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RESEARCH ARTICLE

EFFECT OF DIFFERENT LEVELS OF I.B.A CONCENTRATION ON CLONAL PROPAGATION OF EUCALYPTUS SPECIES IN GHANA WEST AFRICA

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ABSTRACT

Effective rooting and survival of cuttings has become more important owing to increased global wood demands and climate change. This study assesses the survival of ten (10) clones each of *Eucalyptus grandis* x *Eucalyptus urophylla* (*E. grandis* x *E. urophylla*) and *Eucalyptus pellita* (*E. pellita*) clonal cuttings when treated with different Indole-3-butyric acid (IBA) auxin concentrations of 0 ppm (control), 2000 ppm, 3000 ppm and 4000 ppm respectively. Overall, the mean survival of cuttings with respect to both species (10 clones each) was low (i.e < 50%) for all concentrations of IBA used. *E. grandis* x *E. urophylla* cuttings had the highest mean survival (43.39%) when treated with 2000 ppm of IBA and lowest mean survival (37.86%) when treated with 0 ppm. *E. pellita* (P) cuttings had the highest mean survival (27.14%) when treated with 2000 ppm of IBA. Individual clones however showed higher survival with respect to IBA treatments. *Eucalyptus grandis* x *Eucalyptus urophylla* clone (GH11) achieved a mean survival rate of 75% with 2000ppm of IBA. *E. pellita* clone P2 achieved its highest survival (52.68%) in the control experiment (0 ppm). Although IBA application had significant effect on cutting survival, parent material (individual clones) also plays a vital role in cutting survival as some clones such P51 and P82 achieved survivals of less than 10% given all four levels of IBA used. Individual clones also respond to IBA concentration differently. GH3 and GH10 had their highest survivals (i.e 66.07% and 61.61% respectively) in the control (0ppm of IBA). Results from this study can be used in plus tree clonal selection regimes to identify the best economically rewarding clones in terms of cutting survival to be used in forest plantation establishments.

KEYWORDS

Clone, auxin, adventitious rooting, hybrid, cutting, ortet.

1. INTRODUCTION

Despite the large number of Eucalyptus species existing worldwide, less than twenty are actively used for commercial plantations and this can be attributed to factors such as environmental conditions, wood and pulp quality as well as rooting ability (survival) of cuttings (Almeida et al., 2010). With deforestation rates estimated to be as high as 12 million ha per annum in tropical regions in recent years, there has been growing concern about the sustainability of the world's forest ecosystems (Sohngen et al., 2008). For many plants especially with long germination, vegetative propagation is a fast and economic method of multiplication and it is the only practically applicable method available (Pacurar et al., 2014). Research evidence suggests that auxins play a central role in the determination of rooting capacity, by enabling the faster production of rooted cutting material which is essential for vegetative propagation

(Fogaça and Fett-Neto, 2005). IBA induces changes in the metabolism of enzymes, carbohydrates, RNA, DNA and proteins which in effect may either inhibit or promote regeneration of adventitious roots during cell division and differentiation (Ebling et al., 2012).

Traditional propagation of Eucalyptus species mainly occurs through seed germination, most of the planting stock is still produced from seeds of unselected genotypes (Husen and Pal, 2006). The application of exogenous plant growth regulators, as the auxins group, can increase the success of adventitious rooting in vegetative propagule collected from selected genotypes of Eucalyptus and the indole-3-butyric acid (IBA) is the most used (Ebling et al., 2012). Although, clonal propagation programmes have achieved some successes in the past, issues of cuttings not being able to achieve successful rooting and desired survival still persist. Over recent years, the focus of cloning has also shifted to industrial requirements,

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rather than remaining limited to disease resistance and volume increase (Sivarajan et al., 2014). Unrooted cuttings cause a lot of financial loss to commercial forestry ventures and as such improving the survival of *Eucalyptus pellita* and *Eucalyptus grandis* × *Eucalyptus urophylla* cuttings has been a major concern to foresters in recent years as this would in turn greatly improve the quality and yield of propagated plants. Cloned progenies have been employed in breeding programmes over the years to increase selection intensities and save time (Högberg et al., 2018).

Researchers have sought over the years to find out the implications of various treatment factors on the rooting of stem cuttings for clonal propagation programmes (Malling, 2001., Štefančič et al., 2005; Husen and Pal, 2006). Some of these factors being leaf area of cutting type, auxin used, and age of the stock bank stand of trees to be propagated. Breeding for clonal selection requires some extra effort, because individual performance evaluation in progeny trials is not robust enough to predict performance of the same individual as a clone over a range of different environmental and silvicultural conditions (Reis et al., 2011). It is out of these nursery survivals and trials that on-field tree planting regimes are proposed (Weng et al., 2013). This study therefore examines how IBA concentration affects to the survival of *Eucalyptus* clonal cuttings.

2. MATERIALS AND METHODS

This experiment was carried out at the plant nursery site of Miro Forestry Company Limited which is located in Agogo in the Asante-Akim North Municipal District of the Ashanti Region of Ghana. The plantation area is located about 7° north of the equator and has a tropical climate with average monthly temperatures ranging between 24°C and 28°C and annual rainfall between 1,300 mm - 1,650 mm. A pair of mini shears was used to excise the cuttings from the parent plant (ortet). The cuttings were then placed immediately in a bucket of water and thereafter trimmed with a pair of scissors. 99% concentrated Indole-3-Butyric Acid (IBA) and talcum powder both purchased from Dizengoff Ghana Limited were mixed in specific ratios with the aid of a weighing balance to give the desired hormone concentrations in parts per million (ppm).

Ten clones each of *Eucalyptus grandis* × *Eucalyptus urophylla* and *Eucalyptus pellita* were used in this research. IBA concentrations of 0 ppm (control), 2000 ppm, 3000ppm and 4000 ppm were applied to apical cuttings of the two tree species used in this research. Indole-3-Butyric Acid (IBA) was the only rooting hormone used in this research. In preparing the different concentrations of IBA, the weighing balance was first calibrated with a known weight of 50 g which was made of steel. Both concentrated IBA powder and talcum powder were gently weighed on the balance with respect to each of the concentrations used in this research. Measured amounts of IBA and talcum powder for each respective hormone were thoroughly mixed. For 0 ppm cuttings were just planted in substrates after trimming without hormone treatment. Mixtures were done as follows to obtain desired hormone concentrations for the research:

- 0.2 g of IBA into 10g of talcum powder = 2000 ppm of rooting hormone.
- 0.3 g of IBA into 10g of talcum powder = 3000 ppm of rooting hormone.
- 0.4 g of IBA into 10g of talcum powder = 4000 ppm of rooting hormone.

The basal tip of the cutting where excision was made was dipped into the prepared hormone powder with respect to the different concentrations used, after which the cutting was planted in the cocopeat (planting medium) filled trays.

Eucalyptus pellita had a code name beginning with the letter 'P' and ending with a number (P1, P2, P11, P15, P46, P56, P82, P83, P95 and P98) while *Eucalyptus grandis* × *Eucalyptus urophylla* clones had their code names beginning with 'GH' and ending with a number (GH3, GH7, GH10, GH11, GH14, GH19, GH27, GH29, GH31 and GH32). In this study twenty-four cuttings each of the clones were treated with the above-mentioned concentrations of rooting hormones with four replications each making a

total of 112 cuttings per experiment. Cuttings which had visible roots and were green and healthy 28 days after planting (DAP) were said to have survived. Cuttings were collected from a two-year-old clonal hedge stand. Two- to three-week-old sprouts were collected and trimmed to single-stemmed apical and basal cuttings respectively and before treatment with a determined concentration of IBA. Cuttings were immersed in water immediately after excision from ortets to reduce environmental shocks on the plant.

2.1 Statistical analyses

Survival results of the clones were subjected to Analysis of variance (ANOVA) tests to find out if differences between survivals with respect to levels of IBA concentrations were significant. Results were also further subjected to Tukey's Test of Multiple Comparisons to find out the exact treatments at which significant differences occurred.

3. RESULTS

3.1 *Eucalyptus grandis* × *Eucalyptus urophylla* cutting survival with respect to hormone concentration.

Figure 1: GH Clone survival with respect to four levels of IBA.

Cuttings which had visible roots and were green and healthy 28 DAP planting were said to have survived. Mean survival of cuttings from all 10 GH clones were low (i.e < 50%) for all concentrations of IBA used in this research. Survival rates of individual clones however varied from clone to clone as shown in figures 1 and 2 respectively. GH7 cuttings achieved highest survival (68.75%) when treated with 2000 ppm of IBA and lowest survival (41.96%) when treated with 4000 ppm of IBA.

Figure 2: GH Clone survival with respect to four levels of IBA.

For all 10 GH clones, the lowest survival (7.14%) was recorded when GH31 cuttings were treated with 0 ppm (IBA). GH 3, GH 10 and GH32 also recorded their highest survivals (i.e 66.07%, 61.61% and 31.25%) respectively when treated with 0 ppm Clones GH29 and GH32 achieved survival rates of less than 40% for all levels of IBA concentrations used.

Figure 3: *E. pellita* (P) Clone survival with respect to four levels of IBA

In this study, *E. pellita* cutting survival was also assessed with respect to varying hormone concentrations used in this research. Figures 3 and 4 display results of survival rates in terms of percentages for each of the 10 clones of *E. pellita* used in this research. From Figure 3 clone P2 achieved the highest survival (52.68%) when treated with 0 ppm of IBA (control experiment) and lowest survival (37.50%) was also recorded when clone treated with 3000 ppm of IBA.

Figure 4: *E. pellita* (P) Clone survival with respect to four levels of IBA concentration.

The lowest survival (1.79%) for all 10 *E. pellita* (P) was recorded when P82 was treated with 0 ppm IBA. Mean survival as displayed for all concentrations of IBA used in this research was low (i.e. < 50%). Clones P11, P51 and P82 recorded survival rates less 20% for all concentrations of IBA used.

Table 1: Summary of Tukey's test conducted for GH-IBA concentration interaction.		
Tukey's multiple comparisons test	Mean Diff.	Adjusted P Value
GH27:2000 ppm vs. GH27:4000 ppm	13.5	0.0003*
GH27:2000 ppm vs. GH27:Controlppm	12.5	0.0015*
GH31:2000 ppm vs. GH31:4000 ppm	10.25	0.0393*
GH31:2000 ppm vs. GH31:Controlppm	11	0.0144*

*= significant difference between interaction

Table 1 displays some significant differences as indicated by the Tukey's test of multiple comparisons between clones with respect to cutting-IBA interactions. Some individual clones such as GH27 and GH31 exhibited significant differences with respect to the interaction being tested for. Tukey's test results as shown indicates the corresponding adjusted P values between clones GH27 and GH31.

4. DISCUSSION

Four out of the ten *E. grandis* × *E. urophylla* clones used in this research (i.e. GH7, GH14, GH27, GH31) had the highest survival rates when treated with 2000 ppm of IBA. Survival results for *Eucalyptus pellita* cuttings did not

vary from the trend of *E. grandis* × *E. urophylla* as survival rates continued to vary with respect to alterations in IBA concentration. This is in agreement with reports that; the propagation of cuttings is dependent on the concentrations and application methods of exogenous rooting hormones (Carter and Slee, 1993; Maile and Nieuwenhuis, 1996). Overall mean survival rate was computed based on each of the concentrations of IBA used and 2000 ppm of IBA yielded the highest survival rate (i.e. 43.39%) followed by the control (37.86%), 3000 ppm (36.52%) and then lastly 4000 ppm (31.70%) which goes agrees with findings by that exogenous auxins are vital for rooting capacity as well as survival of cuttings (Kotis et al., 2009).

Clone GH3 treated with IBA concentrations 2000 ppm, 3000 ppm, 4000 ppm and 0 ppm, had survival rates of 45.54%, 47.32%, 45.54% and 66.07% respectively. Clone P1 survival rates recorded were 36.61%, 50%, 41.07%, and 36.61% for concentrations of 2000 ppm, 3000 ppm, 4000 ppm and 0 ppm respectively. Clone P82 recorded very low survival rates for all four hormone concentrations used. Survival rates for P82 were 5.36%, 5.36%, 2.68% and 1.79% for 2000 ppm, 3000 ppm, 4000 ppm and 0 ppm respectively. Overall survival rates for clone P51 were also low (i.e. <10%) for all hormone concentrations used which shows that individual clones of the same species respond differently to IBA treatments and this could be as a result of genetic, provenance and other environmental factors. ANOVA analysis conducted on survival results showed that variations between *E. grandis* × *E. Urophylla* cuttings were significant.

These significant differences occurred within specific clones in relation to IBA concentration whereas Tukey's test of multiple comparisons identified specific interactions that gave these significant differences. This suggests that although survival may be influenced by hormone concentration in some clonal species, survival also is dependent on the type of clone which is being treated with different concentrations of IBA. Rooting is maximized when the optimal combinations of factors is achieved (Dick and Dewar, 1992). Clone GH27 exhibited significant variations when treated with different concentrations of rooting hormone while GH3 and GH11 exhibited no significant differences.

5. CONCLUSION

This study was conducted to find out the survival rates of *Eucalyptus pellita* and *Eucalyptus grandis* × *E. urophylla* cuttings in response to IBA concentrations of 0 ppm, 2000 ppm, 3000 ppm and 4000 ppm. Overall, both species exhibited highest mean survival rates when 2000 ppm of IBA was applied. The mean survival rate of *E. grandis* × *E. Urophylla* was 43.39% while that of *Eucalyptus pellita* was relatively low at 27.14%. These mean calculations were based on ten clones of the two species used. Some individual clones survived best when no IBA was applied (i.e. GH10-61.61%, GH32-31.25%, and P2-52.68%) and this sets a precedent for processing specific clonal cuttings without the exogenous rooting hormone application by Miro Forestry Limited. Further studies should be conducted to assess the performance of *E. grandis* × *E. urophylla* and *Eucalyptus pellita* clones with respect to cutting type and hormone concentration and also go on field trials in order to compare in-greenhouse survival rates to on-field survival rates. With regards to findings from this research, the assessment of the effect of the genetic compositions of individual clones on overall cutting success will be examined in the near future.

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