



Mini-cutting rooting and plantlet growth in *Erythrina crista-galli* L.¹

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ABSTRACT

In this study, we evaluated the adventitious rooting competence of mini-cuttings and plantlet growth of two clones of swamp cork (*Erythrina crista-galli* L.). Experiments were carried out in a factorial of two clones to study the need of application of indolebutyric acid (IBA), substrate composition for mini-cutting rooting and plantlet growth, and rooting curves of the clones 15SM05 and 15SM08, in the completely randomized design. Mini-cuttings of swamp cork clones showed high rooting competence, with mean rooting percentage above 80% even without IBA application. Vermiculite and its combination with commercial substrate resulted in the highest percentage of rooting, and number and length of roots and shoots. The clones showed differences regarding their adventitious rooting competence and the increment in rooted mini-cuttings with time in a humidity chamber, being the clone 15SM05 the most suitable for plantlet production.

Keywords: native species; vegetative propagation; substrate; plantlet quality.

INTRODUCTION

Erythrina crista-galli L., commonly referred to as swamp cork, is a deciduous tree occurring in swampy or marshy floodplains, from Maranhão to Rio Grande do Sul in Brazil. Under these conditions, swamp cork hosts epiphytic plants and attracts several birds, such as hummingbirds, and insects (Gratieri-Sossella *et al.*, 2008), such as bees, which are its main pollinating agents (Galleto *et al.*, 2000). The swamp cork has great ornamental potential, as well as utilization in unprotected and degraded areas. Plantations or natural regeneration are usually from seminal seedlings. Seeds produced from self-fertilization, common in fragmentations of original forests, present lower fresh weight and germination than those from cross-fertilization (Galleto *et al.*, 2000).

Vegetative propagation is an alternative for plantlet production that allows multiplication of selected individuals and establishment of uniform populations by

cloning. Clonal plants can be produced from different vegetative propagation techniques. In the case of forest species, cutting or its variations (mini-cutting and micro-cutting), are the most used technique for mass production of plantlets. This is due to the fact that vegetative propagules are produced in clonal hedges established from seminal seedlings or rooted cuttings. Although these aspects are more notable for exotic species, scientific and technological development in vegetative propagation by mini-cutting of native species has been improved (Pimentel *et al.*, 2016; Rodrigues *et al.*, 2017; Burin *et al.*, 2018).

The success of plantlet production by mini-cutting depends on adventitious rooting. Several factors are involved in this process, such as the species and the genotype to be propagated, the quality of the root system being formed, and the growth of the produced plantlet (Neves *et al.*, 2006). In addition, moisture and temperature in the rooting environment, composition of the substrate,

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use and concentration of auxin, and type and size of the propagule (Pimentel *et al.*, 2016; Rodrigues *et al.*, 2017) affect the rooting percentage. Studies on native tree species have shown that the type of mini-cutting affects adventitious rooting and growth in *Cedrela fissilis* (Xavier *et al.*, 2003), while the mini-cuttings origin, leaf area and size, and concentration of indolebutyric acid affect rooting competence in *Handroanthus heptaphyllus* (Rodrigues *et al.*, 2017). Therefore, improvements in adventitious rooting competence at genotype level depends upon fine-tuning of some of these factors.

Considering that only herbaceous mini-cuttings of swamp cork plants up to one-year-old have high rooting potential (Gratieri-Sossella *et al.*, 2008), and no reference was found discussing factors affecting mini-cutting rooting, this research was carried out to evaluate the application need of different concentrations of indolebutyric acid (IBA) for adventitious rooting, the different substrate composition for mini-cutting rooting and plantlet growth, and the rooting curve of two swamp cork clones.

MATERIAL AND METHODS

The experiments were conducted in an acclimatized greenhouse of the Center for Plant Breeding and Vegetative Propagation, Plant Science Department of the Federal University of Santa Maria, State of Rio Grande do Sul, Brazil. The mini-clonal garden was established in August 2016, with plantlets originated from rooted mini-cuttings of the clones 15SM05 and 15SM08. The mini-clonal garden was established and managed according to the methodology used for other native species, such as *Cabralea canjerana* (Burin *et al.*, 2018) and *H. heptaphyllus* (Rodrigues *et al.*, 2017).

After the establishment, the plantlets were coppiced and periodically pruned for mini-stump formation. The collected shoots were used for preliminary experiments and definition of treatments. For all experiments, approximately 20 cm long shoots of the 15SM05 and 15SM08 clones were collected in the mini-clonal garden and sectioned in mini-cuttings of approximately 3 cm in length with a single bud and two leaflets with 50% of the total area. For rooting, mini-cuttings were planted in 110 cm³ polyethylene tubes and placed in a humidity chamber, equipped with a misting system to maintain relative humidity above 80%. The humidity chamber is localized in an acclimatized greenhouse.

The experiments were carried out to study the need of indolebutyric acid (IBA) application (March 2017), substrate composition for mini-cutting rooting (April 2017) and plantlet growth (May 2017), and rooting curves of the clones 15SM05 and 15SM08 (August 2017). In one experiment, initiated in March 2017, mini-cuttings were

immersed in a hydroalcoholic solution of 0, 1000, 2000, or 3000 mg L⁻¹ of IBA for 10 s. The mini-cuttings were planted in polyethylene tubes containing a mixture of a commercial substrate with pine bark and vermiculite of medium granulometry in the proportion of 2:1 (v/v) and placed in the humidity chamber. The percentages of survival, rooting, and shooting, number and length of roots, and number and length of shoots were evaluated at 30 days. The experiment was conducted as a factorial (clones × IBA concentrations) in the completely randomized design, with six replications of five mini-cuttings.

In another experiment, initiated in April 2017, mini-cuttings were planted in tubes containing different substrates. For rooting, expanded vermiculite of medium granulometry, coarse sand, a commercial substrate with pine bark, and the combination of commercial substrate and vermiculite of medium granulometry (proportion 2:1 - v/v) were evaluated. At 30 days of cultivation in the humidity chamber, mini-cuttings were subjected to the same evaluations described in the previous experiment. The experiment was conducted as a factorial (clones × substrates) in the completely randomized design, with seven replications of five mini-cuttings.

The rooted mini-cuttings obtained in the previous experiment were planted in 110 cm³ tubes in May 2017 to evaluate the plantlet growth in different substrate compositions. The commercial substrate with pine bark and vermiculite of medium granulometry (proportion 2:1 - v/v), commercial substrate and soil of subsoil (proportion 1:1 - v/v), and commercial substrate and soil of subsoil (proportion 2:1 - v/v) were evaluated. In addition, 5 g L⁻¹ of fertilizer Osmocote™ (N = 15, P₂O₅ = 9 and K₂O = 12) was added to all substrate compositions. The plantlets were kept in an acclimatized greenhouse for 120 days. Every 30 days, the plantlets were evaluated for survival percentage, number of leaves, shoot height, stem diameter, and shoot height:stem diameter ratio. The experiment was a factorial (clones × substrates) in the completely randomized design, with six replications of four plantlets.

In August 2017, mini-cuttings were also planted in polyethylene tubes containing expanded vermiculite of medium granulometry in order to study the rooting curve of each clone and determine the ideal time of mini-cuttings cultivation in the humidity chamber. The percentage of cuttings undergoing rooting and the number and length of formed roots were evaluated every seven days, until 42 days of cultivation in the humidity chamber. The accumulated rooting refers to the total number from the beginning until the evaluated day. Therefore, the total number was gotten at 42 days of cultivation in the humidity chamber for the percentage of rooting and the number and length roots. The daily current increment (DCI) and the daily mean increment (DMI) of rooting were

calculated as $DCI = X_{(i+1)} - X_{(i)}$ and $DMI = X_{(i)} / T_{(i)}$, respectively, where: i = time of evaluation; $X_{(i+1)}$ = percent of rooting, number, or length of roots at time $(i+1)$; $X_{(i)}$ = percent of rooting, number, or length of roots at the evaluation time; and $T_{(i)}$ = number of days at evaluation (Ferreira *et al.*, 2004), which intersection determines the ideal time of mini-cuttings cultivation in the humidity chamber. The experiment was conducted in the completely randomized design, with five replications of ten mini-cuttings.

The analysis of variance was used to test the effects of the treatments. The different levels of any factor that were found to lead to significant differences in the tested traits by the ANOVA F-test ($p < 0.05$) were further compared using Tukey's test, with a 5% type-I error probability ($p < 0.05$). To meet the assumptions of normality and homogeneity, the percentage data were transformed to arcsine of $\sqrt{x/100}$ and counting data to $\sqrt{x+0.5}$. All analyses were done with the aid of the software Sisvar, version 5.6 and Excel 365.

RESULTS AND DISCUSSION

There was no interaction between clones and concentrations of IBA for all evaluated traits (Table 1). Mini-cuttings presented a high percentage mean of rooting (80.8%), with all coefficients of variation below 25%, except for the percentage of shooting. Clones did not differ for percentages of survival and rooting and number of roots, but the clone 15SM05 was superior to 15SM08 for the length of roots and percentage, number, and length of shoots. The IBA application only affected the percentage of rooting. Increasing the concentration of IBA negatively affected the percentage of rooting, while the application of 1000 mg L^{-1} did not differ from the control treatment. Thus, mini-cutting rooting of swamp cork did not depend upon the IBA application.

The clones of swamp cork presented high adventitious rooting competence, which was expected since these clones were previously selected for mini-cutting rooting. The clone 15SM05 showed a 7-fold higher percentage of shooting than the 15SM08, which is important in a new plant for reaching an equilibrium between root and shoot growth and development. These selected clones presented high rooting competence without the need of IBA application, which is very important for plantlet production by mini-cutting as IBA application is laborious and costly. Similar results were observed for other tree species, such as *Vochysia bifalcata* (Rickli *et al.*, 2015), *Eucalyptus grandis* (Wendling & Xavier, 2005), *Peltophorum dubium* (Mantovani *et al.*, 2017) and *H. heptaphyllus* (Rodrigues *et al.*, 2017; Oliveira *et al.*, 2015), for which the application of AIB did not improve the percentage of the mini-cutting rooting.

There was no interaction between clones and substrate compositions for all the evaluated traits (Table 2), showing that the requirements for substrate composition were similar for both clones. High percentage mean of rooting (81%) and low coefficient of variation were also found for different substrate compositions. Clones of swamp cork did not differ for the percentages of rooting and survival, but the clone 15SM05 was superior than the 15SM08 one for the number and length of roots and the percentage, number, and length of shoots. Vermiculite and its combination with commercial substrate showed the best results for the percentage of rooting and the number and length of roots and shoots. Commercial substrate presented the highest percentage of shooting and, together with vermiculite, the highest percentage of rooting.

Although sand is a substrate that provides some aeration and porosity, and it is easy to acquire at low cost, it resulted in lower rooting percentages, probably due to

Table 1: Percentages of survival (S), rooting (R) and shooting (SH), number (NR) and length (LR) of roots, and number (NS) and length (LS) of shoots of mini-cuttings of two clones of *Erythrina crista-galli* treated with different concentrations of indolebutyric acid (IBA) and cultivated in a humidity chamber for 30 days

Treatments	S (%)	R (%)	NR	LR (cm)	SH (%)	NS	LS (cm)
Clones							
15SM05	92.5 a*	80.0 a	3.4 a	4.9 a	36.7 a	0.9 a	0.8 a
15SM08	88.3 a	81.7 a	3.9 a	3.6 b	5.0 b	0.3 b	0.2 b
IBA (mg L ⁻¹)							
0	96.7 a	85.0 ab	3.6 a	5.1 a	26.7 a	0.7 a	0.6 a
1000	98.3 a	95.0 a	3.3 a	4.1 a	25.0 a	0.7 a	0.5 a
2000	83.3 a	75.0 ab	3.7 a	4.3 a	21.7 a	0.5 a	0.4 a
3000	83.3 a	68.3 b	4.0 a	3.5 a	10.0 a	0.4 a	0.4 a
Mean	90.4	80.8	3.7	4.3	20.8	0.6	0.5
CV (%)	19.3	24.6	12.6	17.8	67.5	21.0	19.8

* Treatments followed by the same letter did not significantly differ ($p > 0.05$) according to Tukey's test. CV = coefficient of variation.

its high bulk density. The bulk density is directly affected by compaction, which results in mechanical resistance, reducing the porosity of the substrate and thus limiting the infiltration and redistribution of water, making it difficult to aerate (Mateus *et al.*, 2017). The physical characteristics of the substrate, such as high density and small particle size, reduce aeration and affect respiration (Garcia *et al.*, 2012), which is essential for rooting. In this experiment, vermiculite and its combinations with commercial substrate have lower bulk density than the combination of commercial substrate, vermiculite and coarse sand (Pimentel *et al.*, 2016). As vermiculite has a very low bulk density (130 kg m^{-3}), it contributes to lower substrate density and, therefore, increasing the percentage of rooting and the number and length of roots. The greatest rooting of *H. heptaphyllus* mini-cuttings was also obtained with the combination of commercial substrate and vermiculite, corresponding to a substrate with low bulk density and high aerial space, total porosity, and water retention capacity (Pimentel *et al.*, 2016). Besides improving rooting percentage, vermiculite also favored the number and length of roots and shoots. Longer roots allow absorption of water and minerals at higher depths, increasing the adaptability to unfavorable soil and climatic conditions, such as greater resistance to water deficit periods.

Rooted mini-cuttings were then planted in 110 cm³ tubes with different substrate compositions for growth evaluation in the greenhouse. Switching vermiculite to soil from subsoil increases the bulk density of the substrate composition from 297 kg m^{-3} to 590 kg m^{-3} and reduces the percentage of porosity from 78 to 73 (data not shown), without affecting any of the evaluated traits during 120 days of cultivation of swamp cork plantlets in the greenhouse (Figure 1). This should be associated with the adaptation of swamp cork plants to different soil types

and conditions. Since vermiculite was important for mini-cutting rooting, the combinations of commercial substrate and vermiculite (CS+V 2:1 – v/v) should be the choice of substrate composition for plantlet production of swamp cork. In this experiment, the clone 15SM05 presented the highest number of leaves, shoot height, and shoot height by stem diameter ratio already at 30 days of cultivation in the greenhouse. After 120 days of cultivation, the clone 15SM05 was superior to 15SM08 for all evaluated traits, with 89% of plantlets alive. The relationship between shoot height and stem diameter of the plantlets at 120 days of cultivation was 6.8, 2.8, and 4.8 for the clones 15SM05, 15SM08, and average substrates, respectively. These values indicate a high morphological quality of the swamp cork plantlets, considering that the relationship should be less than 10 (Birchler *et al.*, 1998), and presented a balanced growth between shoot height and stem diameter, which provides greater resistance and better fixation to the soil when planted in the field (Artur *et al.*, 2007).

The cork swamp clones also differed for the current and mean daily increments, and the accumulated rooting percentage (Figure 2). After 21 days of cultivation in the humidity chamber, the clone 15SM05 already presented 87.5% of rooted mini-cuttings compared to 0% of the 15SM08 clone. The percentage of rooting at 28 days of the clone 15SM05 was 91.7%, whereas that of the clone 15SM08 was 39.6%, reaching 87.5% after 42 days. The intersection between the current and mean daily increments determines the ideal time for the removal of mini-cuttings from the humidity chamber, as indicated by Ferreira *et al.* (2004) and confirmed by Melo *et al.* (2011). The interception occurred at 28 days of cultivation for the clone 15SM05, seven days after the maximum daily current increment. This extra time of cultivation in the humidity chamber should improve mini-cutting rooting and plantlet survival during acclimatization (Melo *et al.*, 2011).

Table 2: Percentages of survival (S), rooting (R) and shooting (SH), number (NR) and length (LR) of roots, and number (NS) and length (LS) of shoots of mini-cuttings of two clones of *Erythrina crista-galli* cultivated in different substrates for 30 days in a humidity chamber

Treatments	S (%)	R (%)	NR	LR (cm)	SH (%)	NS	LS (cm)
Clones							
15SM05	99.1 a*	83.0 a	4.4 a	3.9 a	67.0 a	1.0 a	0.8 a
15SM08	94.6 a	78.9 a	3.1 b	2.9 b	25.9 b	0.4 b	0.1 b
Substrates							
CS	98.2 a	82.1 ab	3.1 b	2.7 b	73.2 a	0.9 a	0.5 a
S	92.9 a	69.1 b	3.3 b	2.5 b	23.2 c	0.5 b	0.3 a
V	98.2 a	96.4 a	5.1 a	4.5 a	48.2 b	0.6 ab	0.6 a
CS+V	98.2 a	76.2 b	3.5 b	4.1 a	41.1 bc	0.6 ab	0.4 a
Mean	96.9	81.0	3.8	3.4	46.4	0.7	0.5
CV (%)	12.0	24.0	13.4	17.6	47.7	16.5	16.4

* Treatments followed by the same letter did not significantly differ ($p > 0.05$) according to Tukey's test. Substrates: CS = commercial substrate; S = coarse sand; V = expanded vermiculite; CS+V (2:1 v/v) = combinations of the commercial substrate with vermiculite.

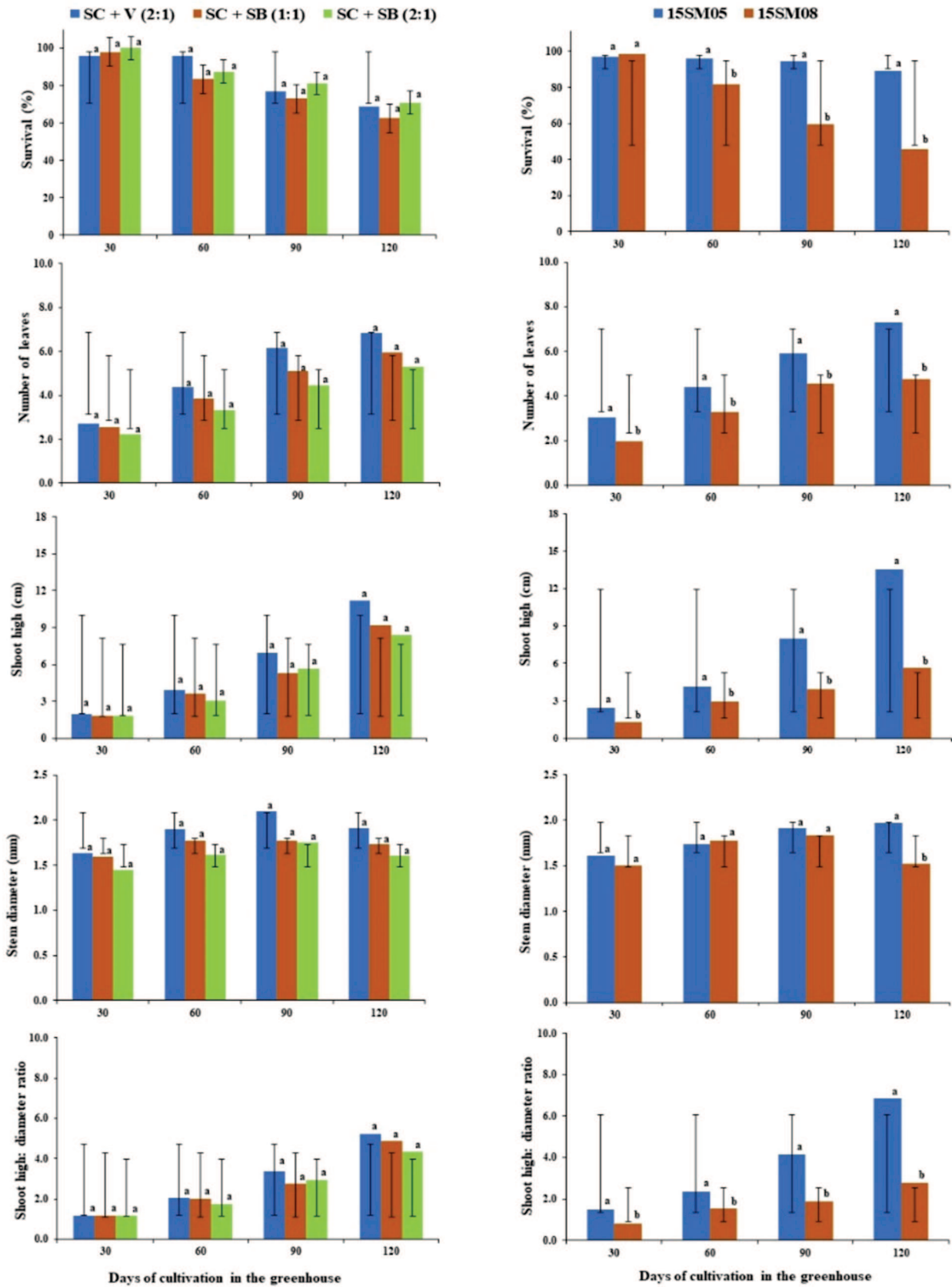


Figure 1: Percentage of survival, number of leaves, shoot height (cm), stem diameter (mm), and shoot height:stem diameter ratio in plantlets of two clones of *Erythrina crista-galli* cultivated in different substrates for 120 days in the greenhouse. Treatments followed by the same letter did not significantly differ ($p > 0.05$) according to Tukey's test. Substrates: combinations of a commercial substrate and vermiculite (CS+V 2:1 – v/v), the commercial substrate and soil from subsoil (CS+SS 1: 1 v/v) and the commercial substrate and soil from subsoil (CS+SS 2:1 v/v).

Therefore, mini-cuttings of the clone 15SM05 should be maintained in the humidity chamber for 28 days for rooting. The current daily increment did not intercept the mean daily increment until the mini-cuttings of the clone 15SM08 were removed from the humidity chamber. Therefore, mini-cuttings of the clone 15SM08 were transferred for the acclimation in an earlier rooting stage compared to 15SM05. These probably explain the differences between clones for mini-cuttings rooting and shooting (Tables 1 and 2) and plantlet survival and growth (Figure 1). These results confirmed that mini-cuttings should be removed from the humidity chamber after the interception of current and mean daily increments (Ferreira *et al.*, 2004; Melo *et al.*, 2011), since early removal may affect plantlet survival and growth (Melo *et al.*, 2011).

The number of roots followed the same pattern as the rooting percentage for both clones, with the maximum daily current increment at 21 days for 15SM05 and 28 days for 15SM08 (Figure 2). Regarding the length of the roots, the maximum daily current increment was at 35 days of cultivation for both clones. Based on the percentage of rooting and the number of roots, mini-cuttings of the clone 15SM05 should be cultivated in a humidity chamber for 28 days, while those of the clone 15SM08 should be cultivated for 42 days. Therefore, differences in rooting competence of the swamp cork clones (Tables 1 and 2 and Figure 1) were more associated with the day of evaluation, as they presented similar percentages of rooting after 42 days of cultivation in the humidity chamber.

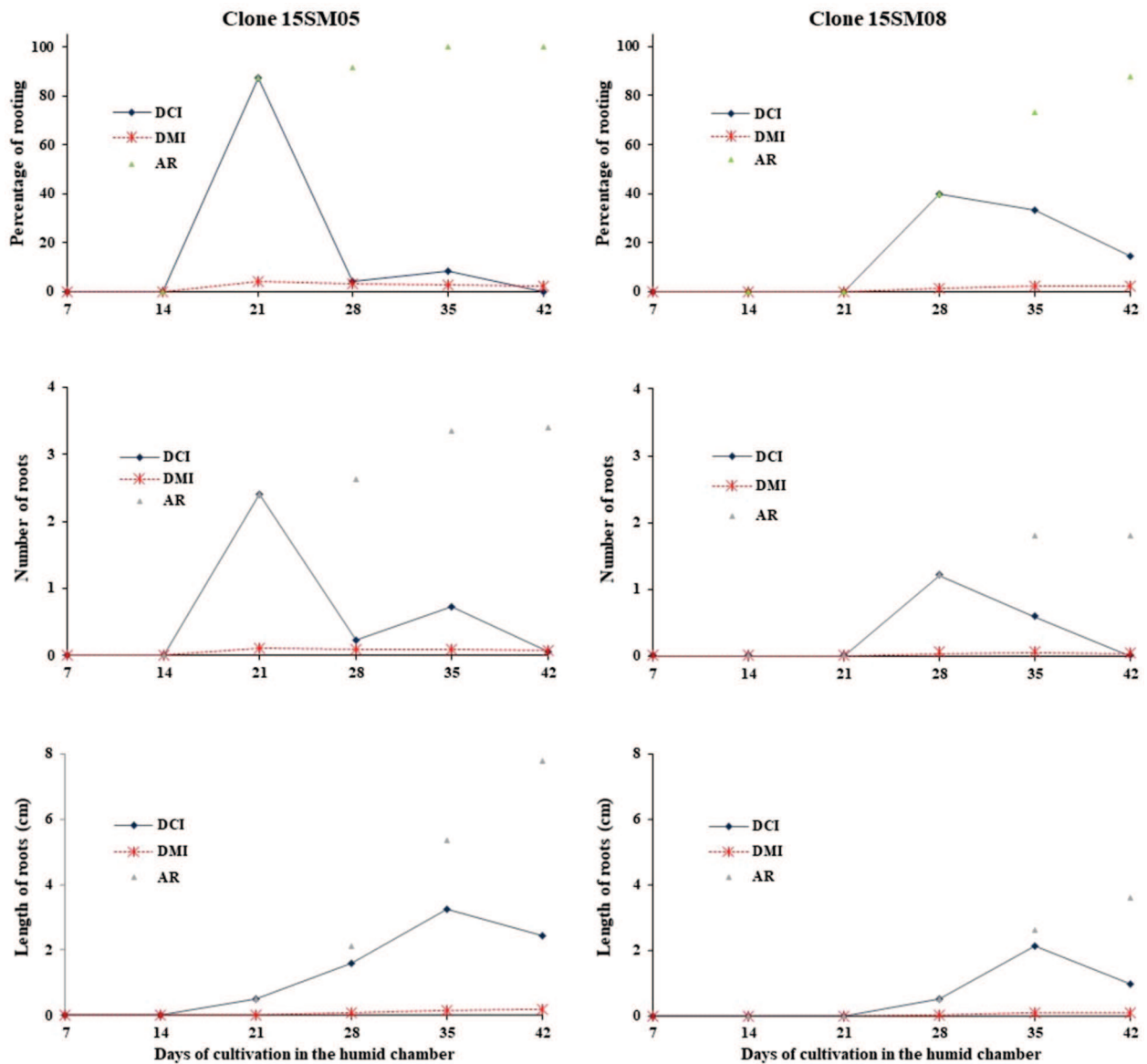


Figure 2: Daily current (DCI), daily mean (DMI) increments, and accumulated rooting (AR) of the percentage of rooting, and number and length of formed roots in mini-cuttings of two clones of *Erythrina crista-galli* cultivated in a humidity chamber for 42 days.

These differences in the adventitious rooting competence between swamp cork clones were also found in other forest species. Variations among clones and the position on the trunk where epicormic shoots were collected were found in *Ilex paraguariensis* (Bisognin *et al.*, 2018). The number of rooted mini-cuttings per mini-stump allowed an early selection of clones for the propagation of *C. canjerana* by mini-cutting (Burin *et al.*, 2018). Variations among clones were observed for IBA application and serial mini-cutting technique in *E. grandis* (Wendling & Xavier, 2005). These differences among clones corroborate the existence of genetic variability for adventitious rooting competence, enabling the identification of clones with higher production of rooted mini-cuttings in order to increase the multiplication rate (Burin *et al.*, 2018).

The swamp cork clones also showed differences in the increment of rooted mini-cuttings with time, which is very important for defining the ideal time for the removal of the mini-cuttings from the humidity chamber. The clone 15SM08 needed 50% more time in a humidity chamber than 15SM05, increasing the possibility of disease incidence and the demand for this specialized environment for producing the same number of plantlets. In addition to the greater competence for adventitious rooting, the clone 15SM05 also showed a higher rate of induction and formation of roots than 15SM08. Similar results were found in the progenies of *Anadenanthera macrocarpa* half-siblings, indicating genetic differences in the ideal time of mini-cuttings to complete the rhizogenic process in the humidity chamber (Dias *et al.*, 2012). Furthermore, in hybrid clones of *E. benthamii* × *E. dunnii*, the ideal time for mini-cutting rooting in the humidity chamber varied between 35 and 42 days, depending on the evaluated clone (Brondani *et al.*, 2012).

The results of these experiments show that mini-cuttings of swamp cork clones showed high rooting competence, with mean rooting percentage above 80% even without IBA application, and resulted in plantlets with high morphological quality. Vermiculite and its combination with commercial substrate resulted in the highest percentage of rooting, and number and length of roots and shoots. The clones showed differences regarding their adventitious rooting competence and the increment in rooted mini-cuttings with time in the humidity chamber. It probable happened because adventitious root formation is strongly controlled by a developmental program involving cell specification and reprogramming, initiation, and cell division leading to primordia formation, and emergence, and root outgrowth (Lakehal & Bellini, 2019). Therefore, for vegetative propagation by mini-cuttings, both percentage of rooting and spare time for rooting are very important traits, and

strategies should be designed for the selection of clones with high rooting competence and rate of adventitious root formation. The swamp cork clone 15SM05 combines high rooting competence, rate of adventitious root formation, and vigorous growth of the plantlets, and is therefore most suitable for the mass production of plantlets.

CONCLUSIONS

Mini-cuttings of *Erythrina crista-galli* clones showed high rooting competence even without the application of IBA. Vermiculite and its combination with commercial substrate resulted in the highest percentage of rooting, number and length of roots and shoots. The clones showed differences regarding the adventitious rooting competence and the increase of rooted mini-cuttings over time. Clone 15SM05 combines high rooting competence, adventitious root formation rate and vigorous plantlets growth.

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