Original Article

Propagation of juvenile cuttings of clones of (tropical Eucalyptus) Eucalyptus urugrandis: Examination of the role of auxins in adventitious rooting

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Abstract

The objective of the research was to evaluate the vegetative development of cuttings of tropical Eucalyptus clones (Eucalyptus urugrandis) with auxin application. A factorial arrangement (7 x 6), consisting of cuttings of tropical Eucalyptus clones (EC16, EC18, EC45, LA157, LA41, LA85, LA86) and auxin doses (1500 mg kg^{-1} AIB, 1000 mg kg^{-1} AIB, and 0 mg kg^{-1} AIB, 1500 mg kg^{-1} ANA, 1000 mg kg^{-1} ANA, 0 mg kg^{-1} ANA, 0 mg kg^{-1} ANA), respectively, was used; for a total of 42 interactions, with three replications and four observations per experimental unit. The variables evaluated were: survival percentage, mortality percentage, rooting percentage, seedling height, number of leaves, and number of roots. The analysis of the behavior of clone cuttings in E. urograndis in the variable survival (66.74%), number of leaves (14), plant height (13.48), with better response to ($p<0.05$) was in clone EC166 and the hormone concentration of1500 mg kg^{-1} AIB, not so for the number of roots (119) which showed better response to ($p<0.05$) with 1000 mg kg^{-1} ANA. The highest rooting percentage ($p<0.05$) was presented by clone EC 16 with 27.06%. The clonal propagation of eucalyptus with the use of auxins is a valuable milestone to promote genetically homogeneous plantations.

Keywords: rooting, hormones, survival, growth.

Introduction

Currently, the cultivation and production of Eucalyptus L'Hér (Myrtaceae) is considered an important economic, social and environmental alternative in several countries (Cabezas & Chila, 2021). Eucalyptus species consist of 747 native to Australia, Papua, New Guinea, Indonesia and the Philippines (Leicach et al., 2012), although over the years they have been distributed in a wide variety of environments and ecosystems (Beech et al., 2017). E. urograndis is in high demand in the international market. (Quispe & Ramos, 2019). Among the uses of E. urograndis is cellulose for paper production and wood products for furniture, in the pharmaceutical and cosmetological industry, among others (Azad et al., 2018b; González-Candia et al., 2016; Quispe & Ramos, 2019).

The efficiency of eucalyptus in silviculture is mainly based on clonal propagation of selected genotypes both in pure species and interspecific hybrids (Vilasboa et al., 2019). The tropical interspecific hybrid type between Eucalyptus grandis and Eucalyptus urophylla popularly known as Eucalyptus urograndis has been established over the years (Benedito & Freitas, 2022).

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E. urograndis established itself as a global benchmark for clonal productivity because it combines rapid growth and excellent rooting ability with a high degree of productivity (Rezende et al., 2014).

Tree cloning is a practical, economical and viable process. The advantages of this method over sexual reproduction include greater genetic stability and speed of clone generation (Azad et al., 2018b). The methodology of vegetative propagation on an operational scale produces certain positive impacts on breeding strategies. This involves productive advantages in both quantity and delivery time of the gain produced by a breeding program (Soria & López, 2014). Vegetative propagation is facilitated by the use of propagules either from young plants or basal epicormic shoots (Wendling & Brondani, 2015; Dias et al., 2015).

In addition to the effect of genotype, the most important factors in achieving adequate rooting levels are juvenility and stress. Aging, or physiological maturity, is a phenomenon that affects woody plants and is common in the genus Eucalyptus. Juvenile materials generally root adequately but lose the ability to form roots into propagules as the number of cell divisions of the apical meristems increases (Francisco de Asis & Rodríguez, 2014). In most cases, protocols cannot be reproduced with the same success in different environments and with different genetic materials. Therefore, it is difficult to indicate a common protocol for the species (Soria & López, 2014).

Failure or loss of competence to form adventitious roots is a frequently faced problem in breeding programs, which hinders the propagation of some important genotypes, with high-quality interesting attributes for commercial production (Vilasboa et al., 2019). The use of auxins has been very useful in the generation of clones, among them, the most used are indo-3-acetic acid (IAA), indo-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) (Nakhooda & Jain, 2016). IAA as well as its concentration are factors influencing the rooting of forest species (Azad et al., 2016, 2018a; Azad & Matin, 2015).

The use of auxins becomes a key strategy to overcome the problems of sexual propagation of Eucalyptus urograndis. Sexual reproduction is difficult due to the production time of seedlings, higher costs in the production process, and lack of availability of selected seeds. In this order of ideas, this work was focused on evaluating the vegetative development of tropical Eucalyptus (Eucalyptus urugrandis) clone cuttings with the application of ANA and AIB auxins.

Materials and Methods

Location

The research was carried out at the “Los Angeles” farm of the Novopan company, Buena Fe canton, Los Rios, Ecuador. The sector is located at 1200 m a.s.l. (Latitude: S 0° 40' / S 0° 30' and Longitude: W 79° 30' / W 79° 15'). The climatic characteristics of the nursery have a minimum relative humidity of 95%, a maximum relative humidity of 90%, a minimum relative temperature of 58 °C and a maximum relative temperature of 26 °C.

Experimental design

A Completely Randomized Design (CRD) was applied with a seven by six factorial arrangement with 42 treatments, three replications and four observations per experimental unit. The single effect factor A was constituted by seven clones (EC16, EC18, EC45, LA 157, LA41, LA85, LA86 and factor B by auxin doses: 1500 mg kg^{-1} AIB, 1000 mg kg^{-1} AIB, and 0 mg kg^{-1} AIB , 1500 mg kg^{-1} ANA, 1000 mg kg^{-1} ANA, 0 mg kg^{-1} ANA, 0 mg kg^{-1} ANA.

Statistical analysis

The data obtained were analyzed by ANOVA analysis of variance and processed with Infostat statistical software. In order to establish statistical differences, Tukey's multiple range test was applied (P= 0.05). Data with zero values were transformed using the following formula: $\sqrt{(x+0,5)}$.

Selection of microstakes from the mother plant

The mini cuttings (12 cm in length and 0.5 cm in diameter) were obtained from three-month-old plants maintained in the greenhouse. The donor plants were pruned during the first months of age. After three months of age, rejuvenated propagules were extracted based on their good morphological characteristics and in a waning lunar calendar.

Stake preparation

The cuttings were immersed in a container with water, nitrogen, 34% hydrogen and ammonia at 7.5 ml/L, to avoid dehydration and contamination. The bases of the stakes were bevel cut and rooting hormones were applied. The stakes were placed in the substrate at a depth of 4 cm in containers formed by 140 round cavities, with dimensions of width 38.5 cm, length 58 cm, height 17 cm, and net weight 1057 g.

Rooting powder preparation

The preparation of the powders was carried out as described by (Carranza et al., 2013). The hormone indolbutyric acid (IBA) and naphthalene acetic acid (NAA) with 99% purity was weighed according to the concentrations proposed in the treatments, then mixed with 30 g of magnesium silicate $Mg_3 (SiO_{10}) (OH)_2$ (talc), and 98% potable alcohol until a pasty mass was formed, then allowed to dry at room temperature and packaged in labeled glass jars until use.

Substrate preparation

With the help of cauldrons, the substrate was disinfected at a temperature of 100°C for one hour. The substrate raw material was made from Pinus bark, to which a mixture of urea (CH_4N_2O), sucrose 60%, fructose or levulose 5%, dextrose 9%, and polyhydroxybutyric acid ($C_9H_{18}O_6$) was added.

The substrate was stored for four months and then mixed with rice husk. In the mixing phase with rice husk, the substrate used was mixed with nitrogen at 12% and phosphorus at 52% (NP_2O_5), 3 kg per cubic meter. The mixing ratio of Pinus bark with rice husk was 2240 kg of Pinus bark and 53 kg of rice. Before filling the germination trays, the substrate was dispersed at soil level and 5% sodium hypochlorite (NaCIO) was applied; once the substrate was disinfected and dried at room temperature, it was placed in the germination trays.

Growing conditions and variables evaluated on cuttings

The application of fertilizer was carried out eight days after the stakes were established, with the mineral salts proposed by Murashige & Skoog (1962)which were applied at half concentration every eight days at 5:00 pm.

The structure of the house has an automated aerial irrigation system every two hours for 15 minutes, with micro sprinklers that operate every five seconds and drains along the structure. The seedlings were taken to the acclimatization house after 20 days to obtain good rustification (Carranza et al., 2013). After 25 days, the percentage of survival, mortality and rooting, number of leaves, number of roots, height and root length (cm) were evaluated.

Results

Effect of clones

In the simple effect analysis, significant differences were observed ($P \leq 0.05$) for all the variables analyzed. In the control treatments to which no hormone concentrations were applied, all variables were not considered in the analysis since none of the plants survived.

For the effect (Eucalyptus clones) in the variables percentage of survival and rooting, the highest average was obtained by clone EC 16 with 66.74% and LA 85 with 51.05%. The clone with the highest percentage of mortality was LA 157 with 65.76%. In the variable number of leaves, the highest average was obtained by clone EC 16 with 11 leaves per plant. Regarding height, the highest average was in clone EC 18 with 11.54 cm. In the variable number of roots, the highest average was obtained by clone LA 86 with 55.00. In the variable root length, the highest average was obtained by clone EC 18 with 12.24 cm and LA86 with 9.74 cm (Table 2).

Effect of hormone concentrations

In the effect (auxin dose) on the variable percentage of survival and rooting, the stimulant with the highest average was 1500 mg kg^{-1} AIB with 76.76%. In the mortality percentage variable, the highest average was obtained by clone EC 45 with an average of 67.73% while the lowest average was that of clone EC 16 with 27.06%. In the variable number of leaves the highest averages were observed in the concentration and 1500 mg kg^{-1} AIB and 1000 mg kg^{-1} AIB with 8.56 AND 7.17 leaves in their order. The stimulant with the highest average in height was 1000 mg kg^{-1} ANA with 9.49 cm and 1500 mg kg^{-1} AIB with 8.56 cm. The highest average root heights were with auxin 1500 mg kg^{-1} AIB with 87.30 and 1000 mg kg^{-1} AIB with 50.77. The stimulant with the highest average root length was 1500 mg kg^{-1} AIB with 11.40 cm 1000 mg kg^{-1} AIB with10.66, as shown in Table 1.

Table 1. Average single effect of Eucalyptus clones and auxin doses on vegetative propagation of E. urograndis.

Factor A	**Percentage of survival**		**Mortality rate**		**Percentage of rooting**		**Number of leaves**		**Height (cm)**		**Number of roots**		**Root length (cm)**	
EC 16	66.74	a	27.06	c	66.74	a	11.06	a	10.26	a	42.80	b	8.86	ab
EC 18	45.20	b	35.74	c	45.20	b	8.20	bc	11.54	b	50.48	ab	12.24	a
EC 45	30.30	b	67.73	ab	30.30	b	6.52	cd	8.68	b	46.56	ab	9.17	ab
LA 157	29.53	c	65.76	a	29.53	c	4.21	b	6.95	bc	26.23	b	9.80	ab
LA 41	44.66	b	45.06	b	44.66	b	7.40	bcd	10.52	cd	38.69	b	9.17	ab
LA 85	51.05	a	40.08	b	51.05	a	5.45	from	6.79	cd	55.00	a	8.80	b
LA 86	30.08	b	44.79	b	30.08	b	2.70	e	4.08	d	40.08	b	9.74	a
Factor B														
1500 mg kg^{-1} AIB	76.76	a	14.32	c	76.76	a	8.56	a	8.56	a	87.30	a	11.40	a
1000 mg kg^{-1} AIB	49.20	a	46.42	c	49.20	a	7.17	a	7.17	b	50.77	a	10.66	a
1500 mg kg^{-1} ANA	30.30	b	65.92	b	30.30	b	4.88	b	7.74	b	26.85	b	9.61	b
1000 mg kg^{-1} ANA	26.02	c	71.07	a	26.02	c	4.79	b	9.49	a	19.12	c	7.23	b

Interaction of clones by hormonal contractions.

The analysis of variance for each clone confirmed the results with the full model regarding the effect of the clone and its interaction with hormone concentrations on the rooting capacity of E. urugrandis cuttings. The clone type by hormone contractions interaction had a significant effect on all variables analyzed in Figure 1.

The clone with the highest average in survival percentage was 99.90%, rooting percentage 99.90%, with the hormone concentration 1500 mg kg^{-1} AIB, root length 13.64 with the stimulant 1000 mg kg^{-1} ANA was EC18. In their order, the one with the lowest average was LA 86 obtained 14.55% survival and rooting with the 1500 mg kg^{-1} ANA hormone was LA 86. The lowest plant height with 2.85cm with a 1000 mg kg^{-1} ANA ratio. The lowest number of roots with 3.22 with a ratio of 1500 mg kg^{-1} AIB. And length with 5.95 cm with stimulant 1000 mg kg^{-1} AIB, as shown in Figure 1.

The highest number of leaves was observed for clones LA 85 with 13.79 average number of leaves with the hormone 1500 mg kg^{-1} AIB. The highest average height was obtained by clones LA 41 with 13.04 cm with the stimulant 1000 mg kg^{-1} ANA. The number of roots was higher in LA 41 clones with 118.98 with the 1500 mg kg^{-1} ANA hormone.

Figure 1. Effect of clone and stimulant interaction on rooting evaluation of tropical eucalyptus clones.

Note: a) Percentage of survival, b) Percentage of rooting, c) Root length, d) Number of leaves, e) Height of shoots, f) Number of roots.

Discussion

The use of ANA and AIB hormones has given a positive response to vegetative propagation of E. urograndis in the different clones. These results are corroborated with those expressed by (Azad et al., 2018b) who states that the use of stimulant constitutes the success of vegetative propagation. The results obtained in this research differ from what is expressed by (Gallo et al., 2017). The results obtained in this research differ from what is expressed by the author, who states that when using low concentrations, the rooting responses are better, together with the influence of environmental factors. The percentage of survival obtained in the propagation of E. urograndis in treatment T7 with 100%

(Clone EC 18 with AIB hormone at a ratio of 1500 mg kg^{-1}), allows to differ from what is expressed by those who used a different species but with a different concentration (Delazeri et al., 2017), those who used a different species, but of the same genus that found significantly higher rates for AIB at high concentrations (6000 mg kg^{-1} AIB), with a survival percentage of 80% according to Brondani et al. (2008). In some hybrid clones, the hormone concentration ranges from 1000 to 8000 mg kg^{-1} AIB (De Bona et al., 2012). According to the authors of the study, the high concentration of AIB promotes toxicity for the rooting of the mini-stakes, and the concentration varies depending on the species studied.

The rooting percentage parameters and response as a function of the different clones and hormone concentrations used, allow observing a favorable response of root development in Eucalyptus cuttings since the ranges fluctuated between 21.78 to 99.9%. These results corroborate what was expressed by Sisaro & Hagiwara (2016) and Goulart et al. (2014), who indicate that one of the factors influencing rooting is the characteristics of the genetic material is the species, although there are other factors such as the time of year and the rooting facilities available.

The results obtained in the different treatments of clones to the proportion of the stimulant allow confirming what he expresses (Ayala et al., 2020) that for Eucalyptus species there are genetic differences in the ability to survive among clones, and that this can have an important effect when designing an operational clone propagation scheme. According to Goulart et al. (2014), the concentration of the stimulant depends on each clone. For large-scale production of E. urograndis based on vegetative propagation, the care of the mother plants is crucial; therefore, a protocol for disinfection of the instruments used is proposed, to avoid the presence of fungi and bacteria that could affect rooting.

The mortality percentage obtained in the propagation of E. urograndis in the T17 treatment with 83 (Clone EC 17 with ANA hormone in proportion 1000 mg kg^{-1}) allows differing from that expressed by Ramos & Lombardi (2020), who used the same species and found a mortality rate of 30%. According to Morales (2018), the survival capacity of the seedlings is mainly attributed to the genetic characteristics of the mother plant, as well as each of the techniques used in the nursery. The effect of hormones in those data that are not considered when presenting a mortality percentage of 100% after eight days, shows that the auxin content in the mother plants is not sufficient in their genetic and physiological composition to induce roots, which demonstrates that in this species the application of auxins is very important.

The height of cuttings obtained in the propagation of E. urograndis in treatment T26 with 13.49 cm (Clone LA 41, 1000 mg kg^{-1} ANA) allows differing what is expressed by Pinto et al. (2012), who used a different species (Arracacia xanthorriza Bancroft) that showed a significant difference for ANA at 2000 mg kg^{-1}, obtaining superior results with a height of 55.5 cm. The application of ANA promotes cell elongation, and the related species and hormone percentage are closely related since increasing the concentration of auxins decrease the growth of seedlings, caused by the presence of different organisms in the same concentration. The number of leaves obtained in the propagation of E. urograndis in the T1 treatment with 13 (EC 16, 1500 mg kg^{-1} AIB) is higher than those obtained by those who in their research obtained lower results than those obtained in the T1 treatment with 13 (EC 16, 1500 mg kg^{-1} AIB). Muñoz & Molina (2016) obtained lower results in the T8 treatment with 6 (1000 mg kg^{-1} AIB). According to Alves et al. (2016), the age of the mother plants is of great importance for the development of nursery seedlings, the younger the age, the greater the capacity for leaf development.

The Root number obtained in the propagation of E. urograndis in the T29 treatment with 119 (LA 41, 1500 mg kg^{-1} ANA) differs from the data obtained by Uribe et al. (2012) in a different species (Nothofagus glauca) who obtained in T2 with 5 (3000 mg kg^{-1} ANA) and T4 with 1 (1000 mg kg^{-1} ANA). The data obtained are lower than those presented in this research. (Taiz & Zeiger, 2003). The data obtained are lower than those presented in this research and show that low auxin concentrations are required for root development since the cells of the root meristems contain a sufficient level of auxins from the aerial part for normal elongation. The root length obtained in the propagation of E. urograndis in the T7 treatment with 13.94 cm (EC 18, 1000 mg kg^{-1} AIB), allows for differing what was expressed by More et al. (2021) with a different species (Retrophyllum rospigliosii), which showed lower data at T8 with 3.1 (1000 mg kg^{-1} AIB). Several authors have confirmed that the ideal AIB concentration varies depending on the species. The permanence of the mother plant in the training area is of utmost importance, as well as nutrition, luminosity, solar radiation, pruning, and the number of leaves on the cuttings (Muñoz & Molina, 2016).

Conclusions

Vegetative propagation gives the forest species E. urograndis, higher production of good quality seedlings with genetic attributes preserved from their parent and in less time. The applied methodology shows that the highest percentage of survival and rooting was in clone EC 18 (1500 mg kg^{-1} AIB) with 99%, which shows that the application of rooting hormones induces a favorable response in the E. urograndis species. These clones with a superior root system will have a potential efficiency in the use of genetic resources to be established in commercial plantations.

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