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Vegetative propagation of an endemic species of the Pampa biome

Propagação vegetativa de uma espécie endêmica do bioma Pampa.

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ABSTRACT

The definition of vegetative propagation methodologies is an alternative for the preservation of endangered native species with potential for economic exploitation, as Hesperozygis ringens. Thus, this study aims to verify the possibility of propagating this species through cutting, testing different substrates and types of cuttings, and comparing the propagation through cuttings obtained from atock plants in a greenhouse and directly in the field. Apical and non-apical cuttings, 6.0cm long, obtained from material from the field and greenhouse, were propagated in trays containing carbonized rice husk and a mixture of carbonized rice husk with coconut fiber powder (2:1, v/v) as substrates. Completely randomized blocks were used in a 2x2 factorial design, with four replicates of 10 cuttings. The plantlets production of H. ringens by cutting is feasible, since it presents a high rooting percentage even when propagules are collected in plants of populations in situ. However, when collected from plants in a greenhouse, the root system of plantlets presents a higher quality than that obtained in field, making possible the production and establishment of plantlets in greenhouses.

Keywords: Cutting; Hesperozygis ringens; Rooting

RESUMO

A definição de metodologias de propagação vegetativa é essencial para a preservação de espécies nativas ameaçadas de extinção com potencial para exploração econômica, como Hesperozygis ringens. Assim, este estudo objetivou verificar a possibilidade de propagar a espécie via estaquia, testando diferentes substratos e tipos de estaca e comparar a propagação com estacas obtidas de plantas matrizes em casa de vegetação e diretamente do campo. Estacas apicais e não apicais com 6,0cm de comprimento, obtidas de material oriundo do campo e casa de vegetação foram propagadas em bandejas contendo os substratos casca de arroz carbonizada e uma mistura de casca de arroz carbonizada com pó de fibra de coco (2:1, v/v). O delineamento foi em blocos ao acaso em arranjo fatorial 2 x 2, com quatro repetições de 10 estacas. A produção de mudas de H. ringens por estaquia é viável, pois apresenta elevada taxa de enraizamento, mesmo com propágulos coletados de populações in situ. No entanto, quando coletadas de plantas em casa de vegetação, o sistema radicular apresenta qualidade superior às obtidas diretamente do campo, viabilizando a produção e o estabelecimento de mudas em viveiros.

Palavras-chave: Estacas; Hesperozygis ringens; Enraizamento

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1 INTRODUCTION

The Lamiaceae family is represented by species that have been reported to possess a wide range of biological activity, and a wide diversity of phytochemicals (VLADIMIR-KNEŽEVIĆ et al., 2006). *Hesperozygis ringens* (Benth.) Epling is a shrub species, endemic to the fields of the Southeastern Mountains and Southern Missões Region in the state of Rio Grande do Sul (RS). It is relevant due to the high concentrations of pulegone (79.2%), a volatile compound with antimicrobial, fungicidal, insecticidal and allelopathic actions, and also several oxygen derivatives (oxides of pulegone 1.2%, 8-hydroxy- p-menth-3-one, 1.3% and 8-hydroxy-p-menth-4-en-3-one, 3.7%) (VON POSER et al., 1996).

In a study conducted by Ribeiro et al. (2010), the essential oil of *H. ringens* presented a high larvicidal potential and interfered with the production of eggs of *Rhipicephalus microplus*, constituting an option for its control. This is because the control and elimination of ticks in cattle are carried out with the application of toxins prejudicial to humans and the environment, causing contamination of meat and milk if not used correctly, in addition to contamination of soil and water (DOMINGOS et al., 2013). In addition, the essential oil of *H. ringens* had its use recommended as an anesthetic for fish capture and fish handling procedures (SILVA et al., 2013; TONI et al., 2014). However, antigerminative properties of the alcoholic extract and the essential oil conferred for the species (VON POSER et al., 1996) suggest the possibility that it may be investigated as for the generation of natural herbicides.

The search for methods and substances less aggressive to the environment has stimulated the investigation of new products from plant extracts and essential oils. Your secondary metabolites are less harmful to the environment, more selective and with potent effect on a specific target (CASTILHO et al., 2017) and constitute a promising alternatives because are biodegradable and from renewable sources (BIANCHINI et al., 2017). However, the exploitation of native plant species may only be carried out with the certainty of its preservation. Therefore, it is necessary to investigate vegetative propagation methods that sustainable exploitation of *H. ringens*, since this species is classified as vulnerable according to the Lista da Flora Ameaçada de Extinção do RS (2014).

In this context, cutting is one of the most suitable techniques for large-scale vegetative propagation, as it is a simple, fast and easy-to-perform technique (HARTMANN et al., 2011), guarantees uniformity in varietals and optimizes available plantlets (EMER et al., 2016), increasing productivity and plantlet quality (MATTANA et al., 2009). This study aims to verify the possibility of propagating this species through cutting, testing different substrates, types of cuttings and comparing the propagation through cuttings obtained from matrices in a greenhouse and directly in the field.

2 MATERIALS AND METHODS

Two experiments were conducted in a masonry greenhouse covered with fiberglass, with light interception of 50%. The irrigation system was intermittent misting, and the relative air humidity was kept above 90%.

In the first experiment, apical cuttings and median cuttings of *H. ringens* were collected in situ from a population existing in the locality of Jacaquá, municipality of São Francisco de Assis (geographical coordinates 29º36'85" S and 55º09'79" W), in an area with sandy soil dominated by grasses vulnerable to erosive processes. The fertile material from two specimens of the population was recorded in the HVAT Herbarium of the University of Vale do Taquari, Univates, under record no. 4036 and 4089.

Branches of approximately 25 cm in length were collected early in the morning and immediately wrapped in moistened newspaper and packed in closed polyethylene pots. Then, the material was transported to UFRGS, where, in the afternoon of the same day, the cuttings were prepared, standardized at 6.0 cm in length, and three pairs of leaves were sectioned in half. The apex was kept in part of the cuttings, while the other part comprised the middle cutting, constituting one of the factors analyzed in the experiment. The second factor consisted of two compositions of the substrate, the first one only carbonized rice husks (CRH), and the second a mixture of CRH with coconut fiber (Golden Mix PM®, AMAFIBRA) (CFP) in a ratio of 2:1 (v/v).

In the second experiment, only apical cuttings, also 6.0 cm long, were obtained from branches collected in the same place using the same procedure as described for the experiment 1. Branches emitted by plants kept in a greenhouse at UFRGS were also collected. As a second factor, two substrate compositions were also tested: 100% CRH, and CRH with CFP (2:1, v/v).

Polyethylene multicellular trays (70 mL each cell) containing one cutting per cell were used for both experiments and the base of the cuttings was inserted into the substrate at about 2.0 cm depth.

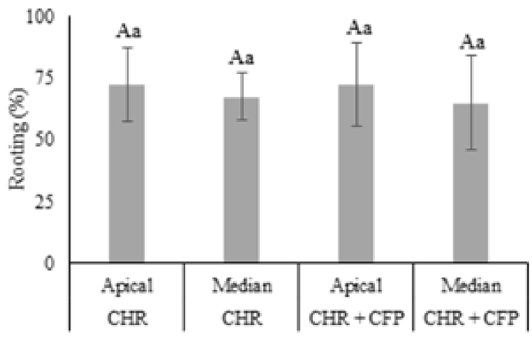
The evaluation of cuttings was performed at 25 days after experiment installation. The following data were determined: rooting percentage (%R), number of buds (NB), shoot height (SH), root length (RL), fresh mass of rooted plants (FMRP), fresh root mass (FRM), root volume (RV), dry matter of rooted plants (DMRP) and root dry matter (RDM). For the measurement of root and shoot dry matter, the percentage difference of mass after drying the material in a greenhouse at 65 ºC until reaching a constant weight was considered. The analyzed variables were measured using a precision scale (SHIMADZU) and a millimeter ruler.

Completely randomized blocks were used in a 2x2 factorial design, with four replicates containing 10 cuttings. For the first experiment, two types of cuttings were evaluated (apical and median cuttings) x two types of substrate. In the second experiment, apical cuttings of two different origins (field and greenhouse) x two types of substrates were considered. The data of both experiments were submitted to analysis of variance (ANOVA) and comparison of means by the DMS test at a 5% level of error probability using the software Costat 6.4 and SigmaPlot 11.0.

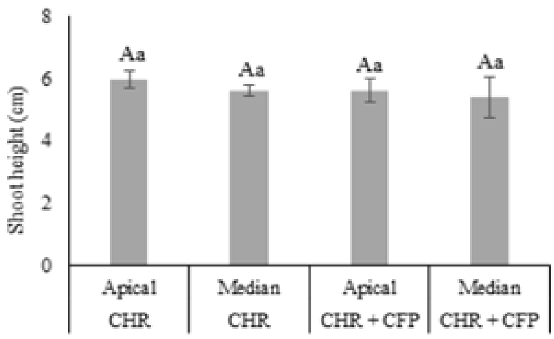
3 RESULTS AND DISCUSSION

The average rooting percentage was 77%, not statistically differing between the variables origin of cuttings, type of cutting and composition of the substrate. In the experiment 1, there was no influence of the substrate and cutting type on all variables analyzed, except for root volume ($p = 0.043$), which was higher when using medium cuttings (Figure 1).

Figure 1: Statistical significance of the variables analyzed for rooting, growth and development of shoots and roots of apical and non-apical cuttings (cutting types) of H. ringens using carbonized rice husk (CRH) and mixture of carbonized rice husks with coconut fiber powder (2:1, v/v) (CRH + CFP) as substrate 25 days after the experiment was installed. (a) Rooting percentage; (b) Number of buds per cutting; (c) Shoot Height; (d) Root length; (e) Fresh mass of plants obtained from rooted cuttings; (f) Dry matter of plants obtained from rooted cuttings; (g) Fresh root mass; (h) Root dry matter; (i) Root volume. Bars with different upper case letters indicate a statistical difference between the types of cuttings and lowercase letters between the substrates by DMS test at a 5% of error probability.



(a)



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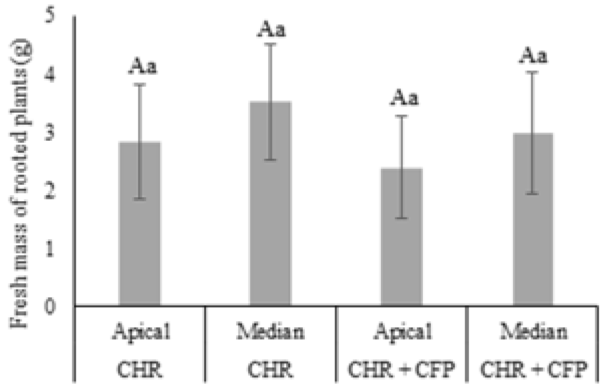
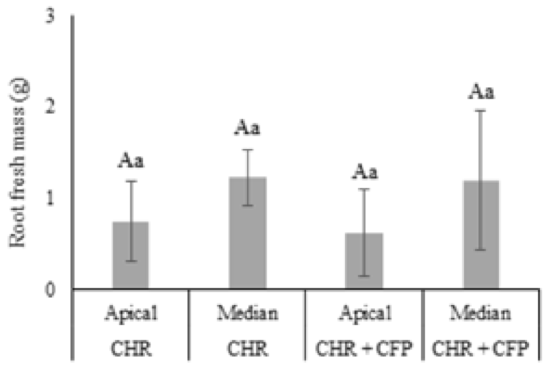
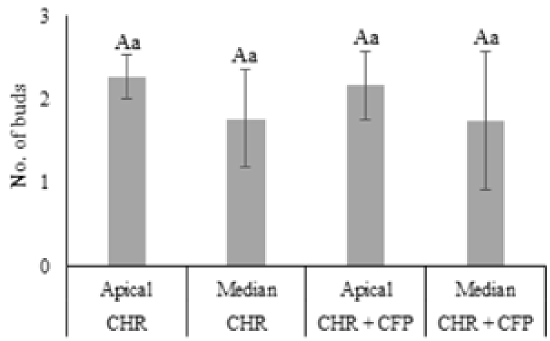

Fresh mass of rooted plants (g)
0
1
2
3
4
5
Aa
Aa
Aa
Aa
Apical CHR
Median CHR
Apical CHR + CFP
Median CHR + CFP

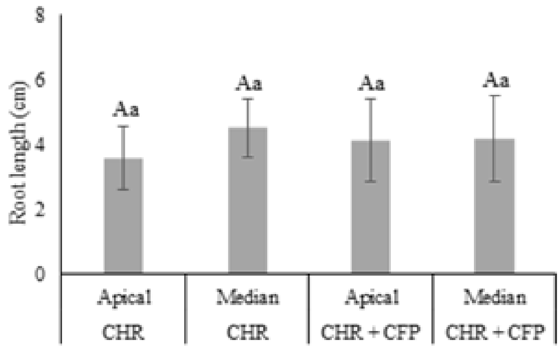
(e)


Root fresh mass (g)
0
1
2
3
Aa
Aa
Aa
Aa
Apical CHR
Median CHR
Apical CHR + CFP
Median CHR + CFP

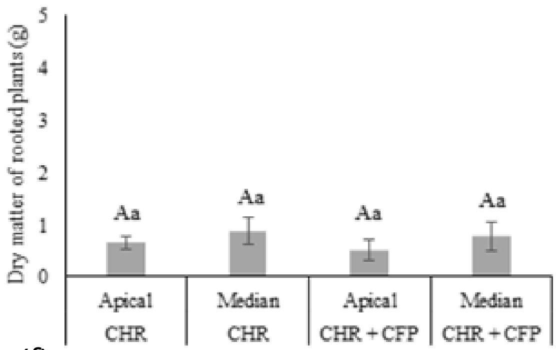
(g)


No. of buds
0
1
2
3
Aa
Aa
Aa
Aa
Apical CHR
Median CHR
Apical CHR + CFP
Median CHR + CFP

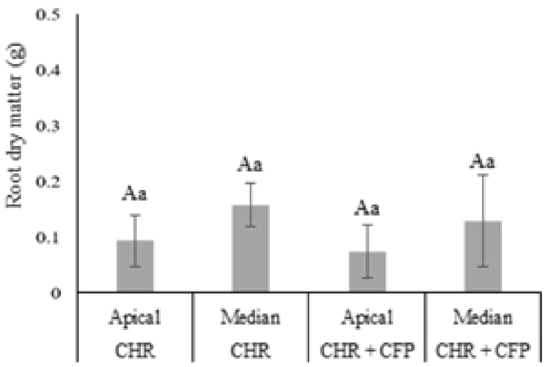
(b)


Root length (cm)
0
2
4
6
8
Aa
Aa
Aa
Aa
Apical CHR
Median CHR
Apical CHR + CFP
Median CHR + CFP

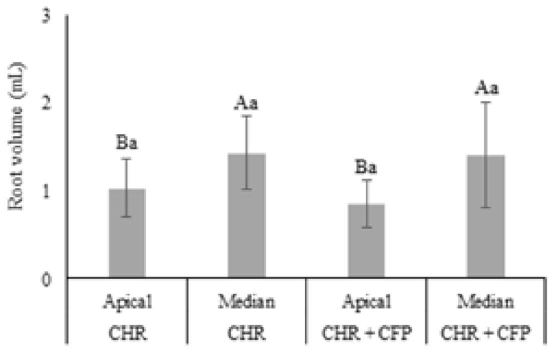
(d)



(f)



(h)



(i)

For some species, the presence of leaves and apexes on cuttings is an important factor for the induction of rooting and accumulation of dry matter in shoots, as verified for *Mentha* sp. (CHAGAS et al., 2008) and *Lippia alba* (ROCHA et al., 2001). This is because apical meristems and young leaves are sites of auxin and carbohydrate synthesis, favoring survival and root formation (PACHECO; FRANCO, 2008; PES; ARENHARDT, 2015). However, the results obtained by this study indicate that, for *H. ringens*, there is no disadvantage in the use of non-apical cuttings in relation to plants with apexes with respect to the quantitative variables evaluated.

However, it was visually verified that the plantlets obtained from apical cuttings showed a qualitative superior aspect, mainly in the appearance of leaves and shoots.

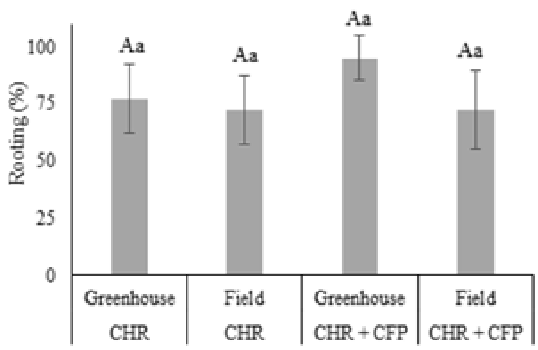
In the experiment 2, where factors originated from cuttings (greenhouse and field) and substrates (carbonized rice husks and coconut fiber powder with 1:1 carbonized husks) were tested, there was a significant interaction for the variable shoot height; the variables root length, fresh mass and dry matter of rooted plants, root fresh mass and dry matter and root volume were influenced only by the origin of collection of the cuttings.

The composition of the substrate did not significantly influence the parameters analyzed. The effects of the substrate depend directly on the irrigation management, mainly due to factors related to water availability and retention (KÄMPF, 2005). In addition, the aeration space of the substrate is also of fundamental importance, especially with regard to propagation by cutting, since it will have a direct effect on the emission and development of roots.

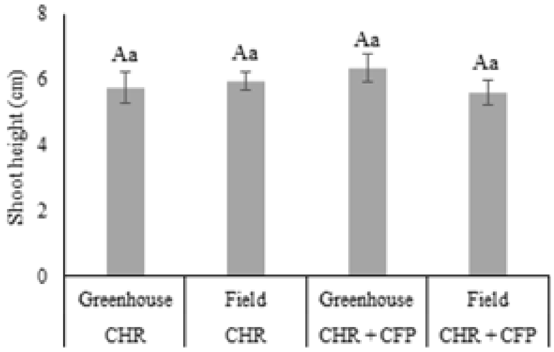
In this study, the use of carbonized rice husks, due to their low water retention capacity and their high porosity, associated with coconut fiber, which, conversely to rice husks, has a high water retention capacity, provided a good relation between water and air, thus favoring the development of roots. The mixture of materials with different physical characteristics, such as that used in this study, results, in most cases, in a substrate composition with water retention at adequate levels for the formation of plantlets, as already observed by Souza et al. (2006) for passion fruit cuttings. These results are corroborated by Zorzeto et al. (2014), who stated that coconut fiber presents a particle size with intermediate and fine fractions, whereas in rice husks there is a predominance of large and intermediate fractions. However, according to these authors, the mixture of both materials results in the desirable porosity, aeration and drainage characteristics for success in vegetative propagation.

Apical cuttings obtained from mother plants kept in greenhouse had better results for most of the analyzed variables, mainly involving the evaluation of the root system (Figure 2). In addition, the variables fresh mass and dry matter of rooted plants also presented higher averages for cuttings from matrices kept in the greenhouse (Figures 2E and F). Probably, the results are because these plants, besides being protected from meteorological agents such as rain, wind and frost, are also less susceptible to pests and diseases, thus providing better conditions for the development of plants. In addition, because of the partial shading in the greenhouse due to the polyethylene cover, cuttings from branches emitted under such conditions tend to root more easily due to the preservation of auxins and other endogenous substances to the detriment of phenolic compounds harmful to rooting (FACHINELLO et al., 2005).

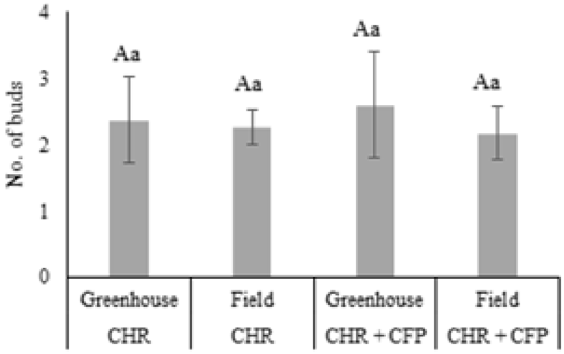
Figure 2: Statistical significance of the variables analyzed as for rooting, growth and development of shoots and roots of apical of H. ringens from plants kept in a greenhouse or collected in situ (field), using carbonized rice husks (CRH) and mixture of carbonized rice husks with coconut fiber powder (2:1, v/v) as substrates 25 days after the experiment was installed. (a) Rooting percentage; (b) Number of buds per cutting; (c) Shoot height; (d) Root length; (e) Fresh mass of plants obtained from rooted cuttings; (f) Dry matter of plants obtained from rooted cuttings; (g) Fresh root mass; (h) Root dry matter; (i) Root volume. Bars with different upper case letters indicate a statistical difference between the origin of cuttings and lowercase letters between the substrates by DMS test at a 5% of error probability.



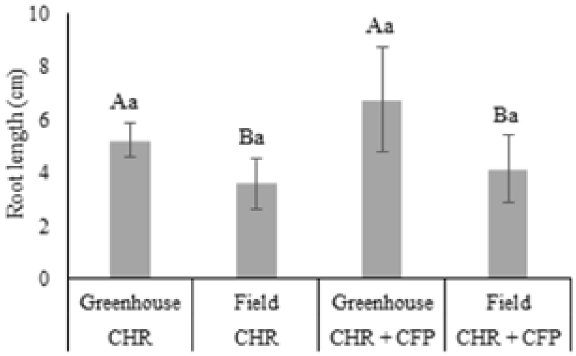
(a)


Shoot height (cm)
0
2
4
6
8
Aa
Aa
Aa
Aa
Greenhouse CHR
Field CHR
Greenhouse CHR + CFP
Field CHR + CFP

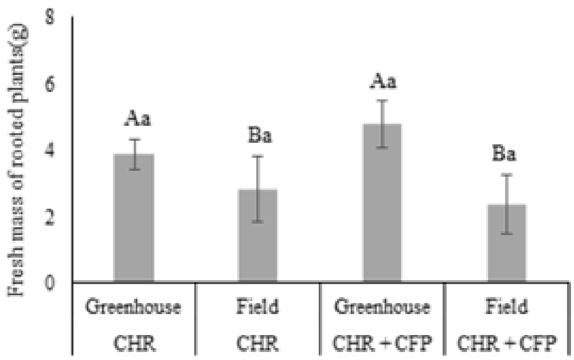
(c)


No. of buds
0
1
2
3
4
Aa
Aa
Aa
Aa
Greenhouse CHR
Field CHR
Greenhouse CHR + CFP
Field CHR + CFP

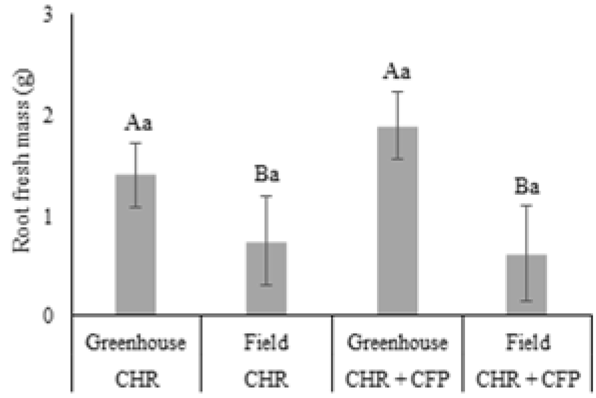
(b)


Root length (cm)
0
2
4
6
8
10
Aa
Ba
Aa
Ba
Greenhouse CHR
Field CHR
Greenhouse CHR + CFP
Field CHR + CFP

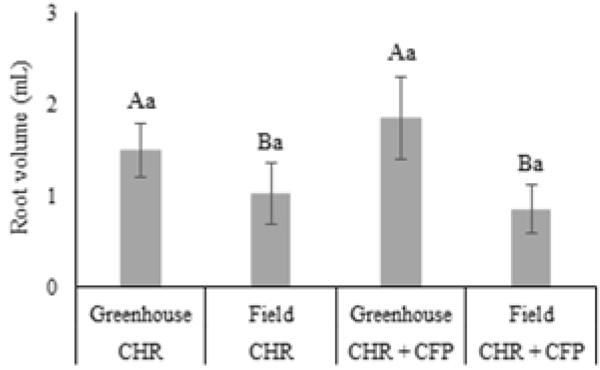
(d)


Fresh mass of rooted plants(g)
0
2
4
6
8
Aa
Ba
Aa
Ba
Greenhouse CHR
Field CHR
Greenhouse CHR + CFP
Field CHR + CFP

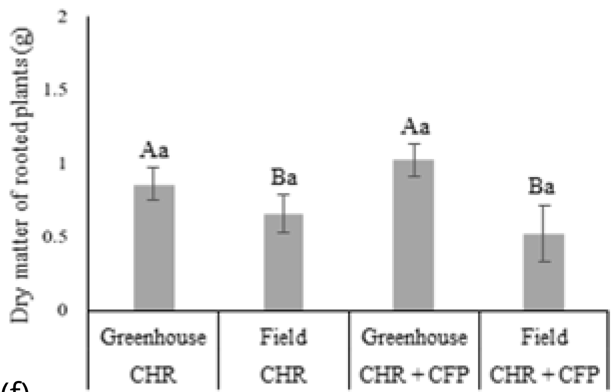
(e)


3
2
1
0
Root fresh mass (g)
Aa
Ba
Aa
Ba
Greenhouse
CHR
Field
CHR
Greenhouse
CHR + CFP
Field
CHR + CFP

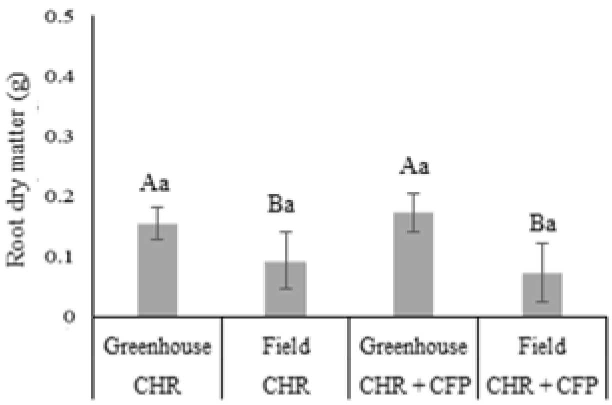
(g)


3
2
1
0
Root volume (mL)
Aa
Ba
Aa
Ba
Greenhouse
CHR
Field
CHR
Greenhouse
CHR + CFP
Field
CHR + CFP

(i)


2
1.5
1
0.5
0
Dry matter of rooted plants (g)
Aa
Ba
Aa
Ba
Greenhouse
CHR
Field
CHR
Greenhouse
CHR + CFP
Field
CHR + CFP

(f)


0.5
0.4
0.3
0.2
0.1
0
Root dry matter (g)
Aa
Ba
Aa
Ba
Greenhouse
CHR
Field
CHR
Greenhouse
CHR + CFP
Field
CHR + CFP

(h)

The hypothesis of the viability of the propagation of *H. ringens* by cutting was confirmed. This is an important factor for the study of this species with a potential for extraction of compounds of secondary metabolism, as is the case of the species of this study. According to Xavier et al. (2009), cuttings allow reducing the time of production, besides the multiplication of individuals with desirable genotypes. The use of apical cuttings, obtained from matrices originating from the field or cultivated in greenhouses, can be used as propagules for cutting.

Considering the potential of the species under study, the restricted occurrence and the fact that it is on the official list of species threatened with extinction, the importance of this work is highlighted. The possibility of a sustainable production of plantlets for planting is evidenced here, whether it is the development of researches on new products and commercial exploitation or the maintenance and expansion of populations in order to preserve the species, even considering the restrictions of the genetic base by using vegetative propagation.

4 CONCLUSIONS

The plantlets production of *Hesperozygis ringens* by cutting is feasible, since it presents a high rooting rate even when propagules are collected from plants of populations in situ. However, when collected from plants kept in a greenhouse, the root system of formed plantlets presents a higher quality than that obtained in the field, a fact that makes possible the production and establishment of plantlets in greenhouses.

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