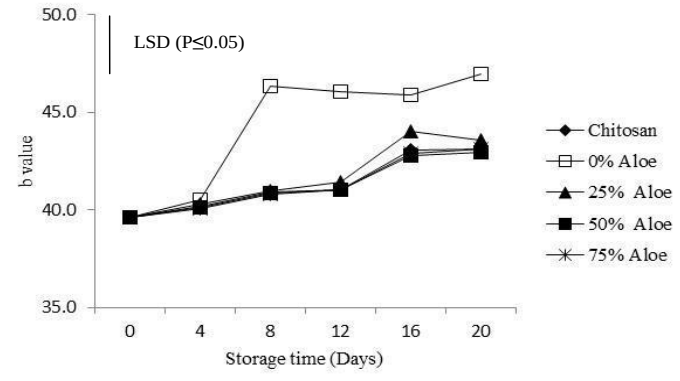

Figure 4. Effect of different *Aloe vera* gel concentrations on Chromatic b* of the peel color of mango fruits var. 'Ngowe'.

trials registered some changes in chromatic L*, a* and b* colour values during the storage period (Figures 2, 3 and 4 respectively).

Lightness (L*) increased significantly (P≤0.05) during storage, changes in L* value for control (L* values increased from 68.1 to 75.5) which was higher than what was recorded in the coated fruits (Figure 2). L* values increased over time irrespective of the coatings from 68.1 to 75.5 for negative control, from 68.1 to 74.8 for fruits coated with 25% *A. vera* gel, from 68.1 to 74.0 for 50% *A. vera* gel coated fruits, from 68.1 to 74.1 for 75% *A. vera* gel coated fruits and from 68.1 to 74.0 for chitosan coated fruits while no significant difference (P≤0.05) were recorded for chitosan and 50% *A. vera* gel coated treatments at the end of the storage period.

Chromatic a* value from mango fruits also increased over time irrespective of the treatments. There was a gradual significant increase (P≤0.05) in peel a* value beginning on day eight (Figure 3). Before the storage, the a* value was -17.7, and after the storage the value reached to -2.9 for negative control fruits, to -8.3 for 25% *A. vera* gel coated fruits, to -11.2 for 50% *A. vera* gel coated fruits,-11.2 for 75% *A. vera* gel coated fruits and to -10.3 for chitosan coated fruits. The increase in a* value was however, slower for fruit coated with 50 and 75% *A. vera* compared to control or 25% *A. vera* gel treatments.

Chromatic b* value similar to L* and a* value for fruits slightly increased over time regardless of the coatings, and it was significantly higher (P≤0.05) on day eight for the negative control (Figure 4). Initial b* value was 39.6, afterwards the value gradually increased, reaching to 46.9 for negative control fruit, to 43.5 for 25% *A. vera* gel coated fruits, to 42.9 for 50% *A. vera* gel coated fruits, 43.1 for 75% *A. vera* gel coated fruits and to 43.1 for chitosan coated fruits. The increase in a* value was however, slower in fruits coated with 50 and 75% *A. vera* gel and chitosan compared to negative control.

Generally the peel color of the mango fruits coated with 50 and75% *A. vera* gel was significantly less developed than those coated with other treatments. The coatings were effective on flesh colour change of the mangoes under room temperature conditions and 13°C (colour change during ripening for this variety 'Ngowe' is from green to yellow). Color changes in peel are presented as L*, a*, b* and were expressed as lightness (L), greenness (-a), blueness (+a), yellowness (+b), colour space coordinates. Fruits from each treatment for both trials registered some changes in chromatic L*, a* and b* colour values during the storage period (Figures 5, 6 and 7 respectively).

The L* (lightness) decreased during storage, L* values for control decreased from 88.1 to 72.5 which was lower than the coated fruits (Figure 5). L* values decreased over time irrespective of the treatments from 88.1 to 72.5 for negative control, from 88.1 to 75.8 for 25% *A. vera* gel coated fruit, 76.0 for 50% *A. vera* gel coated fruits, 76.6 for 75% *A. vera* gel coated fruits and 76.0 for chitosan coated fruits. There was no significant difference (P≤0.05) between fruits coated with chitosan and 50% *A.*

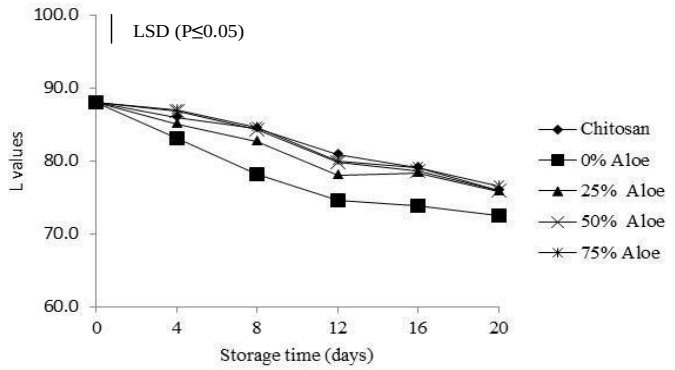

Figure 5. Effect of different *Aloe vera* gel concentrations on Chromatic L* value of the flesh color of mango fruits var. 'Ngowe'.

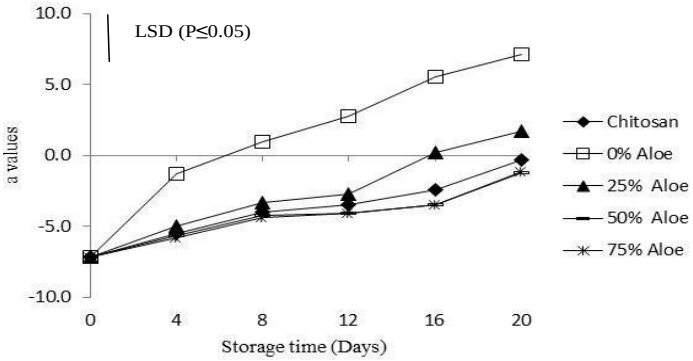

Figure 6. Effect of different *Aloe vera* gel concentrations on Chromatic a* of the flesh color of mango fruits.

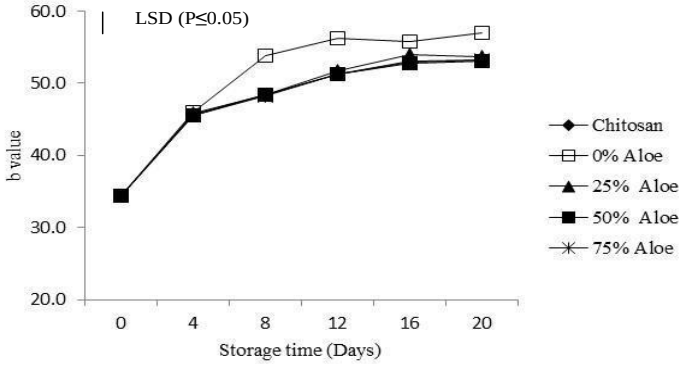

Figure 7. Effect of different *Aloe vera* gel concentrations on Chromatic b* of the flesh color of mango fruits var. 'Ngowe'.

vera gel coated treatments at the end of the storage period.

Chromatic a* value from mango fruits also increased over time irrespective of the treatments. There was a gradual increase in flesh a* value beginning on day eight (Figure 6). Before storage, a* value was -7.2, and after the storage the value increased to 7.1 for negative control fruits. It also increased to 1.7 for fruits coated with 25% *A. vera* gel, -1.2 for fruits coated with 50% *A. vera* gel, -1.2 for 75% *A. vera* gel coated fruits and to -0.3 for chitosan coated fruit. The increase in a* value was however, slower for fruit coated with 50 and 75% *A. vera* compared to negative control or 25% *A. vera* gel treatments. There was a significant difference (P≤0.05) among the treatments at the end of the storage period.

Chromatic b* value gradually increased over time regardless of the treatments, and it was significantly higher (P≤0.05) on day eight for fruits coated with 0% *A. vera* gel (Figure 8). Initial b* value was 34.4 and it gradually increased, reaching to 57.0 for fruits coated with 0% *A. vera* gel, to 53.6 for 25%, to 53.0 for 50% , 53.2 for 75% *A. vera* gel and to 53.2 for chitosan coated fruits. The increase in b* value was however, slower for fruit coated with 50 and 75% *A. vera* and chitosan compared to 0% *A. vera* gel. There was a significant difference (P≤0.05) among the treatments at the end of the storage period.

Generally the flesh color of the mango fruits coated with 50 and75% *A. vera* treatments was significantly less developed than flesh colours in the other treatments. Color is related to the presence of different pigments. Changes in colour are mainly due to chlorophyll transformation into other pigments and to the synthesis of carotenoids and anthocyanins. Chlorophyll retention was higher in the fruits coated with *A. vera* gel and chitosan coatings and least of it was seen in the uncoated fruit. *A. vera* gel treatment and chitosan delayed the green colour loss on the fruit skin. Coatings applied to fruit act as a barrier, altering permeability to gases. This results in increased internal CO_2 contents, slowing down the external and internal colour change of the fruit in return delaying chlorophyll degradation and carotenoid synthesis (Ergun and Satici, 2012). Similar results of colour retention in coated fruit had been reported in carambola fruits (Neeta et al., 2013).

Ascorbic acid

The ascorbic acid content in the mango fruits decreased significantly during the ripening storage period (Fig.8). A significant decrease ($P \leq 0.05$) in ascorbic acid contents was observed in all the treatments. Initially, the ascorbic acid was 26.6 mg/100 g. At day four, there was significant difference ($P \leq 0.05$) between the negative control (0% *A. vera* gel) and the other treatments but there was no significant difference among 50 and 75% *A. vera* gel concentrations and those coated with 1% chitosan (the positive control). At day eight, 50% *A. vera gel* was the most effective in reducing decrease in ascorbic acid followed by chitosan while the 0% aloe had the least ascorbic acid. At day twelve, there was

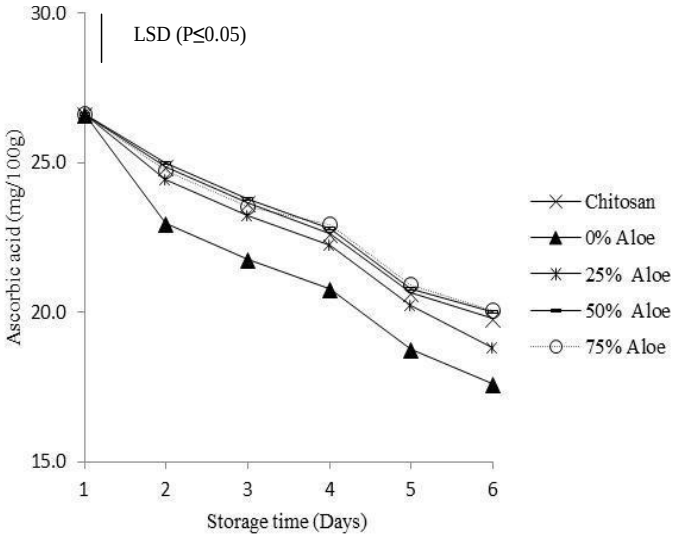

Figure 8. Ascorbic acid of mango fruits var. 'Ngowe' as affected by *Aloe vera* gel coatings.

significant difference between the control and the rest of the treatments, 75% *A. vera* gel had the highest ascorbic acid.

At day sixteen, there were significant effects between the control and the other treatments 25, 50 and 75% *A. vera* gel and chitosan treatments. The negative control had the lowest ascorbic acid while75% *A. vera* gel had the highest ascorbic acid value. At day twenty, 0% *A. vera* gel had the lowest ascorbic acid while 75% *A. vera* gel had the highest ascorbic acid among the other treatments. Generally, all treatments had a decrease in ascorbic acid from the initial value. There was a gradual decrease in all days although the decrease was reduced by *A. vera* treatments. Ascorbic acid is one of the most abundant antioxidants present in fruits. Results suggest that *A. vera* gel and chitosan coating caused lower losses of antioxidant capacity by the end of storage, when coated fruits were compared to the negative control. Application of *A. vera* gel and chitosan modified internal atmosphere, more concentration of CO_2 resulting to lower concentration of O_2 hence the oxidation process was retarded which caused reduction in conversion of ascorbic acid to dehydro ascorbic acid. Lal et al. (2003) reported similar results on mango using various chemicals under different storage period.

Anthracnose incidence

None of the *A. vera* gel concentration tested inhibited the growth of *Colletotrichum gloeosporioides* as compared to the control, and grew almost similarly with all treatments through the 7-day incubation period. Mango fruits treated with 1% chitosan had the lowest disease severity index. The highest fungicidal effect was observed in those mangoes coated with 1% chitosan.

Conclusion AND Recommendation

Findings of this study demonstrate the potential of using *A. vera* gel as a coating for improved postharvest shelf life and maintaining quality of mango fruits hence reduced postharvest losses. The results showed that at both temperatures, 50 and 75% aloe concentrations significantly increased the shelf life evidenced by reduced decrease in titratable acidity. Fruit colour and ascorbic acid were also maintained for twenty days in these treatments. Since *A. vera* is an edible plant, does not pose any environmental hazard and is easily available in Kenya and other tropical regions*, A. vera* at 50% concentration can be used as an alternative fruit coating for mangoes.

Conflict of Interest

The authors have not declared any conflict of interest.

AKNOWLEDGEMENTS

Author wish to express his sincere thanks for the financial support received from National Council of Science Technology and Innovation (NCST) without which this work would not have been a success. Am also grateful to the Department of Crops, Horticulture and Soils of Egerton University for their support.

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Vol. 7(1), pp. 8-15, January, 2015
DOI: 10.5897/JHF2014.0367
Article Number: B77B67549548
ISSN 2006-9782

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Journal of Horticulture and Forestry

Full Length Research Paper

Propagation of Cabralea canjerana by mini-cutting

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Received 27 August, 2014, Accepted 16 October, 2014

Canjerana (*Cabralea canjerana* (Vell.) Martius) is a tree species indigenous to Brazil that when grown and managed in plantation systems are of great ecological and economic importance. Due to the difficulty of producing seminal seedlings, we examine the possibility of vegetative propagation by evaluating the rooting potential of canjerana mini-cuttings with different concentrations of indolbutyric acid (IBA) and substrate combinations. Mini-cuttings were treated with 2000 mg/L of IBA and planted in commercial substrate; coarse sand; carbonized rice husks; and a combination of the two. Apical and nodal mini-cuttings were treated with 0, 1000, 2000 and 3000 mg/L of IBA and planted in a combination of commercial substrate, coarse sand and carbonized rice husks. A mini-clonal hedge was formed with three clones of canjerana to evaluate mini-stump productivity and mini-cutting rooting. The combination of commercial substrate, coarse sand and carbonized rice husks maximized mini-cuttings rooting. Nodal mini-cuttings had higher rooting capability than apical ones. The application of 3000 mg/L of IBA improved rooting differentiation and growth of canjerana mini-cuttings. Canjerana clones differ in rooting capability and survival rates in vegetative propagation systems, but the use of a mini-cutting propagation system is a feasible production technique for this important species.

Key words: Vegetative propagation, miniclonal hedge, mini-cutting rooting, indolbutyric acid.

INTRODUCTION

Cabralea canjerana (Vell.) Martius, known as canjerana in southern Brazil, is a native tree species that belongs to the Meliaceae family. Canjerana is a common species in some Atlantic Forest fragments, and it is very important for forest regeneration programs in degraded areas (Nobrega et al., 2008). The wood is considered to be one of the most valuable, with excellent quality characteristics and high resistance to the attack of xylophage insects. The stem bark can be used for the extraction of a commercially important red dye. Both the stem bark and the roots have medicinal properties and can be used as a purgative, febrifuge, abortifacient, antidyspeptic, astringent and emetic (Carvalho, 2006). Extracts from leaves and seeds can affect the development of *Ascia monuste orseis* larvae, reducing leaf consumption in cabbage (Mata and Lomonaco, 2013).

The seeds of canjerana present recalcitrant storage behavior, with a drastic reduction in germination after 15 days of beneficiation (Grunennvaldt et al., 2014), which is an obstacle for the production of seminal seedlings. Vegetative propagation enables the production of plantlets in any season and the establishment of specific

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and favorable combinations of important characters, as well as the possibility of fixing superior genetic interactions and high levels of heterozygosity (Zobel, 1993; Gaspar et al., 2005). As canjerana provides wood and other products, the development of efficient clonal propagation techniques for superior genotypes will increase management and productivity of commercial plantations, thereby contributing to local economies.

The use of mini-cuttings is one of the most recent techniques for vegetative propagation. The mini-cutting technique can enhance propagation efficiency and was developed to overcome the rooting problems of some adult plants of the genus *Eucalyptus* (Wendling et al., 2003). Mini-cuttings from rejuvenated propagules are used for regeneration and mass production of woody species plantlets (Hartmann et al., 2011). In some clones of *Eucalyptus* the mini-cuttings from rejuvenated plant material develop a better root system than is found in mini-cuttings from non-rejuvenated tissue (Wendling and Xavier, 2005). Research results with other tree species indicate the viability of using rooted mini-cuttings for commercial propagation (Dumroese et al., 2003; Xavier et al., 2003; Wendling et al., 2007; Lima et al., 2009; Silva et al., 2010; Dias et al., 2012).

In the plantlet production from mini-cuttings, the substrate plays a fundamental role in root initiation and growth. It keeps the mini-cuttings in an upright position, provides a matrix for needed water and nutrients, and improves root differentiation and growth. Because of these specific but multifaceted requirements, the best substrate is usually composed of a variety of different materials that fulfill these physical functions and facilitate the hormonal stimulation of the desired growth responses, as well as providing availability of needed assimilates for rooting and plant health (Fonteno et al., 1981; Smart et al., 2003). Frequently in forest species, the inclusion of exogenous indolbutyric acid (IBA) has been shown to improve root differentiation (Wise et al., 1985; Rosier et al., 2004; San José et al., 2012). However, mini-cuttings of some woody species have been shown to root even without the application of IBA (Silva et al., 2010; Ferreira et al., 2010).

The choice of the substrate for the production of plantlets should take into consideration physical factors, such as texture and density, which interfere with aeration, water retention capacity and aggregation of the substrate (Wendling and Gatto, 2002). A good substrate must provide sufficient porosity to allow good aeration and have a high water retention capacity (Hartmann et al., 2011). Recommendations in the literature vary from an addition of 20 to 40% or a reduction of 60 to 80% of porous material (carbonized rice husks, coconut fiber, pine needles, compost, etc.) for the substrate composition for different species and propagation conditions (Wendling and Gatto, 2002). Substrates with air-filled pore space of 30%, with total porosity of 85% and water availability between 24 and 40% were considered as ideal conditions for seedling production (Schimitz et al., 2002), but no such information was found for mini-cuttings rooting. The three individual substrates, carbonized rice husks, commercial substrate, and coarse sand, and their combinations exhibit different physical properties from those considered ideal by Schimitz et al. (2002) for seedlings production. Carbonized rice husks increase the total porosity and air-filled pore space in the combination of substrates, but not to the optimum level suggested by Schimitz et al. (2002). This probable explains why the best substrate is usually obtained by using a mixture of components to ensure nutritional balance and diverse microbial flora (Wendling and Gatto, 2002). Given the ecological and economic importance of canjerana and the difficulty of producing seminal seedlings, this study was intended to examine the possibility of vegetative propagation by mini-cuttings. The objective was to evaluate the rooting potential of canjerana mini-cuttings with different concentrations of IBA and substrate combinations.

MATERIALS AND METHODS

The study was carried out in an acclimatized greenhouse of polycarbonate 10 mm panels with maximum temperature set to 32°C. For the experiments, one-year-old seedlings of canjerana were placed in plastic pots containing 300 cm^3 of a commercial substrate (organic pine bark base) and submitted to drastic pruning (coppice) to produce the sprouts that were used for mini-cutting preparation after 60 days. The coppice seedlings were manually irrigated twice a week with a nutrient fertilizer solution with 50% of the salt concentrations as described by Wendling et al. (2007). The mini-cuttings were grown in a vertical position in polyethylene trays (55 × 34 × 15 cm) containing the substrate and maintained in a greenhouse with a system of intermittent misting, mean air temperature of 25°C, and relative humidity of approximately 80%. The high relative humidity was maintained by nebulizers, triggered by a timer. After evaluating rooting and survival in a mist chamber, the rooted mini-cuttings were acclimatized in a greenhouse for 30 days and used for the formation of the mini-clonal hedge.

The first experiment evaluated the effect of the substrate on mini-cutting rooting and survival. The bases of single bud mini-cuttings, 1.5 to 2.0 cm long containing a half leaflet, were immersed for 10 s in a 50% water/ethanol solution containing 2000 mg/L of IBA and placed in one of the four substrates: commercial (organic pine bark base); coarse sand; carbonized rice husks; and the combination of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks. Samples of these substrates were collected, dried at 65°C for 48 h and submitted for physical analysis (Fermino, 2003). Carbonized rice husks and the mixture of commercial substrate, coarse sand and carbonized rice husks were the substrates with air-filled pore space that came closer to the optimal value of 30% (Table 1). After 60 days of cultivation, mini-cuttings were evaluated for rooting and survival and the number and total length of roots (cm). The experiment was a complete random design with five replicates of four mini-cuttings.

The second experiment evaluated the type of mini-cuttings (apical and nodal) of 1.5 to 2.0 cm in length containing a half leaflet. The bases of mini-cuttings were immersed for 10 s in a 50% water/ethanol solution containing either 0, 1000, 2000 or 3000 mg/L of IBA. The control treatment consisted of a 50% water/ethanol solution. A mixture of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks was used as substrate. The rooting and survival of the shoots and the number

Table 1. Physical properties of the substrates used for the evaluation of the rooting capacity of canjerana.

Substrates[1]	Dry density (kg/m^3)	Total porosity (%)	Aeration space in saturated substrate (%)	Water availability (%)
Comercial	335	50	15.4	5.4
Coarse Sand	1505	27	12.6	11.5
CRH	167	41	31.4	2.8
Comercial+CS+CRH	56	43	23.9	4.4

[1]Commercial: organic pine bark base; CS: coarse sand; CRH: carbonized rice husks.

Table 2. Percentage survival and rooting and number and length of roots of mini-cuttings of canjerana treated with 2,000 mg/L of indolbutyric acid and grown in different substrates after 60 days of cultivation.

Substrates[1]	Survival (%)	Rooting (%)	Number of roots	Length of roots (cm)
Comercial	70.0[b2]	15.0[b]	1.8[a]	3.3[a]
Coarse sand	70.0[b]	45.0[b]	1.8[a]	2.5[a]
CRH	85.0[b]	30.0[b]	1.8[a]	2.7[a]
Comercial+CS+CRH	100.0[a]	75.0[a]	1.7[a]	3.0[a]
Mean	81.2	41.2	1.8	2.9
CV (%)	14.1	39.4	14.4	8.6

[1] Commercial: organic pine bark base; CS: coarse sand; CRH: carbonized rice husks. [2] Means values followed by the same letter in a column are not significantly different by the Tukey's test at the probability of 5%.

and total length of roots (cm) per mini-cutting were evaluated after 60 days of cultivation. The experiment was a 2 x 4 factorial (apical and nodal mini-cuttings by IBA concentrations) in a complete random design with five replicates of four mini-cuttings.

The third experiment evaluated the productivity of individual mini-stumps and rooting of mini-cuttings of three clones of canjerana. The mini-clonal hedge was established in a soilless system subirrigated with nutrient solution (Bandinelli et al., 2013). Twelve rooted mini-cuttings (complete plantlets) per single stock plant of canjerana were planted at 10 x 10 cm spacing in one tray to form the mini-clonal hedge and given a 15 min daily irrigation with a nutrient solution at 50% concentration of salts as described by Wendling et al. (2007). The pH of the nutrient solution was maintained between 5.5 and 6.0 and an electrical conductivity in 1.5 dS m^{-1}. The sprouts of three consecutive harvesting times of the three clones were used to prepare mini-cuttings of 1.5 to 2.0 cm in length containing a half leaflet. The mini-cuttings were treated with 3000 mg/L of IBA solution for 10 s and planted in a combination of equal proportions by volume of commercial substrate, coarse sand and carbonized rice husks. The number of mini-cuttings per mini-stump was recorded and the percentage of survival and rooting were evaluated at 60 days after planting. The experiment was a complete random design with eight replicates of six mini-cuttings.

Data were submitted to analysis of variance and for those variables with significant differences ($p \leq 0.05$), treatment means were compared by Tukey test or polynomial regression, as appropriate. For purposes of analysis, percentage data were transformed to arcsin $\sqrt{x/100}$ and counting data to $\sqrt{x+0.5}$ to attend the statistical presuppositions. All analysis well done with the ESTAT (UNESP - Jaboticabal) program.

RESULTS AND DISCUSSION

The different substrates affected the rooting and survival percentages of canjerana mini-cuttings ($p \leq 0.05$). The combination of commercial substrate, coarse sand and carbonized rice husks resulted in the highest percentage of mini-cutting rooting (Table 2). The number and length of roots per mini-cutting did not differ significantly across substrates. On average, rooted mini-cuttings produced 1.8 roots with an average total length of 2.9 cm. In the experiment using individual substrates (soil, sand, carbonized rice husks, peat, decomposed residue of black wattle bark) and their combinations, only the sand showed a value of available water within the ideal range for its use as a substrate for seedlings production, which implies that this material can ensure high water availability (Schimitz et al., 2002). The increased survival in combined substrate (Table 2) may reflect the effect of the coarse sand resulting in increased water availability to the mini-cuttings of canjerana. For *Maytenus ilicifolia* (Mart. ex Reissek) a higher rooting percentage (94.3%) was obtained with mini-cutting cultivation in pure commercial substrate (Lima et al., 2009). Rooting of *Calophyllum brasiliense* (Camb.) mini-cuttings was lower in commercial substrates (bark composted pine) and carbonized rice husks when compared with vermiculite (Silva et al., 2010). Clearly then, the optimal substrate varies according to species and conditions of rooting, and substrate evaluation is essential in optimizing rooting protocols given their determinant role in defining the range of root induction. Mini-cuttings from nodal segments showed a higher percentage of rooting and an increased number and length of roots in comparison to

Table 3. Percentage of survival and rooting and number and length of roots of apical and nodal canjerana mini-cuttings after 60 days of cultivation.

Mini-cuttings	Survival (%)	Rooting (%)	Number of roots	Total length of roots (cm)
Nodal	80.0[a1]	50.0[a]	2.2[a]	6.3[a]
Apical	74.0[a]	17.0[b]	1.4[b]	3.0[b]
Mean	77.0	34.0	1.8	4.6
F value[2]	0.2[ns]	5.3**	4.5**	36.6**
CV (%)	22.7	57.1	21.0	13.6

[1] Means values followed by different letters in a column are significantly different by the F test at the indicated probability. [2] ([ns]) no significant, (*) significant at 5% of probability, and (**) significant at 1% of probability.

Table 4. Percentage survival and rooting and number and length of roots of mini-cuttings of canjerana treated or untreated with indolbutyric acid (IBA) after 60 days of cultivation.

Mini-cuttings	Surviv[a]l (%)	Rooting (%)	Number of roots	Tot[a]l length of roots (cm)
Treated with IBA	79.3[a1]	43.3[a]	2.0[a]	5.2[a]
Untreated	70.0[a]	5.0[b]	1.1[b]	2.6[b]
Mean	74.6	24.1	1.5	3.9
F value[2]	1.3[ns]	12.9**	14.8**	70.0**
CV (%)	17.8	52.1	13.6	10.5

[1] Means values followed by different letters in a column are significantly different by the F test at the indicated probability.
[2] ([ns]) no significant, (*) significant at 5% of probability, and (**) significant at 1% of probability.

the apical ones (Table 3). The increment in rooting percentage of nodal segments was 2.9 folds. No significant difference in survival was observed between apical and nodal mini-cuttings. Interestingly, there were no significant differences ($p \geq 0.05$) in the responses of nodal versus apical explants to the IBA treatments, so data from the apical and nodal mini-cuttings were combined in Table 4. Given the lack of IBA treatment effects in this experiment, the differences found between nodal and apical mini-cuttings may be associated with the levels of carbohydrates, amino acids and other substances that promote adventitious rooting of certain tissues of the mini-cuttings, as noted by Hartmann et al. (2011). Thus, the mini-cuttings from nodal segments may have more appropriate levels of reserves than the apical ones, which would improve rooting capability. In addition, cells that have hormones or other compounds that confer an endogenous potential for root formation, such as auxin, quickly react to specific stimuli, like light and temperature (Sorin et al., 2005). It is possible that mini-cuttings from nodal segments have a better competence for rooting, but it does not affect mini-cutting survival.

The mini-stumps of canjerana had adequate nutritional level and the mini-cuttings for comparisons between clones were taken from the same sprouting period, with differences thus being an indication of existing genetic variation between canjerana clones, as expected. These are the first results of canjerana vegetative propagation by mini-cuttings and clearly indicate the effect of nodal and apical mini-cuttings on root initiation and growth. These differences are very important in developing a system for mass production of plantlets, because of economic and labor usage issues. It is possible that these differences are minimized with the advancing age of the shoots and the size of the mini-cuttings (greater than 2.0 cm for example), which should be the aim of future studies.

In other species, such as *Calophyllum brasiliense* (Camb.), apical and nodal mini-cuttings did not differ for rooting (Silva et al., 2010). These results were attributed to the good nutritional status of stock plants of the mini-clonal hedge, as well as to the physiological age of the two types of mini-cuttings, which makes them similar in the degree of juvenility and tissue lignification. In a study with mini-cuttings of *Anadenanthera macrocarpa* (Benth) Brenan, apical mini-cuttings were more responsive to rooting than nodal ones (Dias et al., 2012), being attributed to the greater degree of juvenility and less lignification of apical tissues (Xavier et al., 2003). Furthermore, apical mini-cuttings have a higher concentration of endogenous rooting promoters by virtue of their proximity to both the sites of auxin synthesis and to less differentiated tissues, potentially resulting in increased dedifferentiation of cells to their meristematic condition, which is essential for root initiation (Gehlot et al., 2014).

The application of IBA increased ($p \leq 0.05$) the percentage of rooting and the length and number of roots per canjerana mini-cutting (Table 4). The application of IBA did not significantly affect the survival of the mini-

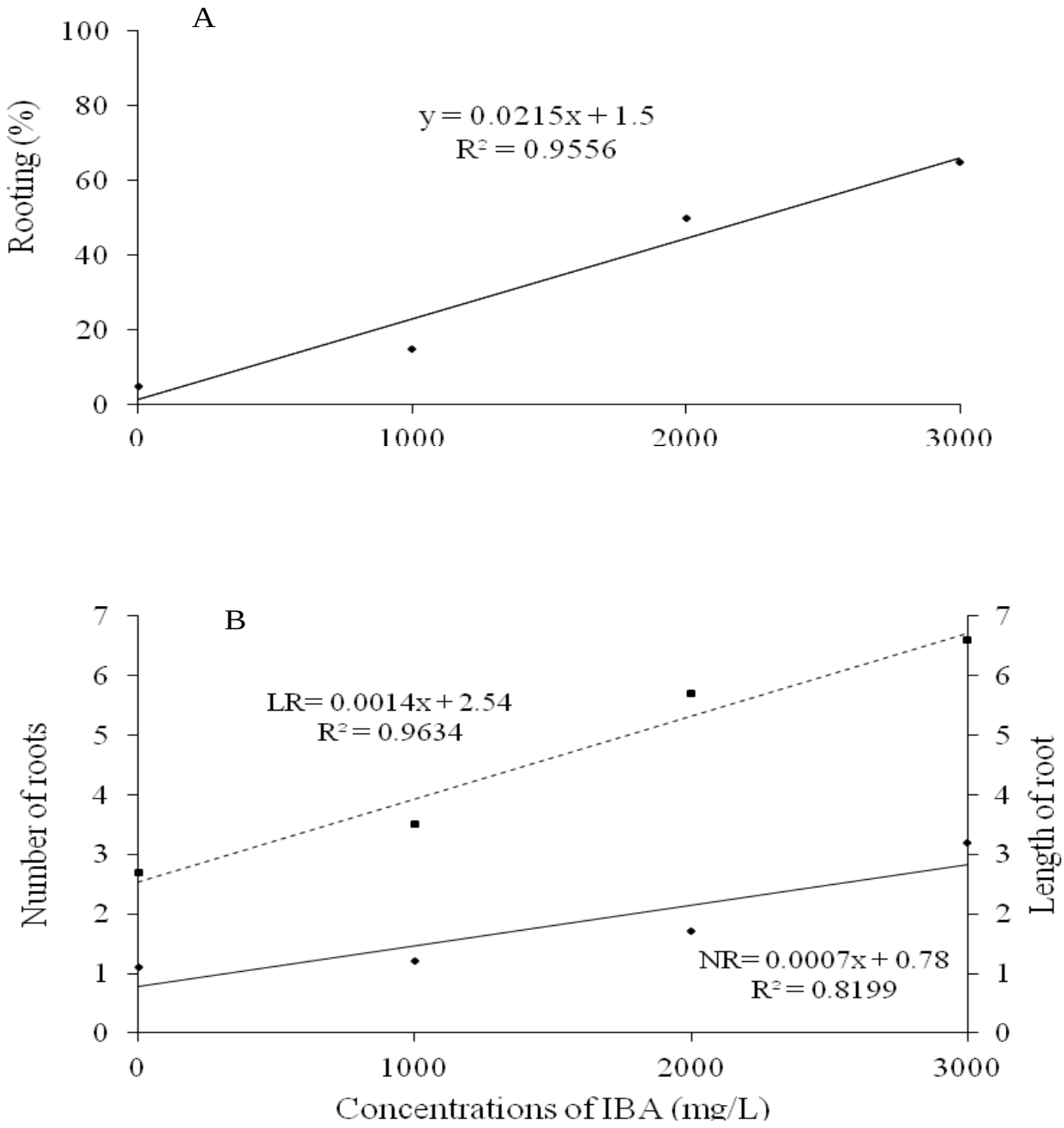

Figure 1. Rooting percent (A) and number (NR) and length of roots (LR) (cm) (B) of mini-cuttings of canjerana treated with different concentrations of indolbutyric acid (IBA) after 60 days of cultivation.

cuttings. Increasing the concentration of the IBA treatment improved the percentage of rooting and increased both the number and length of roots (Figure 1). At 60 days, canjerana mini-cuttings treated with IBA showed a higher percentage of survival and rooting, as well as increased root growth compared with mini-cuttings with no IBA treatment (Table 4). Auxin application enhances root formation in species with low rooting response, as governed by either genotype or physiological stage (Xavier et al., 2003). It is possible that low rooting response is caused by low content of endogenous auxin, which often requires the exogenous application of growth regulators to establish the competence and determination of target cells (Taiz and Zeiger, 2008). Therefore, canjerana fits in this group of species, because the application of IBA increased both

Table 5. The average number of mini-cuttings (NM) and percentages of survival (S) and rooting (R) of mini-cuttings of canjerana harvested at three different harvested dates from mini-stumps formed from mini-cuttings that were rooted in a soilless system subirrigated with nutrient solution.

Clones	Harvest I			Harvest II			Harvest III		
	NM	S (%)	R (%)	NM	S (%)	R (%)	NM	S (%)	R (%)
SM1	2.5[a1]	84[c]	53[b]	2.7[a]	73[c]	69[a]	2.8[a]	72[a]	72[b]
SM3	2.4[a]	98[a]	50[b]	2.5[a]	79[b]	51[b]	2.6[a]	55[b]	52[b]
SM13	1.8[a]	93[b]	67[a]	2.9[a]	90[a]	68[a]	3.0[a]	81[a]	80[a]
Mean	2.2	92	57	2.7	81	63	2.8	69	68
CV (%)	12.04	3.69	3.84	11.05	3.47	4.37	10.52	3.23	4.99

[1] Means values followed by the same letter in a column are not significantly different by the Tukey's test at the probability of 5%.

rooting percentage and the number and length of the new roots (Table 4 and Figure 1), which explains the higher rooting of canjerana mini-cuttings treated with IBA.

The observed differences in average root length may be associated with the increase in rooting competence promoted by IBA application that resulted in a rapid differentiation of the tissues for the formation of adventitious roots in canjerana mini-cuttings. In this study, only the nodal mini-cuttings treated with 2000 or 3000 mg/L of IBA had already developed adventitious roots at 30 days after cultivation (data not shown). The use of IBA resulted in an increased percentage of rooting and both the number and length of roots in the canjerana mini-cuttings (Figure 1). Also, the use of IBA favored rooting and did not affect the survival of mini-cuttings (Table 4), indicating that there was no phytotoxic effect in any of the tested concentrations (Figure 1). Mini-cuttings responded to an increase in IBA concentration up to 3000 mg/L, which can be considered a high concentration for mini-cuttings. At that concentration, 65% of mini-cuttings rooted, with an average of 3.2 roots per mini-cutting which averaged 6.6 cm in length at 60 days following cultivation. This contrasts with some previous mini-cutting studies in which the use of IBA concentrations above 2000 mg/L had negative effects on rooting due to the high degree of juvenility of the new shoot (Titon et al., 2003). It is thought that this is due to the fact that the juvenile mini-cuttings already have tissues with an endogenous hormonal balance that is favorable for rooting, which then leads to no (Silva et al., 2010; Ferreira et al., 2010; Wendling et al., 2010) or even a negative response to exogenous application of IBA (Xavier et al., 2003).

The production of mini-cuttings per mini-stump did not differ among the clones of canjerana that were evaluated in this study. The lowest production of mini-cuttings per mini-stump was 1.8 in the first harvest period and the highest was 3.0 in the third harvest (Table 5). The survival of mini-stumps was 95% and did not differ among harvest dates or clones (data not shown). In mini-cuttings from the mini-clonal hedge grown in a soilless system using subirrigation with a nutrient solution, the average production of mini-cuttings per harvesting date was 2.2 for the first harvest period, 2.7 for the second harvest period, and 2.8 for the third harvest period. These values were higher than has been reported for *Cedrella fissilis* (Vell.) (Xavier et al., 2003) and lower than those observed in *Ilex paraguariensis* (St. Hil.) (Wendling et al., 2007). There were significant differences in rooting ($p \leq 0.05$) among clones and harvest dates. The rooting percentage was 50% or greater in all treatments and showed an increase during the successive harvest periods. Interestingly, survival percentage dropped as rooting percentage increased (Table 5). In a study with *Liquidambar styraciflua* (L.), the survival percentage of rooted minicuttings was influenced by relative humidity and sunlight during the acclimatization period in the greenhouse and outdoor conditions (Wendling et al., 2010). Therefore, increasing the harvesting period of canjerana mini-cuttings can enhance the productivity of mini-stumps, and the maintenance of mini-cuttings in the greenhouse conditions may increase survival and rooting, as mentioned by Wendling et al. (2010). The decline in survival was particularly evident in the clone SM3. This interplay between survival, rooting percentage, and clone indicates the need for tailoring production systems specifically for each clone that will be propagated.

The results of this study clearly show that vegetative propagation by mini-cuttings is a feasible alternative for the mass production of canjerana plantlets for commercial plantation establishment (Figure 2). Apical and nodal mini-cuttings with 1.5 to 2.0 cm in length containing a half leaflet may be planted in media consisting of the same proportions of commercial substrate, coarse sand and carbonized rice husks. Clearly, though, nodal mini-cuttings have a higher competence for rooting. Based on this work, mini-cuttings should be treated with 3000 mg/L of IBA for the greatest rooting efficiency. Given clonal differences in these studies, it is possible that mini-cuttings with different amounts of reserves and/or from other clones might have a greater competence for rooting or higher survival rates during the propagation process, which would facilitate the mass production of plantlets, possibly with lower

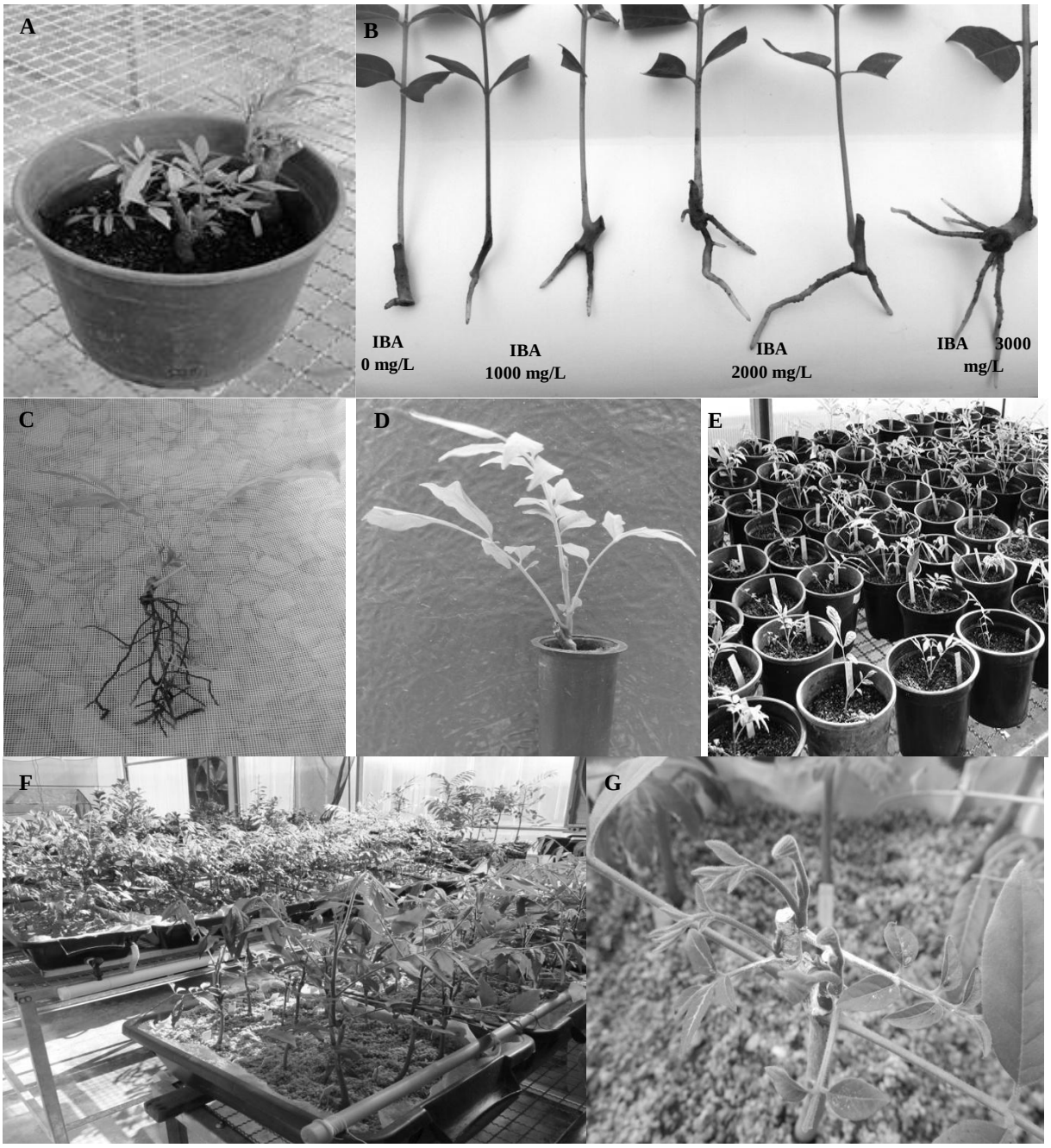

Figure 2. Shoots in mini-stump of canjerana (A), mini-cutting (nodal segment) rooted with different concentrations of indolbutyric acid (IBA) (B), seedling with four months of age (C), acclimatized seedling in tube (D), rooted mini-cuttings being acclimatized in pots (E), mini-clonal hedge in a soilless system (coarse sand as substrate) subirrigated with nutrient solution (F), and mini-stumps with shoots (G).

concentrations or even without the application of IBA. Once the rooted mini-cuttings are acclimatized in the greenhouse they may be transferred to either the mini-clonal hedge or to a full sun area for further acclimatization (Figure 2). The new plantlets can then be effectively planted in the field for growth and wood quality evaluations. This work shows that the use of mini-cuttings is a feasible technique for mass production of canjerana plantlets for plantation establishment from selected clones in order to enhance management and growth of this important species.

Conflict of Interest

The authors have not declared any conflict of interest.

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