

Brown seaweed extract enhances rooting and roots growth on *Passiflora actinia* Hook stem cuttings⁽¹⁾

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ABSTRACT

Passiflora actinia Hook (common name: ‘maracujá do mato’) is an important medicinal species due to significant sedative and anxiolytic activities. In order to commercially exploit the plant, however, studies on propagation to improve rooting in stem cuttings are needed. The present study was designed to evaluate the effect of the brown seaweed (*Ascophyllum nodosum* (L.) Le Jol.) extract when applied on *P. actinia* stem cuttings bases. Five concentrations of the extract in distilled water were evaluated: 0% (pure distilled water), 10%, 20%, 30% and 40%. The experimental design was completely randomized with 4 repetitions and 12 cuttings per experimental unit. Cuttings were evaluated 45 days from planting. Data were analyzed through polynomial regression analysis and Pearson’s correlation coefficients were calculated for all the variables. On average, 51.27% rooting was achieved. Rooting percentage increased linearly according to the brown seaweed extract concentrations. When compared to the control treatment, about 10% increase in rooting was observed at the treatment with 40% seaweed extract. Leaf retention response to *A. nodosum* increasing concentrations was also adjusted in a positive linear model. A 15.6% increase in leaf retention was observed at the 40% seaweed concentration when compared to the control. Positive correlations were observed for leaf retention and rooting percentage and leaf retention and roots length. The immersion of *P. actinia* stem cuttings bases in *A. nodosum* extract at a concentration of 40% promote positive effects on rooting and facilitate the species propagation.

Keywords: Acadian®, *Ascophyllum nodosum* (L.) Le Jol., biostimulant, maracujá-do-mato.

RESUMO

Extrato de alga marinha estimula o enraizamento e crescimento radicial em estacas caulinares de *Passiflora actinia* Hook

Passiflora actinia Hook (nome popular: ‘maracujá-do-mato’) é uma espécie medicinal relevante devido às suas propriedades sedativas e ansiolíticas. Para uma exploração comercial da planta, no entanto, são necessários estudos de propagação, especialmente visando melhorar o enraizamento em estacas caulinares. O presente estudo foi desenvolvido para avaliar o efeito do extrato da alga marinha parda (*Ascophyllum nodosum* (L.) Le Jol.) aplicado na base de estacas caulinares de *P. actinia*. Foram avaliadas cinco concentrações do extrato diluído em água destilada: 0% (água destilada pura), 10%, 20%, 30% e 40%. O delineamento experimental foi o inteiramente casualizado com 4 repetições e 12 estacas por unidade amostral. As estacas foram avaliadas 45 dias após o plantio. Os dados foram analisados por meio de análise de regressão polinomial e coeficientes de correlação de Pearson foram calculados para todas as variáveis. Em média, verificou-se 51,27% de enraizamento. A porcentagem de enraizamento aumentou linearmente de acordo com as concentrações de extrato de alga marinha. Quando comparado ao tratamento controle, observou-se aumento de 10% no enraizamento quando as estacas foram tratadas com 40% de extrato de alga marinha. A resposta de retenção foliar às concentrações de *A. nodosum* também foi ajustada em modelo linear positivo. Um aumento de 15,6% na retenção foliar foi observado na concentração de 40% de extrato de alga quando comparado ao controle. Correlações positivas foram observadas para retenção de folhas e porcentagem de enraizamento e retenção de folhas e comprimento de raízes. A imersão das bases de estacas de *P. actinia* em extrato de *A. nodosum* em uma concentração de 40% pode promover efeitos positivos no enraizamento e facilitar a propagação desta espécie.

Palavras-chave: Acadian®, *Ascophyllum nodosum* (L.) Le Jol., bioestimulante, maracujá-do-mato.

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1. INTRODUCTION

Passifloraceae family comprises about 19 genera and 530 species dispersed in tropical and subtropical regions, especially in America and Africa. The genus *Passiflora* is the most representative of the family, with approximately 400 species (BERNACCI, 2003). *Passiflora* species are widely used in traditional medicine, mainly as sedatives, anxiolytics and anticonvulsants (DHAWAN et al., 2004). Studies report the presence of diverse phytochemical compounds in *Passiflora*, such as flavonoids, phenolic acids, coumarins, phytosterols, cyanogenic heterosides, maltol and alkaloids (ZUANAZZI and MONTANHA, 2004). Among the bioactive compounds conferring the calming effects attributed to the genus, the C-glycosyl flavonoids (SANTOS et al., 2016), chrysin (ZANOLI et al., 2000) and maltol (AOYAGI et al., 1974) are some of the most important.

Passiflora actinia Hook (common name: ‘maracujá-do-mato’) is a Brazilian native species widely distributed in Southern states (SANTOS et al., 2016). This species stands out due to significant sedative and anxiolytic activities, mainly related to the leaves major compound isovitexin, which acts on benzodiazepine gamma-aminobutyric acid-A receptors (SANTOS et al., 2016; LOLLI et al., 2007). In addition to the secondary metabolites of medicinal interest, the species is extensively appreciated for human and animal feeding (LIMA et al., 2007) and also widely recognized for its use as a rootstock for commercial passionfruit farming, due to its tolerance to low temperatures (PIRES et al., 2009).

The study of suitable methods for propagation is one of the primordial aspects for the agronomic exploration of vegetal species (NUNES GOMES and KRINSKI, 2016b). Vegetative propagation by stem cuttings is one of the most widely used propagation methods in commercial production of various medicinal, fruit and ornamental crops. Among the advantages of this type of propagation, the reproduction of stock plants characteristics, uniformity of populations and operational ease stand out (HARTMAN et al., 2011). Several factors can influence vegetative reproduction success. Types of cuttings, substrates, environment humidity, hormonal balance and stock plants health, physiological conditions, and genetic characteristics are some of the main aspects pointed out as influent for stem cuttings adventitious rooting (ZUFFELLATO-RIBAS and RODRIGUES, 2001; NUNES GOMES and KRINSKI, 2016a; BISCHOFF et al., 2017; PIGATTO et al., 2018).

In addition, exogenously applied plant regulators may have positive effects on stem cuttings rooting. In a previous study on *P. actinia* vegetative propagation, the use of ethanol or indolebutyric acid (IBA) did not promote increasing in neither rooting nor roots development (KOCH et al., 2004). In this context, the study of different plant growth regulators with auxin-like effects can provide important tools to improve this species propagation. Some biostimulants are reported to have these effects, promoting rooting and roots growth in several plant species, and represent a growing tendency for use in sustainable agriculture (NARDI et al., 2016).

The brown seaweed *Ascophyllum nodosum* (L.) Le Jol. extract is classified as a plant growth regulator and its effects on plant growth and development vary with the extract concentration, mode of application and plant species (CRAIGIE, 2011). In terms of composition, the extract of brown seaweed is a natural source of macro and micronutrients (N, P, K, Ca, Mg, S, B, Fe, Mn, Cu and Zn), amino acids (alanine, aspartic and glutamic acid, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, tyrosine, tryptophan and valine), cytokinins, auxins, and abscisic acid (KOYAMA et al., 2012). Some of these components, mainly auxins and tryptophan (auxin-precursor) and micronutrients like B and Zn, are largely recognized as important tools to improve adventitious rhizogenesis in stem cuttings (ZUFFELLATO-RIBAS and RODRIGUES, 2001; TAIZ and ZEIGER, 2013).

Considering this context, the present study was conducted to evaluate the effects of treating *Passiflora actinia* stem cuttings bases with different concentrations of the brown seaweed extract.

2. MATERIAL AND METHODS

Passiflora actinia plant material (branches with leaves) was collected during morning period on September 18, 2016 in Curitiba, state of Parana, Brazil (25°24'53"S, 49°18'12"W, 934 m altitude). The region climate is classified as Cfb, characterized by mild summers, cold and dry winter, rains evenly distributed throughout the year and frequent occurrence of frosts, according to the Köppen classification.

The plant material was moistened and carefully disposed in black plastic bags to be transported to the greenhouse where the stem cuttings were made. Branches with 2.3±0.3 mm diameter were selected to prepare cuttings 10 cm long, with a straight cut at the apex and a bevel (diagonal) cut at the base. One leaf reduced to half of its original area was kept on the apex of each cutting (Figure 1A and 1C). Subsequently, the propagules underwent phytosanitary treatment in solution with 0.5% sodium hypochlorite during 10 minutes and then were washed in running water for 5 minutes.

After sanitary treatment, stem cuttings had their bases immersed for 2 minutes in solutions of brown seaweed extract diluted in distilled water at the following concentrations: 0%, 10%, 20%, 30% and 40% (e.g.: 40% solution= 0.4 mL seaweed extract diluted in 0.6 mL distilled water). The experimental design was completely randomized, with 5 treatments, 4 replications and 12 cuttings per experimental unit, totaling 240 stem cuttings. The extract used in this experiment was a commercial concentrate water-soluble liquid extract, manufactured by Acadian Seaplants® from the brown algae *Ascophyllum nodosum* (L.) Le Jol.

Following treatments, stem cuttings were planted in 120 cm³ polypropylene containers filled with previously moistened commercial substrate Tropstrato HT® (Vida Verde – Tecnologia em Substratos™, Brazil). After planting, cuttings were kept in a greenhouse with intermittent misting until evaluation. Greenhouse air relative humidity was kept

higher than 80% and the temperature ranged between 20 and 30 °C during the period of the experiment.

After 45 days from planting, plants were evaluated regarding the following variables: rooting percentage (cuttings with roots longer than 0.1 cm), average number of

roots per cutting, average length of roots per cutting (cm), calli formation percentage (cuttings with undifferentiated mass of cells at the base, as seen in figures 1B and 1D), leaf retention (cuttings that kept the original apical leaf) and, ultimately, survival percentage.

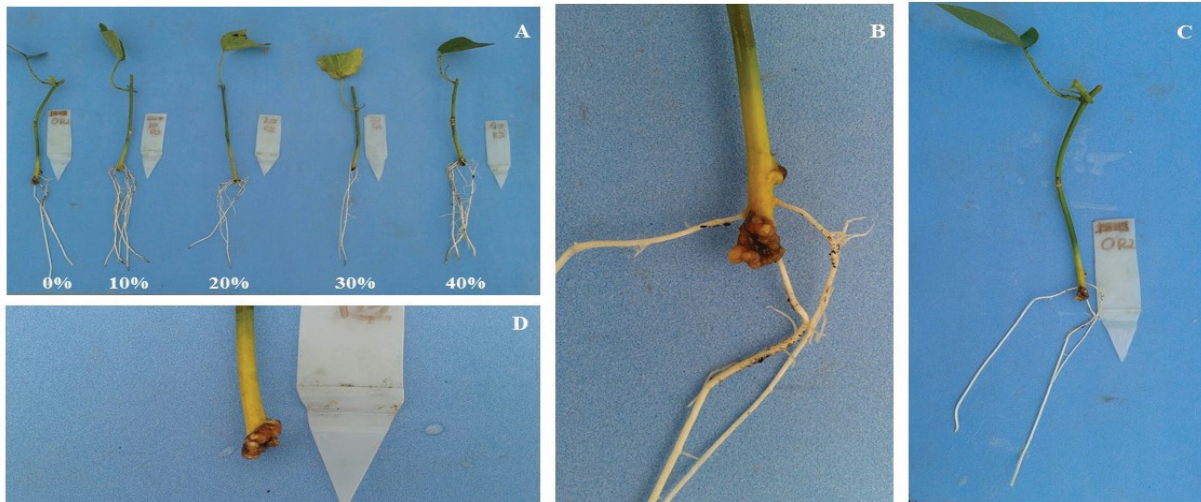


Figure 1. *Passiflora actinia* Hook stem cuttings after 45 days from planting. A: general overview of the effects of different concentrations of brown seaweed extracts. B: calli and roots formation in one stem cutting, evidencing that adventitious roots did not differentiate from calli. C: Rooted stem cutting evidencing the leaf retention. D: Stem cuttings with calli formation and no rooting.

Treatments variances were evaluated regarding homogeneity by the Bartlett test at 5% probability and, when homogeneous, data were submitted to polynomial regression analysis (5% and 1% probability). Assisat 7.7 (SILVA and AZEVEDO, 2016) statistical software was used to perform these analyses. Pearson's correlation coefficients were calculated for all the variables using R software (R CORE TEAM 2016).

3. RESULTS AND DISCUSSION

According to variance analysis, there was a significant dose-dependent effect for all analyzed variables in *Passiflora actinia* Hook stem cuttings treated with the brown seaweed extract (Figure 2). Products derived from marine algae represent a relatively recent technology

in Brazil and are a potential alternative for agronomic applications, mainly by the promotion of plant growth and development (DURAND et al., 2003).

According to the scientific literature, *P. actinia* stem cuttings root easily, achieving rooting rates of at least 40% without exogenously applied plant growth regulators (CHAVES et al., 2004; KOCH et al., 2004; ALBUQUERQUE JUNIOR et al., 2013). The present study corroborates these findings, with an average 51.27% rooting percentage (Figures 1A and 2A). Despite the good performance in the control treatment, rooting percentage increased linearly according to the brown seaweed extract concentrations. When compared to the control treatment, about 10% increase in rooting was observed at the treatment with 40% seaweed extract (Figure 2A).

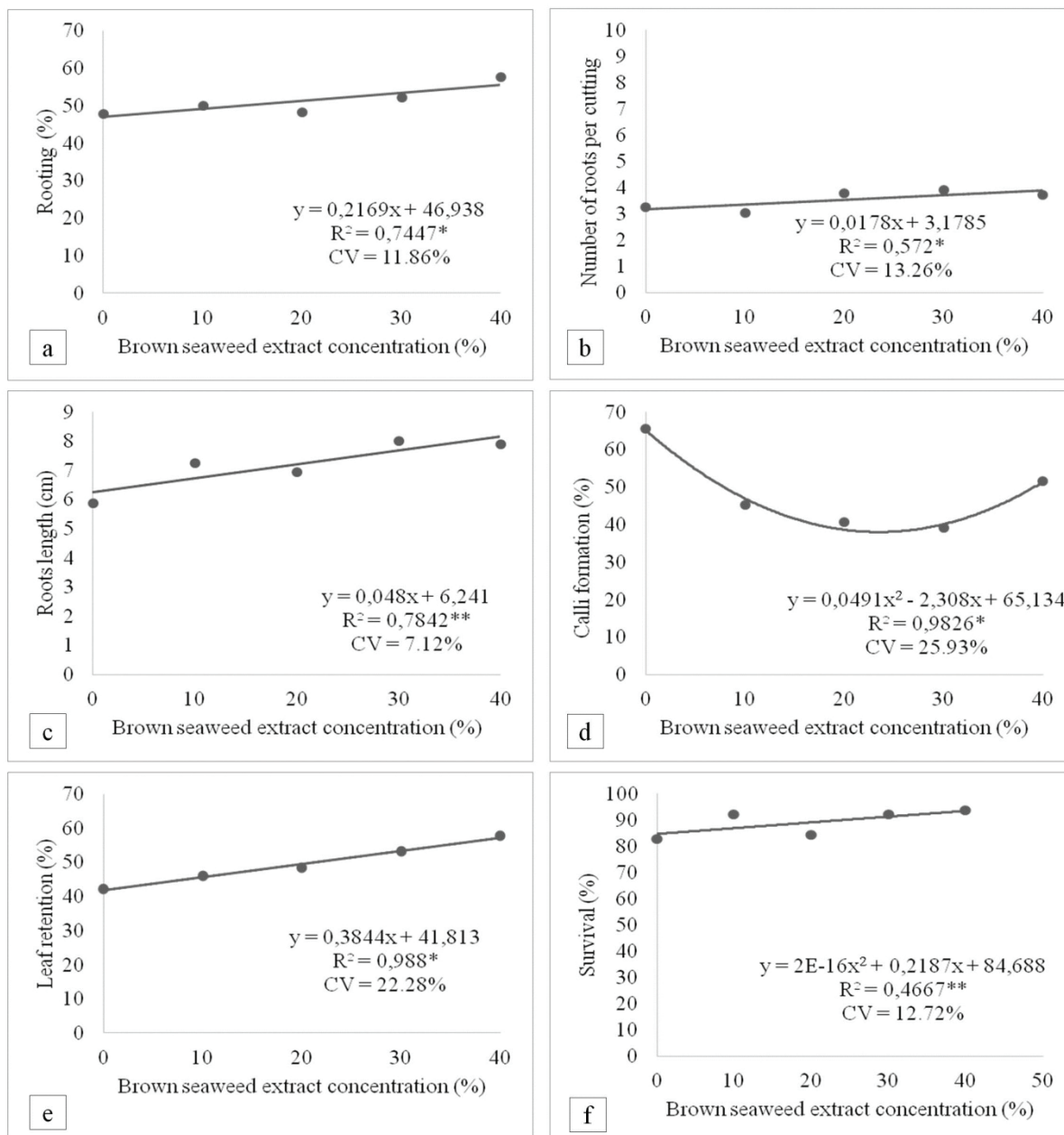


Figure 2. Polynomial regression analysis and respective equations, coefficients of determination (R^2) and coefficients of variation (CV) for the variables rooting percentage (a), number of roots per cutting (b), roots length (c), calli formation (d), leaf retention (e) and survival (f) in *Passiflora actinia* Hook stem cuttings treated with increasing concentrations of the brown seaweed extract. *significant at 5% probability. **significant at 1% probability.

The same linear responses, according polynomial regression analysis, were verified for average roots length and number of roots per cutting (Figures 2B and 2C, respectively). The positive effects of *Ascophyllum nodosum* extracts in stem cuttings rooting and roots growth can be primarily attributed to the presence of auxins, since these extracts are known to contain considerable amounts of indole-3-acetic acid (SANDERSON et al., 1987). It is a well-known fact that exogenously applied auxins act on the activation of vascular cambial cells, promoting adventitious roots emission and growth in stem cuttings (HARTMANN et al., 2011).

In addition to the exogenous auxin source, according to the data reviewed by Koyama et al. (2012), *Ascophyllum nodosum* extracts have significant amounts of amino acids such as aspartic and glutamic acids, glycine, tyrosine, and tryptophan. These amino acids have been reported to enhance rooting and roots number in plants species when applied in culture media and/or are correlated to better rooting performance in stem cuttings (ORLIKOWSKA et al., 1992; DUTRA et al., 2002).

Another important component of brown seaweed extract are macro and micronutrients such as B and Zn (KOYAMA et al., 2012), two of the most used mineral

nutrients to improve rooting in stem cuttings. Zinc can enhance rooting because it is involved in the biosynthesis of tryptophan, an auxin precursor (SCHWAMBACH et al., 2005). Boron, in turn, plays an important role in cell elongation and is considered a rooting cofactor, since it acts synergistically with endogenous auxin, facilitating its transport through the cell membranes (SANTOS et al., 2010; TAIZ and ZEIGER, 2013). These components taken together can explain the better performance for rooting and roots growth in *P. actinia* stem cuttings and also highlight the possibilities for using these extracts as alternative plant growth regulators.

Regarding calli formation, the responses to *Ascophyllum nodosum* extract concentrations were represented in a quadratic model, with a higher callogenesis percentage (65%) in the control treatment (Figure 2D). Despite the high rates of calli formation, it is important to mention that adventitious roots in *P. actinia* did not differentiated from calli, it is, the species underwent direct rhizogenesis. In some cuttings, both calli and roots formation were observed, with no direct correlation between both processes (Figure 1B and 1C). According to Hartmann et al. (2011) the processes of calli and adventitious roots formations are independent and their simultaneous occurrence is explained by the fact that both involve intense cell division and depend on favorable environmental conditions. The high callogenesis, percentage, in this case, can be an indicative of an adequate rooting environment.

The adequate rooting environment can also be observed by the high survival percentages, with an average 89.09% rate. Considering this variable, linear model was, among those tested, the one that presented statistical significance

($p \leq 0.05$) (Figure 2F). However, due to the low coefficient of determination value ($R^2 = 0.46$), the model equation does not present reliability to explain the plant response to the extract, similarly to the results reported by Fragoso et al. (2017) on cherry tree stem cuttings treated with different IBA concentrations. It is possible to affirm for this variable, however, that *P. actinia* cuttings present high resistance to mortality, that the rooting environment allowed adequate conditions for cuttings survival and, ultimately, that the seaweed extract did not jeopardized plant material survival.

Leaf retention response to *A. nodosum* increasing concentrations was adjusted in a positive linear model (Figure 2E). An increase of 15.6% was observed in leaf retention at the 40% seaweed concentration when compared to the control. The physiological response of leaf maintenance in *P. actinia* cuttings may be attributed to the presence of several natural cytokinins in *A. nodosum* extract, mainly zeatin, dihydrozeatin, isopentenyl adenine and isopentenyl adenosine (SANDERSON and JAMESON, 1986). It is a well-known fact that cytokinins coming up through the xylem to the leaves play an important role in retarding leaf yellowing, blade abscission, petiole abscission and, to a lesser extent, pod development (GARRISON et al., 1984).

The effects on leaf maintenance can also be a reasonable explanation for the positive effects of *A. nodosum* in *P. actinia* stem cuttings rooting. The studies from Albuquerque Junior et al. (2013) and Lima et al. (2007) clearly demonstrate a positive correlation between leaf maintenance and rooting in this species. On the present study a significant positive correlation was also observed for leaf retention and rooting percentage and leaf retention and roots length (Figure 3).

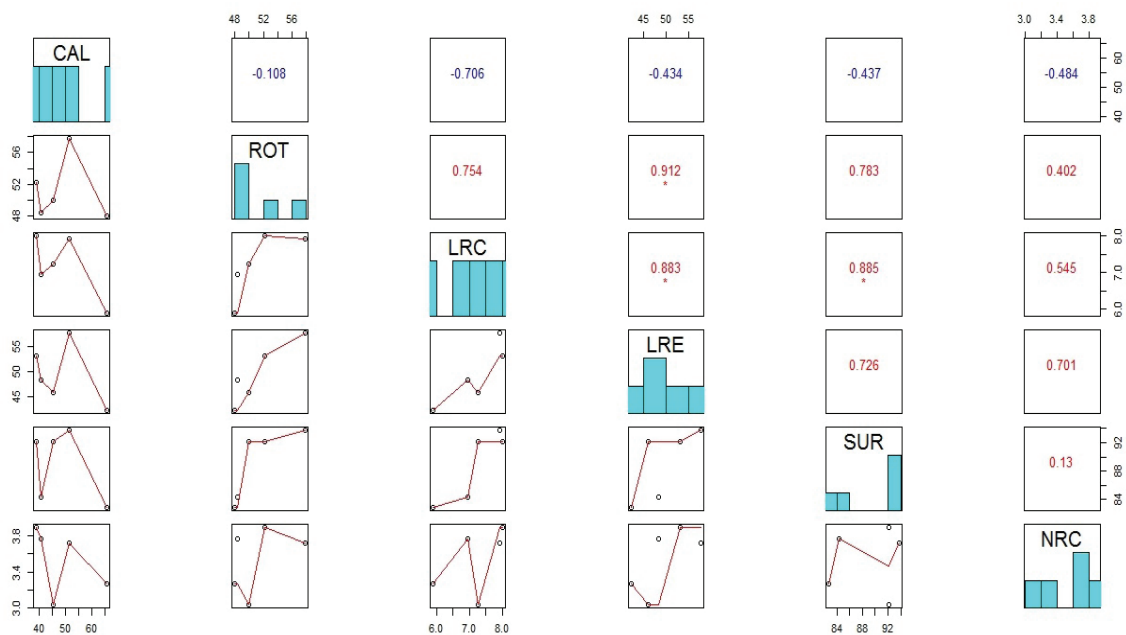


Figure 3. Pearson's correlation coefficients for ROT - rooting (%); CAL- calli formation (%); LRE - leaf retention (%); SUR - survival (%); NRC- number of roots per cutting and LRC - average length of roots per cutting (cm) in *Passiflora actinia* Hook stem cuttings treated with with increasing concentrations of the brown seaweed extract. *significant at 5% probability.








The presence of leaves is an important feature for rooting, especially when using herbaceous or semi-hardwood stem cuttings, because adventitious roots initiation and development are dependent on auxins, carbohydrates, and rooting cofactors that are supplied primarily by the leaves on this type of propagules (BONA and BIASI, 2010).

To the best of our knowledge, the present study is one of the first attempts to use *Ascophyllum nodosum* extract as a plant growth regulator to promote rooting in stem cuttings from medicinal species and demonstrates the versatility of this product for use in several segments of sustainable agriculture.

4. CONCLUSIONS

Passiflora actinia stem cuttings root easily, have elevated survival rates and present direct rhizogenesis. Immersion of stem cuttings bases for 2 minutes in a concentration of 40% *Ascophyllum nodosum* extract enhances rooting percentage, roots number and roots length as well as promotes a higher rate of leaf retention. Leaf retention has a positive correlation with rooting percentage and roots length in *P. actinia* stem cuttings.

AUTHORS CONTRIBUTIONS

E.N.G. 0000-0002-7999-070X: Conception of the study, implementation and final evaluation of the greenhouse study, data analysis and interpretation, drafting and critical revision of the article, final approval of the version to be published. **L.M.V.** 0000-0002-9336-860X: Conception of the study, implementation and final evaluation of the greenhouse study, material and methods section writing. **J.C.T.** 0000-0002-4333-3521: implementation and final evaluation of the greenhouse study, correlation analysis, results and discussion section writing. **M.M.T.** 0000-0002-9946-2701: implementation and final evaluation of the greenhouse study, correlation analysis and introduction section writing. **R.L.G.** 0000-0002-7493-0732: implementation and final evaluation of the greenhouse study, introduction section writing. **C.M.F.** 0000-0003-1224-6772: Final evaluation of the greenhouse study, correlation analysis, results and discussion section writing. **R.C.B.M.** 0000-0002-7793-216X: implementation and final evaluation of the greenhouse study.

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