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Article

Biostimulant and substrates on litchi tree propagation by air layering

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Abstract

The production of quality seedlings is important for the crop cycle and the main propagation type is by the air layering technique, which can be enhanced by using different substrates and plant growth regulators. Therefore, the objective of this study was to evaluate the use of a biostimulant and substrate types in the propagation of litchi tree by air layering. The experiment was installed in a commercial area in the city of Mogi Mirim, SP, Brazil and conducted in a randomized block design with a 5x2 factorial with four replications. The treatments consisted of five Stimulate® biostimulant concentrations (0, 3, 6, 9, and 12 ml L^{-1}) and two substrate types (Plantmax® and earthworm humus). After 120 days, the root length (cm), number of roots, calloused and rooted layers percentage (%), root fresh and dry mass (g) and root volume (cm^3) were evaluated. Based on the results, it was found that the concentrations of 6.1 and 6.9 ml L^{-1} promoted greater success in litchi tree propagation and that the layers can be produced on both evaluated substrates.

Keywords: air layering, humus, *Litchi chinensis*, plant growth regulator

Bioestimulante e substratos na propagação de lichieira por alporquia

Resumo

A produção de mudas de qualidade é importante para o ciclo da cultura, na lichieira a técnica de propagação mais utilizada é a alporquia, que pode ser potencializada com o uso de diferentes substratos e reguladores vegetais. Neste sentido, o objetivo deste trabalho foi avaliar o uso de bioestimulante e tipos de substrato na propagação da lichieira, via alporquia. O experimento foi instalado em área comercial no município de Mogi Mirim/SP, e conduzido em delineamento de blocos casualizados, com fatorial de 5x2 e quatro repetições. Os tratamentos consistiram em cinco doses do bioestimulante Stimulate® (0, 3, 6, 9, e 12 mL L^{-1}) e dois tipos de substrato (Plantmax® e Húmus de minhoca). Após 120 dias avaliou-se o comprimento das raízes (cm), número de raízes, porcentagem de alporques calejados e enraizados (%), massa fresca e massa seca de raízes (g) e volume de raízes (cm^3). Com base nos resultados, verificou-se que as doses estimadas de 6,1 e 6,9 mL L^{-1} promoveram maior sucesso na propagação de lichieira e que os alporques podem ser produzidos nos dois tipos de substratos avaliados.

Palavras chave: húmus, *Litchi chinensis*, mergulhia, regulador de crescimento

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Introduction

The litchi tree (*Litchi chinensis,* Sonn.) is an exotic plant from the *Sapindaceae* family, native from southern China and its fruits have excellent quality, pleasant taste and attractive color (Das & Prasad, 2014). The litchi is a fruit appreciated in all parts of the world, however, it is better known in Asia, where most of the production is concentrated, especially in China (Chand et al., 2014).

In Brazil, data on litchi production and planted area are not well determined. However, it is known that the production of this fruit is concentrated in São Paulo, which in 2006 accounted for 90% of the national production (Lichias, 2015). It is believed that there will be increase in litchi tree productive areas in the coming years due to producer profits and the growing demand for the fruit (Lima et al., 2011). This can already be seen by the increase in the volume marketed by CEAGESP, which grew from 319,025 kg to 2,718,204 kg between the 1999/2000 and 2009/2010 harvests (Gutierrez et al., 2011).

In this scenario, quality seedling production becomes very important since the introduction and success of new cultivation areas depends directly on the propagation material used. However, obtaining market standard litchi seedlings is one of the major obstacles to the commercial cultivation of this fruit (Smarsi et al., 2011).

The litchi propagation by seed is not recommended as it generates plants with a high degree of genetic diversity and a juvenile stage from 10 to 12 years, plus lower quality fruit (Chand et al., 2014). Thus, it is mainly propagated by the air layering technique, since the propagation through stem cuttings does not have good technical feasibility due to rooting difficulty (Smarsi et al., 2008). The success of this technique is improved by the correct choice of inputs used, such as the substrate type and use of plant growth regulators (Dutra et al., 2012a).

The substrate needs to provide water, oxygen and nutrients (Lima et al., 2010) and thus allow root growth and promote structural support to the aerial parts of the layers. Besides the substrate, the root formation process can be related to the use of plant growth regulators.

The exogenous application of synthetic auxin is one of the most studied forms to promote hormonal balance, rooting and uniformity of the root system (Dutra et al., 2012b). Among the growth regulators, there are also biostimulants, which are a mixture of two or more plant growth regulators, or one or more regulators with different compounds of a biochemical nature (Castro & Silveira, 2003). Promising results from the use of biostimulants can already be found in the literature. Abrecht et al. (2014), Dan et al. (2014) and Ferraz et al. (2014) found that biostimulants promoted better performance of pea, corn and passion fruit seeds, respectively.

Given the above, this study aimed to evaluate the use of different Stimulate® concentrations and substrate types in the vegetative propagation of litchi using the air layering technique.

Material and Methods

The experiment was installed in a commercial rainfed planting area, with 10 year-old ‘Bengal’ litchi trees, propagated by air layering and located in the city of Mogi Mirim, São Paulo, Brazil, at coordinates 22° 29'123" S and 47° 1'644" W and an altitude of 665 m, from December, 2012 to April, 2013. The city has warm subtropical climate and dry winter with an Oxisol type soil, typic dystrophic, with a clayey texture, A moderate, hypo-dystrophic, alic (Santos et al., 2006).

The study was conducted in a randomized block design with four repetitions (blocks) in a 5x2 factorial scheme, the factors being represented by five Stimulate® biostimulant doses (0, 3, 6, 9, and 12 mL L^{-1}) and two substrate types (Plantmax® and earthworm humus), totaling 10 treatments. Each of the four blocks consisted of 10 experimental units, individually composed of a plant, representing the 10 treatments, respectively, i.e., each treatment was performed separately in the plants. Five air layers were made in each plant. Considering the ten treatments and four blocks, there was a total of 40 plants and 200 air layers.

The air layers were made in the middle region of semi-woody, vigorous and healthy branches, with a diameter between one and two centimeters. The branches were randomly selected around the four quadrants of the plants and girdled with the aid of a scissor, specific for performing this technique, completely removing a two centimeter width section from the bark.

After the girdling, a 0.5 mL of solution containing biostimulant was immediately applied, using a brush to spread the product across the injured area. The biostimulant dilution was made with water containing 0, 3, 6, 9, and 12 mL of product per liter. The biostimulant, Stimulate®, consists of 0.005% indolebutyric acid (auxin), 0.009% kinetin (cytokinin) and 0.005% gibberellic acid (gibberellin) and has the ability to enhance root development and increase root water and nutrient absorption (Castro et al., 1998).

Following the biostimulant application, the covering of the annular region was conducted with 200 g of substrate (Plantmax® or humus), which was moistened and wrapped in a transparent 25.00 cm x 35.00 cm x 0.02 cm polyethylene bag tied at the ends. All the air layers were then covered with black plastic polythene bags, preventing light from entering and were identified with labels containing the information regarding the treatments.

Thirty days after the air layering preparation, each treatment was irrigated with 25 ml of water using a syringe. This procedure was repeated thereon, every 15 days until the end of the experiment.

After 120 days, the layers were removed from the plants using pruning shears and transported to the field laboratory, where the plastic bags were removed. The air layering area containing the substrate was then placed on a tray with water to facilitate the substrate removal, in order to prevent root loss. Subsequently, they were washed in water and dried at ambient condition.

The variables evaluated were: root length (the longest root in each layer was measured) in centimeters (cm), with the aid of a ruler; number of roots expressed in index from 0 to 5 (0 = no root; 1 = one root; 2 = two to five roots 3 = six to ten roots; 4 = eleven to nineteen roots, and 5 = more than twenty roots), according to the methodology of Smarsi et al. (2008); percentage (%) of calloused and rooted layers; root fresh and dry mass, in grams (g) on an analytical balance; and root volume, expressed in cubic centimeters (cm^3).

To obtain the root dry mass, the entire material was placed in paper bags and dried in an oven with forced air circulation at 65 °C until constant mass. The root volume was measured in a 100 mL graduated cylinder. The roots were inserted into the cylinder containing a known volume of water, and the volume (mL) of water displaced was noted then converted to cubic centimeters.

Data were submitted to variance analysis and when the effect on the type of substrate was significant, the means were compared by the Tukey test at p> 0.05. The effects of the biostimulating doses were analyzed by regression analysis (1st and 2nd degree), considering, when significant, the higher degree equation. All analyzes were performed by System for Analysis of Variance Computer Program - SISVAR (Ferreira, 2011).

Results and Discussion

Based on the variance analysis results, there was no significant interaction between the factors, which implies that the effects of the biostimulant doses on the root development of air layered litchi, does not depend on the substrate types (Table 1).

As in this present study, in an experiment with different types of substrates and indolebutyric acid doses (IBA) on the production of air layering of the umbu fruit tree, Dutra et al. (2012a) found no significant interaction between the substrate types and the plant growth regulator doses. When the isolated effect of the substrates was evaluated, they found significant difference only for one characteristic. Thus, this result was attributed to the productive adaptation plasticity of the species.

In contrast, Smarsi et al. (2008) observed a significant interaction between substracts and IBA doses in the production of litchi layers, especially Plantmax® associated with 2166 and 2430 mg L^{-1} doses and Humus associated with 2175 and 2250 mg L^{-1} doses. These results are different and may be related, not only to the species used, but also the nature of the substrates, growth regulators and soil and weather conditions of the experiment site.

When the isolated effect of the biostimulant doses was evaluated, there was a significant difference for the variables evaluated, except for the number of roots. Differently, regarding the type of substrate, there was no significant difference among the data for any of the characteristics (Table 1). It is noteworthy that the results were satisfactory for both substrates evaluated (Table 2).

Table 1. Summary of the variance analysis for litchi root length, number of roots, rooted and calloused layers percentage, root fresh and dry mass and root volume.

Source of Variation	Mean Square						
	Root Length	Number of roots	Rooted layers (%)	Calloused layers	Root fresh mass	Root dry mass	Root volume
Blocks	1.44^{ns}	0.07^{ns}	531.46*	813.33^{ns}	4.76^{ns}	1.06^{ns}	7.06^{ns}
Bioestimulant	6.11*	0.71^{ns}	467.78*	1585.00**	105.06**	1.83*	33.54**
Substrate	1.83^{ns}	0.48^{ns}	90.00^{ns}	160.00^{ns}	0.10^{ns}	0.01^{ns}	2.40^{ns}
Biostimulant X Substrate	4.59^{ns}	0.29^{ns}	40.00^{ns}	185.00^{ns}	6.04^{ns}	0.16^{ns}	5.33^{ns}
Error	1.76	0.14	171.81	309.63	6.57	0.59	8.01
CV (%)	12.06	8.39	15.45	22.85	25.38	33.71	23.34

* Significant at (p>0.05). ** Significant at (p<0.01). ^{NS} Not significant.

Table 2. Root length, number of roots (NR), rooted layers percentage (RLP), calloused layers percentage (CLP), Root fresh (RFM) and dry (RDM) mass and root volume (RV) of litchi in two types of substrate.

Substrate	Root lenght (cm)	Number of roots (index)	Rooted layers (%)	Calloused layers (%)	Root fresh mass (g)	Root dry mass (g)	Root volume (cm^3)
Plantmax®	11.21 a	3.81 a	86.33 a	75.00 a	10.05 a	2.27 a	11.88 a
Humus	10.78 a	3.59 a	83.33 a	79.00 a	10.15 a	2.30 a	12.37 a
Mean	10.99	3.70	84.83	77.00	10.10	2.29	12.12
LSD	0.86	0.20	8.5	11.42	1.66	0.50	1.84

Same lowercase letters do not differ by Tukey test (p>0.05).

Obtaining a substrate that can meet all the required characteristics for different cultures is not easy, therefore, those that meet the most important characteristics for plant growth should be considered (Ferraz et al., 2005). Evaluating the effect of IBA and substrates on the production of litchi layers, Smarsi et al. (2008) reported that Plantmax® and humus present good conditions for root development.

When evaluating the root length, a quadratic behavior of the means was observed according to the biostimulant doses. The longest root length (11.86 cm) was obtained with the equation peak represented by the 6.4 ml L^{-1} biostimulant dose. From this point on, there was a negative effect of increasing doses on root length (Figure 1A). The application of plant growth regulators, particularly auxin, besides lengthening, also promotes the production of thinner and more numerous roots, characteristic that can help in seedling survival due to better water absorption (Das & Prasad, 2014).

In a study on the effect of Stimulate® doses, the same biostimulant used in this present study, Costa et al. (2012) observed a quadratic increase in the litchi layer root length averages. The highest average observed was 13.36 cm, however the doses used were higher. This result was reached when using 50% biostimulant. It is noteworthy that similar results were found in the present work with lower doses, which can lower the air layering production cost.

Regarding the calloused and rooted layer percentage, as well as that of root length, there was a quadratic increase in the average due to the bio-stimulant dose increase. The highest percentages of calloused (90.48%) and rooted layers (92.65%) were found at the maximum point of the function corresponding to the 6.9 and 6.4 mL L^{-1} estimated biostimulant doses, respectively (Figures 1B and 1C). Doses higher than those reported caused quadratic reduction of calloused and rooted layer percentages. For both variables, the lowest percentages were observed in the absence of Stimulate®.

Likewise, Smarsi et al. (2008) also observed high numbers of calloused and rooted litchi tree layers using IBA and different types of substrate.

However, the average behavior was linear using Plantmax® as substrate and quadratic using humus, which was not observed in this present work. The rooting percentage has extreme importance in the production of seedlings by air layering, since the presence of roots is essential for their development.

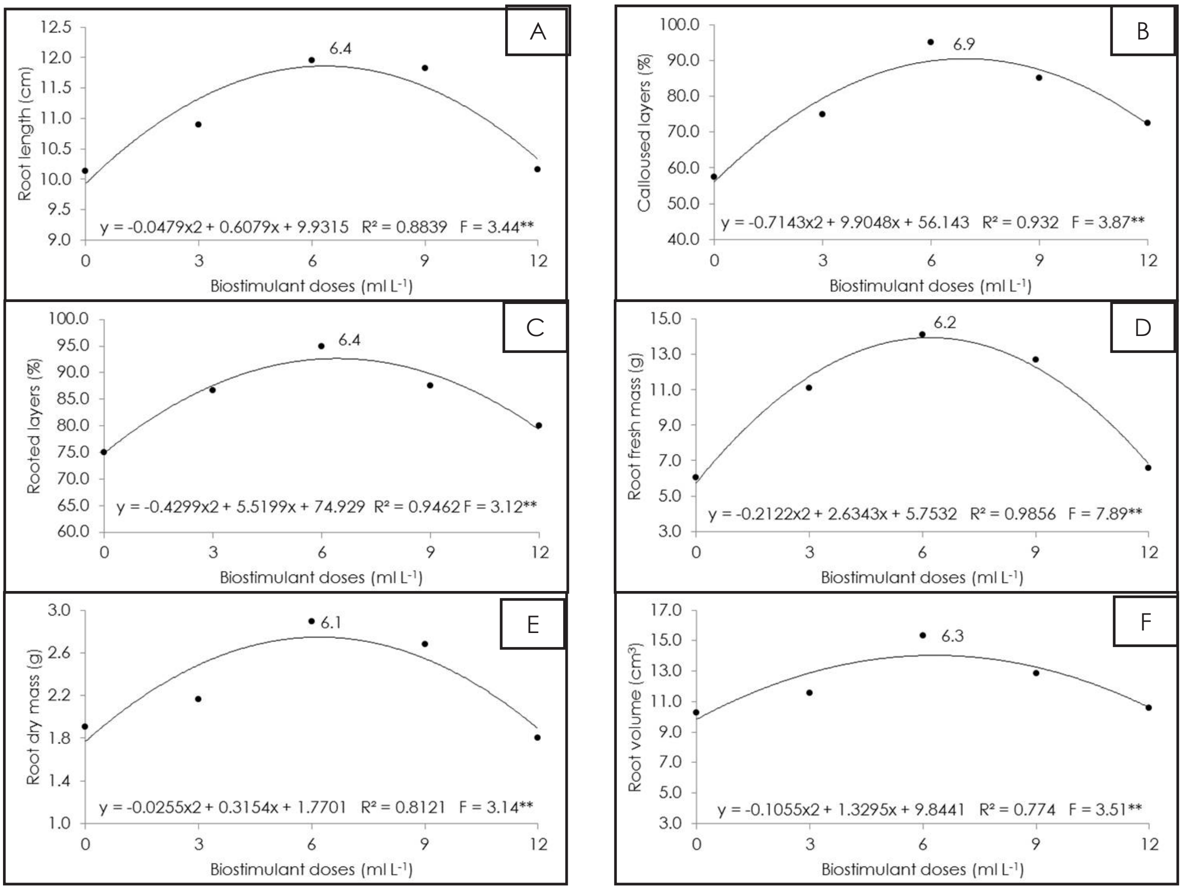

Figure 1. Root length (A). Calloused (B) and Rooted (C) layers percentage. Root fresh Mass (D) and Root dry mass (E) mass and the root volume of Bengal cultivar litchi layers submitted to doses of the biostimulant Stimulate®.

According to Timm et al. (2015), the emission of roots is related to endogenous factors and also to the environmental conditions. In an experiment with annatto layers, Mantovani et al. (2010) reported that the use of black plastic film, although having allowed a darkened environmrnt for rooting, caused an increase of the substrate temperature and made the root development of the layers unfeasible. However, in the present study the use of plastic film covering has not prevented the development of litchi layers, since the percentage of rooted layers obtained can be considered high for litchi.

The higher root fresh weight (13.93 g), dry weight (2.75 g) and volume (14.03 cm^3) values were obtained at the maximum points of the equation represented by the 6.2, 6.1 and 6.3 mL L^{-1} estimated doses, respectively. For these variables, as well as for the others, increasing doses up to the maximum point of the equation provided quadratic increase in the average (Figures 1D, 1E and 1F). Similar behavior was observed by Costa et al. (2012) for litchi layer root dry mass in response to biostimulant, however, the average was 9.41 g. It should be emphasized that this result can be related to the fact that higher biostimulant doses were used.

The use of plant growth regulators promotes higher fresh and dry mass averages due to the increased number of roots and their greater length (Das & Prasad, 2014). However, in this present experiment, the best fresh and dry mass results can be attributed only to the root lengths, since there was no significant difference between the root number average.

Lately, the plant dry matter production has been used as a relevant factor in assessing seedling quality, however, there is the necessary inconvenience of destroying the plant in order to take the measurements, which makes it unfeasible in many nurseries (Arriel et al., 2006). Similarly, larger root volume is fundamental to seedling success after transplanting to the field, as this factor is directly linked to the plant survival rate (Franco et al., 2005).

Conclusions

The use of the biostimulant between the estimated doses of 6.1 and 6.9 mL L^{-1} promotes greater success in the Bengal cultivar litchi propagation by air layering. The layers can be satisfactorily produced in both types of substrates evaluated.

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