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Effects of rooting media and indole-3-butyric acid (IBA) concentration on rooting and shoot development of *Duranta erecta* tip cuttings

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***Duranta erecta* is popularly grown as an ornamental plant in tropical and semi-tropical gardens. Current demands for the shrub have prompted the need for effective propagation methods. In this study, the influence of indole-3butyric acid (IBA) hormone concentration and media on rooting of *D. erecta* propagated from tip cuttings under greenhouse conditions were investigated. IBA hormone was used in three concentrations (2500, 5000, and 7500 ppm) and 0 ppm was the control. The study consisted of three media types (river sand, pine bark, a mixture of peat and perlite at a 1:1 ratio). The experiment was arranged in a 3×4 factorial layout in Randomized Complete Block Design replicated 3 times. Cutting survival, root length and number, shoot length and number were analysed. There was no interaction (P>0.001) between IBA concentration and media for all the measured parameters. The results showed that the optimum concentration of IBA is 5000 ppm, beyond which were inhibitory in all parameters except on root length. Type of media influenced survival and shoot number with pine bark giving the highest but did not affect root number, root length and shoot number.**

Key words: *Duranta erecta*, rooting, cuttings, media, river sand, pinebark, peat-lite.

INTRODUCTION

Duranta erecta is a species of flowering shrub in the Verbenaceae family originally native to Central and South America and is popularly grown as an ornamental plant in tropical and semi-tropical gardens (Singh et al., 2014; Said, 2016). The shrub is native to Mexico, South America and the Caribbean (Okunlola, 2013). *D. erecta* plays a significant role in environmental beautification and management; making public parks, gardens and houses more conducive for relaxation and enjoyment as an ornamental plant (Day and Loveys, 1998; Said, 2016). *D. erecta* increases the economic value of a property if properly placed in a landscape it can provide practical solutions for physical site problems with their durable aesthetic satisfaction (Okunlola, 2013). The shrub offers

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a variety of noticeable effects such as screening, cooling, enhancement of architectural lines, enframement of views, soil erosion management, sun and wind control, sound deadening and horticultural focus (Okunlola, 2013). *D. erecta* is mainly propagated vegetatively from stem cuttings.

Although *D. erecta* can be traditionally propagated using stem cuttings without any manipulations, this method can improve rooting when rooting hormones are used to hasten root initiation (Leonardi et al., 2001). The rooting of cuttings depends on several factors which include the type of cutting, rooting media, the type and concentration of rooting hormone used (Yeshiwas et al., 2015; Akram et al., 2017) and thickness of cuttings (Aminah et al., 2006). Rooting media is considered an integral part of the propagation system, percentage rooting and the quality of the roots produced are directly influenced by the medium (Kumar et al., 2015). There is no standard media or mix for cuttings, hence the appropriateness of the medium depends on the species, the cutting type, the season, the propagation system used, the cost and availability of the medium (Sardoei, 2014). Different media vary in their water holding capacities and potential to physically support the cuttings (Kumar et al., 2015). The common media used are sand, pine bark and a mixture of peat and perlite (1:1) (Akram et al., 2017; Sardoei, 2014). Sand consists of small rock particles, 0.05 to 2.0 mm in diameter formed as a result of weathering of many rocks. Sand must be fumigated or pasteurized before use as it may contain weed seeds and various harmful pathogens. Sand contains virtually no mineral nutrients and has no buffering capacity or cation exchange capacity (CEC) (Hartmann et al., 2002). Peat consists of the remains of aquatic, marsh, bog, or swampy vegetation that was preserved under water in a partially decomposed state and is commonly used in mixes (Hartmann et al., 2002). The acidic nature of peat generally excludes pathogens, insects and weeds from growing.

Perlite is another growing media used in the propagation of plants. Perlite is a grey-white salicaceous material, of volcanic origin, is sterile as a result of the high temperature processing. It is essentially neutral with no buffering capacity, no CEC. Perlite is usually mixed with peat for propagation of cuttings (Dole and Gibson, 2006). Pinebark is a product of the shredded or pulverized softwood bark from pine which has a high moisture holding capacity 15 times its dry weight and is acidic (pH 3.2 - 4.5) and contains a small amount of nitrogen about 1% (Akwatulira et al., 2011).

Application of exogenous hormones such as indole-3-butyric acid (IBA) increases the rate of rooting, final rooting percentage and number of roots (Leakey et al., 1990; Pacurar et al., 2014). Synthetic indole-3-butyric acid (IBA) is closely related to the naturally occurring plant growth regulator in terms of structure and function (Pacurar et al., 2014; Wiesman et al., 1988); and the hormone IBA is used on many crops and ornamentals but mainly horticultural plants to promote rooting of cuttings (Gilani et al., 2019; Akram et al., 2017; Li et al., 2009). However, it was observed that higher doses in some species were not beneficial. For instance, IBA concentrations above 2500 ppm were inhibitory in *Rosmarinus officinalis* and 1500 ppm was inhibitory in *Aloysia triphylla* (Costa Junior et al., 2018). While in *Warburgia ugandensis* 8000 ppm was optimum (Akwatulira et al., 2011) and the same concentration was inhibitory in *Ulmus panifolia* (Griffin and Schroeder, 2004). Considering the variability, rooting is IBA sensitive depending with species (Daskalakis et al., 2018).

In the recent years, there has been an overwhelming demand and use of *D. erecta* as an ornamental decorative shrub in Zimbabwe. According to Harare City Parks Division Annual Report (2014), *Duranta* has gained a lot of popularity due to its demand as a hedge and is a feature of more than 80% of flower beds in the Central Business District (CBD). However, Nursery Annual Report (2014) cites that the demand of this shrub has resulted into serious population deteriorations. Given the central importance that *D. erecta* play in landscape design systems, it becomes imperative to identify least cost rooting media and hormone options for ornamental nurseries. Therefore, research on how to come up with quick and convenient methods of propagating *D. erecta* to meet the ever increasing demand is vital. One such potential alternative is the propagation of *D. erecta* stem cuttings using different media types and different IBA concentrations. Considering the economic importance of the *D. erecta* as well as its potential use as a key ornamental decorative shrub in Zimbabwe, the present study was undertaken to assess the productivity of *D. erecta* in response to different IBA concentrations and rooting media.

MATERIALS AND METHODS

Site description

The experiment was conducted at Harare City nursery (Hillside) in the eastern suburbs of Harare, Zimbabwe from March 2015 to May 2015. The station lies in Natural Region IIa of Zimbabwe's Agro-ecological Zones, characterized by an altitude of 1486 m above sea level, mean annual rainfall of 750 to 1000 mm and mean annual temperature of between 15 and 20°C. Harare has latitude of 17°.51` South and a longitude of 31°.04` East. The study area receives an average annual rainfall of 825 mm and daily temperatures range from 7 to 22°C in winter and 16 to 26°C in summer.

Cultural practices

Preparation of rooting media

Milled pine bark and river sand were prepared by pasteurization and fumigation, while peat and perlite (peat-lite) were mixed without manipulation. Sand was pasteurized for 30 min by heating over an open cast metal plate and cooled (Kester et al., 1990) while milled pine bark was fumigated with Dithane M45 at 2000 ppm.

The different rooting media were allocated to different pots which were all filled up to 90%.

Preparation of cuttings

Stem cuttings of approximately 10 cm in length consisting of two or more lateral buds were sequentially harvested from mature *D. erecta* plants specially managed as sources of cuttings. Material with flower buds was avoided. A total of 360 tip cuttings were collected from coppices of selected *D. erecta* plants from Florence Chilshom park populations in their habitat. Leaves at the basal end of the cuttings were removed while those at the top were retained. The cuttings were collected in the morning hours, and kept moist or cool at all times by placing in cool box. The selected cuttings were softwood obtained from young succulent stems with new growth.

The cut bases were dipped in water up to a length of one cm to avoid water loss and wilting (Kester et al., 1990). Indole-3-butyric acid ≥99% (T) Sigma-Aldrich, Saint Louis United States was used and mixed with talcum powder. To get the desired concentrations IBA was first dissolved in 30% ethanol then mixed with talcum powder to a slurry. The slurry mixture was gently dried evaporatively. The dried mix was the ground and sieved to fine powder. A concentration of 2500 ppm was prepared by mixing 2.5 g IBA with 97.5 g talcum powder, the same method was used for all the concentrations. For each cutting, the 5 mm basal end was dipped into the different concentrations for 10 s. Excess powder was tapped off the cuttings. Each set of IBA treated cuttings was inserted into each rooting media type and replicated 3 times. The cuttings were stuck in different rooting media deep enough to stand. The rooting media were then compacted around the bases of the cuttings (in order to give support), gently watered thoroughly to get rid of any remaining air pockets in the media. Each set of ten cuttings was allocated randomly to each pot. The cuttings were checked on a daily basis and any dead leaves were removed.

Irrigation management

The cuttings were planted in propagation pots placed in a raised bed, equipped with overhead small sprinklers. The sprinklers were operated twice daily, in the early morning and in the late afternoon, for a period of 15 min during each time.

Experimental design and treatments

The experiment was carried out under a controlled environment in a greenhouse. Each growing media (pine bark, river sand, peat-lite) was applied in the pots and placed randomly in a greenhouse. The experiment was laid in a 3×4 factorial experiment in a Randomized Completely Block Design (RCBD) with three replications. There were two factors viz; rooting media (three types; river sand, pine bark and peat-lite) and IBA concentrations (three levels 2500, 5000, and 7500 ppm). No hormone was applied in the control treatment.

Parameters measured

Classical growth analysis was conducted based on six measurements: number and length of roots, number and length of shoots and survival of cuttings (rooted and shooted) following a method by Yeboah et al. (2009). The cuttings were uprooted by gentle pulling after loosening the media in the pot to measure root length and root number. Root and shoot number were measured by counting, root and shoot length were measured using a string and a ruler. All data were collected after seven weeks of planting.

Data analysis

The collected data was analysed statistically by Analysis of Variance (ANOVA) using statistical package GENSTAT version 17.1. Differences among treatment means were compared using the least significant difference (LSD) at 1%. All count data was transformed by square root method in GENSTAT.

RESULTS

Effects of hormone concentration (IBA) on root number and root length of cuttings

IBA concentration significantly increased ($P<0.001$) root number. The highest average number of roots was recorded at 5000 ppm, followed by 2500 ppm which was not different from 7500 ppm and the control (Figure 1A). Cuttings grown with an IBA of 7500 ppm had the lowest average number of roots. Rooting hormone concentration influenced root length ($P<0.001$) (Figure 1B). With 5000 ppm giving higher root length than the control and 7500 ppm, however 5000 pm was not statistically different from 2500 ppm.

Effects of IBA concentration on shoot number and shoot length

IBA concentration of 5000 ppm had cuttings with the highest average shoot number ($P<0.001$) (Figure 2A). There were no differences on shoot number among the other three treatments (control, 2500 and 7500 ppm) (Figure 2A).

Significant variations ($P<0.001$) were obtained on shoot length. The longest shoots were recorded at 5000 ppm, followed by 7500 ppm which was not different from 2500 ppm (Figure 2B) and 2500 ppm was not different from the control.

Effects of IBA concentration on cutting survival percentage

Cuttings grown with an IBA concentration of 5000 ppm recorded the highest percentage survival with an average of 81.85%. The survival of cuttings grown using an IBA maximum concentration (7500 ppm) was not significantly different ($P>0.001$) from the number of cuttings grown in the control treatment and the least concentration (2500 ppm) (Figure 3).

Effects of rooting media on survival percentage and number of shoots

Media had significant effects ($P < 0.001$) on survival

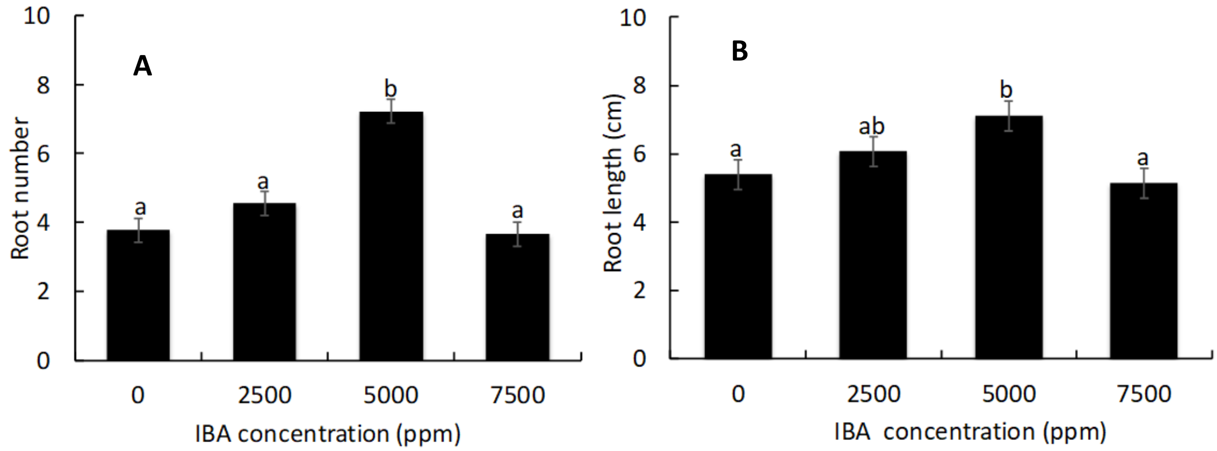

Figure 1. Effects of IBA concentration on root number (A) and root length (B).

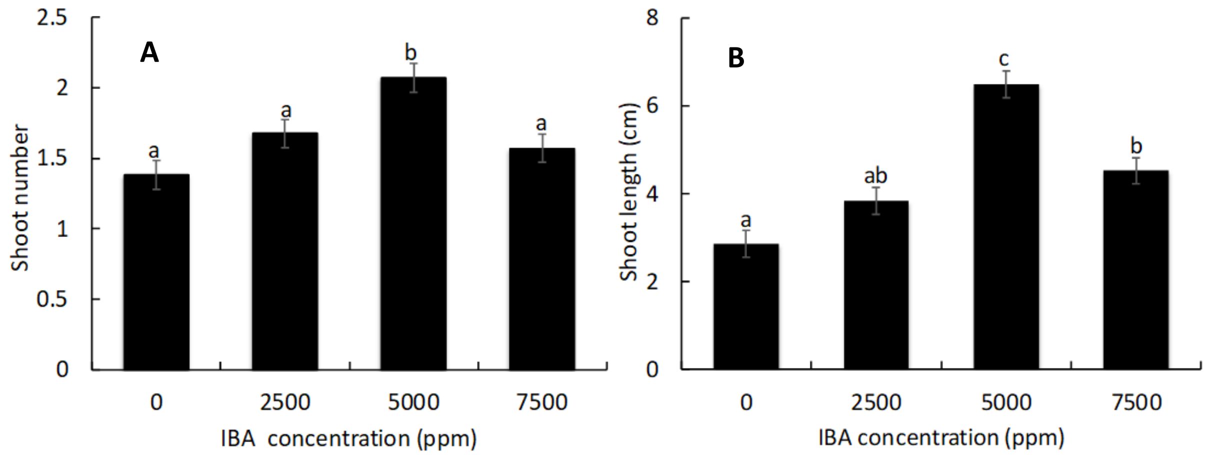

Figure 2. Effects of IBA concentration on shoot number (A) and shoot length (B) of *D. erecta* cuttings.

percentage and number of shoots. Significant variations were obtained on survival of the cutting, with pinebark grown cuttings having the highest survival (Figure 4A). Pinebark grown cuttings had the highest number of shoots, followed by river sand, which was not different from peat-lite (Figure 4B).

DISCUSSION

Effects of IBA concentration on root length and root numbers

The number and length of roots depends on IBA concentration applied during propagation (Figure 1A and B). The results trend on root length can be explained by the fact that proteins from IBA break hydrogen bonds between cellulose micro fibrils promoting cell wall loosening and cells will eventually elongate (Kumar et al., 2015; Qu Yang et al., 2015; Cosgrove, 2000). At optimal exogenous IBA, the rate of cambium de-differentiation is increased, accelerated hydrolytic activity and enhanced callus formation which ultimately gives better root length (Li et al., 2009; Gilani et al., 2019). However, in most species the benefit of exogenous hormones is only realised when they are properly applied, that is the right concentration.

Reduced root length at 7500 ppm can be alluded to the fact that excess IBA can be toxic to the cuttings and reduce callus formation. The current findings are similar to those reported by Sharma et al. (2009) in *Punica garanatum* and Kurd et al. (2010) in olive cuttings; they observed the highest rooting length response at 5000 ppm and a reduced root length at 7500 ppm. Additionally, the trend that comparatively low IBA treatments (2500 ppm) give the least rooting length has been recorded by

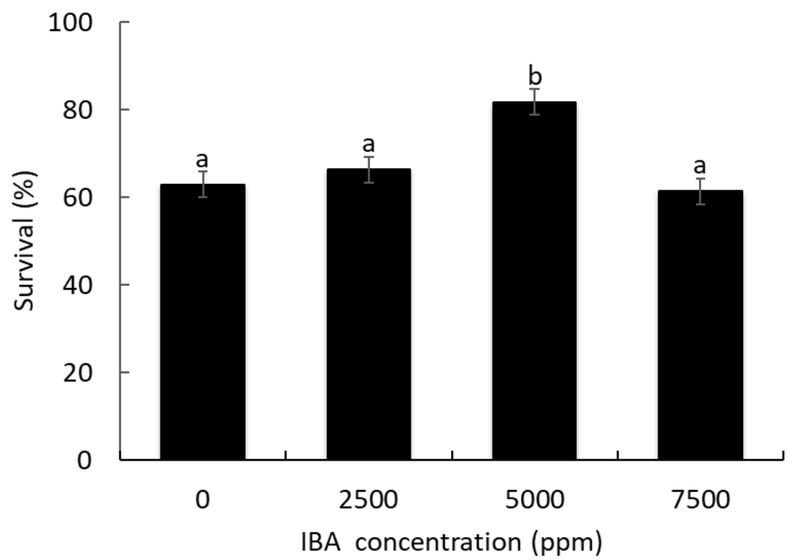

Figure 3. Survival percentage of cuttings from different IBA concentrations.

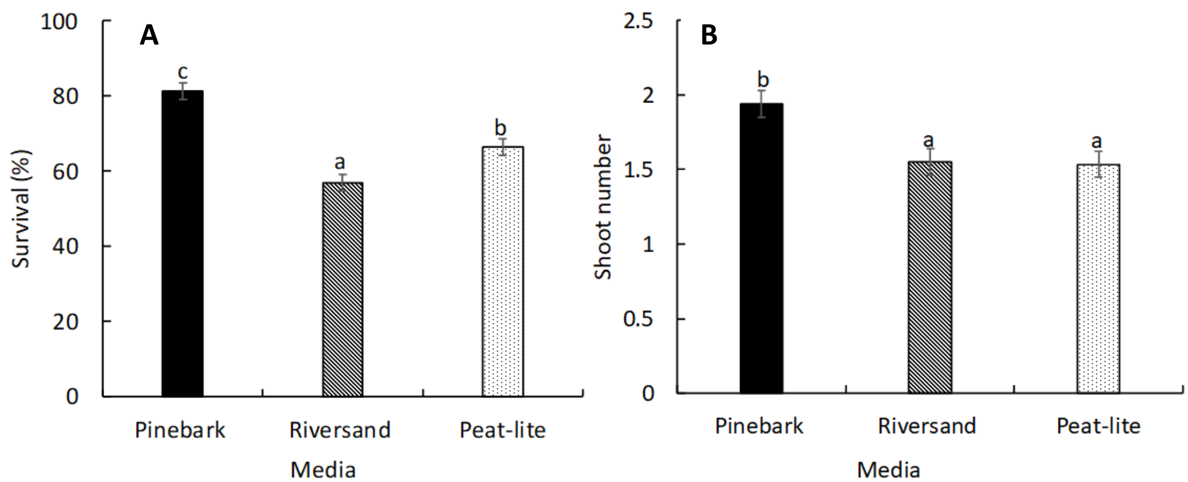

Figure 4. Effects of media on survival (A) and shoot number (B).

many authors like Singh et al. (2014) on *D. erecta* golden stem cuttings Kurd et al. (2010) on Olive stem cuttings, Qaddoury and Amssa (2004) on date palm offshoots.

The highest root numbers at 5000 ppm are in line with the findings of Singh et al. (2014). Singh et al. (2014) explained the higher number of roots at optimum IBA concentration to be a result of less time required to callus formation, thus enhanced cambium dedifferentiation producing numerous cells which will differentiate to form root cells. Optimum IBA increases the rate of amyloplast disappearance, amylolast levels decline naturally during rooting (Singh et al., 2014). Thus our findings indicate that at optimum indole-3-butyric acid, the decline of amylolast can be enhanced and cambium activities are stimulated, that will mobilize stored food material to the root initiation sites (Gilani et al., 2019) hence promoting numerous root formation. This trend is in line with the findings of Mohamed (2005) on *Vitis vinifera* who reported that a concentration of 5000 ppm gives the highest root numbers compared with the highest concentration of 6000 ppm used on medium cuttings.

Effects of IBA concentration on number and length of shoots

Significant variations were observed across various IBA concentrations in terms of shoot number. The high shoot numbers of *D. erecta* cuttings treated with 5000 ppm IBA concentration agree with the findings of Bashir (2009) on *Simmondsia chinensis* stem cuttings. However, auxins being root promoting growth regulators, had no direct impact on shooting of buds as bud shooting is usually influenced by stored carbohydrate in the cuttings. In fact, maximum number of shoots produced from 5000 ppm IBA concentration can be explained by the fact that the same concentration supported better root quality (length and numbers), this increased surface area for nutrient absorption from below ground parts to the above ground parts (Leakey et al., 1990; Gilani et al., 2019).

This trend of results on shoot length can be explained by the same mechanism that influences shoot numbers that is enhancement of mineral nutrients transport by applied IBA to the growing points of the cutting (Akwatulira et al., 2011). Since the same concentration has supported more number of shoots, basically an increase in the number of shoots means more surface area for photosynthesis. More assimilates can translate to an increase in metabolic processes responsible for shoot proliferation (Gilani et al., 2019). However, the mechanism of IBA in promoting shoot growth is not yet clearly understood.

Effects of IBA on survival of cuttings

The results indicate that there is an optimum concentration for IBA beyond which IBA application becomes inhibitory. This was also reported by Anyasi (2011) where maximum IBA concentration resulted in rooting failure of *Chromolaena odorata*. The quantity of IBA applied should be sufficient enough to dissolve the cuticle and provide a tight seal at the basal end of the cutting soon after callusing thus preventing it from decay thus increasing chances of survival. The inhibitory effect by maximum IBA concentration can be explained by the toxicity of potassium (K+) ions which are free radicals. The K+ ions in auxins play a significant role in root initiation by dissolving the epidermal layer. However, when provided in excess instead of dissolving the epidermal layer they actually destroy the epidermal layer and adjacent cells. These findings go in line with the findings of Sharma et al. (2009) who observed that a concentration of 5000 ppm IBA has the highest survival percentage (100%) of *P. garanatum* cuttings whilst 10000 ppm gives the least survival percentage of cuttings.

Rooting media on survival of cuttings and shoot number

The results showed a trend at which pine bark grown cuttings had the highest survival and shoot length. Accelerated survival supported by pine bark can be explained by its low pH range (3-6) (Hartmann et al., 2002). The hydromorphic characteristics of *D. erecta* possibly require acidic conditions to be fully expressed. Also it can be due to the high water holding capacity of pine bark, given the physical properties of pine bark compared to river sand and peat-lite which are loose in texture (Akwatulira et al., 2011). Aeration and water holding capacity of the media are often negatively correlated and therefore a balance between these must be achieved to ensure optimal rooting (Ofori et al., 1996; Kumar et al., 2015). However, the trade-off varies with species, the present findings suggest that *D. eracta* does better in media with a higher water holding capacity compared to better aeration status. High survival of cuttings when rooted in pine bark agrees to the findings of Akwatulira et al. (2011) who observed and recorded a higher survival percentage in pine bark compared with sand and top soil in the rooting *Warburgia ugandensis* stem cuttings. A similar trend was observed in *Prunus africana* where sand grown cuttings had the intermediate survival percentage compared to other media mixtures (Tchoundjeu et al., 2002). Anyasi (2011) also found that sand and a mixture of peat and perlite give the least survival percentage on *C. odorata*.

Pine bark grown cuttings had the highest shoots number, river sand and peat-lite gave almost equal shoot number. These findings are in consistency with the findings of Akwatulira et al. (2011) who observed the maximum number of shoots in *W. ugandensis* grown in pine bark. Given the better water holding capacity of pinebark, we can suggest that pinebark enhances the availability of water and mineral nutrients at the basal end of the cuttings. This makes translocation of water and mineral nutrients to the above ground parts of the cuttings leading to rapid bud break (Akwatulira et al., 2011; Gilani et al., 2019).

Conclusion

The results showed that *D. erecta* propagation is media and IBA concentration dependant. Even though there are various levels of IBA which can used to propagate *D. erecta* cuttings, a concentration of 5000 ppm IBA is the most appropriate concentration in improving survival of cuttings, root number and length, shoot length and shoot numbers. Based on these findings, it can be concluded that 5000 ppm IBA is the optimum concentration for propagating *D. erecta*. Results from the current study also showed that pine bark is an appropriate rooting media as it increases the survival of cuttings and shoot numbers. The study has shown that root length, root number and shoot length are independent of media used. The influence of time of the year when the cuttings are prepared and the effect of cutting length need to be assessed since these have a bearing on the amount of stored carbohydrates which affect the rooting performance of cuttings. Nursery growers are recommended to use a concentration of 5000 ppm IBA and pine bark as the rooting media to maximise production of *D. erecta* cuttings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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