

## ***Rhipsalis grandiflora* Haw. (CACTACEAE) PROPAGATION BY STEM CUTTINGS**

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**ABSTRACT:** The genus *Rhipsalis* is being cultivated as a potted plant since the view of the pendant branches with coloured flowers and fruit is very appreciated. Considering that there is no information about the production of rooted propagules of these species, the goal of this work was to evaluate the development of stem cuttings, stimulated by the application of plant growth regulators. The indolebutyric acid (IBA) was tested at 0; 4.07; 5.81, and 11.63 mmol L<sup>-1</sup> and the naphthaleneacetic acid (NAA) at 0; 4.53; 6.47, and 12.94 mmol L<sup>-1</sup> for their activity in promoting roots on apical stem segments of *Rhipsalis grandiflora* Raw. The base of stem cuttings was dipped into auxin solutions mixed with talc and each one was partially embedded in fir bark (*Pinus* sp) and fern fiber (1:1) in polystyrene propagation flats, placed throughout the rooting period in a greenhouse (50% shading), at 60-90% of relative humidity and temperatures from 20 to 25°C. The experimental design was a completely randomised with eight treatments (twelve replicates), with samplings at 0; 20; 50; 80, and 150 days. The results indicate no differences between IBA and NAA regarding the dry mass of roots, but an increase in for concentrations of 0 and 4.07 mmol L<sup>-1</sup> of IBA and 0 and 4.53 mmol L<sup>-1</sup> of NAA, in relation to treatments with auxins. Stem cuttings treated with 4.07 mmol L<sup>-1</sup> of IBA and 4.53 mmol L<sup>-1</sup> of NAA, had greater dry mass of shoot bud differentiation in relation to those treated with 0 mmol L<sup>-1</sup>.

**Key words:** Rhipsalidinae, articles, plant growth regulators

## **PROPAGAÇÃO DE *Rhipsalis grandiflora* Haw. (CACTACEAE) POR ESTACAS**

**RESUMO:** Plantas do gênero *Rhipsalis* estão sendo cada vez mais cultivadas em vaso, pois o efeito ornamental de seus ramos pendentes, com flores e frutos coloridos, é bastante apreciado. Em vista da falta de informações sobre o procedimento adotado para a formação de mudas, o objetivo deste trabalho foi de avaliar o desenvolvimento de estacas de *Rhipsalis grandiflora* Raw., compostas pelo artículo apical, tratadas com os fitoreguladores ácido indolbutírico (AIB) e ácido naftalenoacético (ANA), nas concentrações de 0, 4,07, 5,81 e 11,63 mmol L<sup>-1</sup> do AIB e 0, 4,53, 6,47 e 12,94 mmol L<sup>-1</sup> do ANA. A base das estacas foi imersa na mistura de talco com as auxinas testadas, sendo colocadas em bandejas de poliestireno contendo casca de *Pinus* sp. e fibra de xaxim (1:1) e mantidas durante o período de enraizamento em casa de vegetação (50% de sombra) com 60-90% de umidade relativa e temperatura na faixa de 20-25°C. Foi empregado o delineamento inteiramente casualizado com oito tratamentos, amostragens aos 0, 20, 50, 80 e 150 dias e doze repetições. Não houve diferença entre os dois reguladores, na quantidade de massa seca de raízes produzida. As estacas cultivadas nos tratamentos 0 e 4,07 mmol L<sup>-1</sup> do AIB e 0, 4,53 mmol L<sup>-1</sup> do ANA apresentaram acúmulo de massa seca de raízes, quando comparado aos demais tratamentos. Nas estacas em que se aplicaram 4,07 mmol L<sup>-1</sup> do AIB e 4,53 mmol L<sup>-1</sup> do ANA, a massa seca das brotações foi significativamente superior, em relação às estacas com 0 mmol L<sup>-1</sup> de fitoregulador.

**Palavras-chave:** Rhipsalidinae, mudas, artículos, fitoreguladores

### **INTRODUCTION**

The genus *Rhipsalis* is included in the Cactaceae-Rhipsalidinae family, and is represented mainly by epiphytic species, and sometimes lithophytic, widely distributed within the Americas, with some species occurring in the tropical Africa and Indian Ocean islands

(Loefgren, 1915; Lombardi, 1991). Since Cactaceae are typically xerophytic, this group is specialized in surviving on trees in the humid tropical forests, in places with higher light intensity. Besides performing photosynthesis, their thickened stems store water and nutrients for their survival (Britton & Rose, 1923; Joly, 1976; Scheinvar, 1985).

The Christmas Cactus and the Easter Cactus of the genus *Schlumbergera* (= *Zygocactus*) are common flower crop, as well as some species of *Rhipsalis*, *Hatiora* and *Lepismium* of the same subfamily Rhipsalidinae. In spite of the commercial interest, little is known about their propagation and cultivation. In the genus *Rhipsalis*, during the evolution process, regardless of its leaf-like shape, the stem became photosynthetic tissue, and also became a storage organ: this modified stem is formed by segments called articles. Propagation techniques use vegetative portions of the plant, such as the stem, roots or leaves (Hartmann et al., 1997).

In addition to genetic factors, adventitious root formation on the stem also depends on the presence of substances in cells and buds, which can be auxins (Válio, 1979), carbohydrates, nitrogenous compounds, vitamins and other substances. Multiplication by cutting can be achieved with or without the use of plant growth regulators and the cutting has to be dipped in a substrate. When this propagation technique is used, light intensity and quality are very important factors for root formation as semi-hardwood and herbaceous cuttings root better in the presence of light (Hartmann et al., 1997).

The objective of this study was to determine the effect of exogenous application of plant growth regulators (auxins) on the induction and formation of root and shoot bud differentiation, by evaluating the dry mass produced, using the apical article of *Rhipsalis grandiflora* Haw. as stem cutting.

## MATERIAL AND METHODS

The activity of indolebutyric acid IBA, at the concentrations of 0; 4.07; 5.81, and 11.63 mmol L<sup>-1</sup> and naphthaleneacetic acid and NAA, at 0; 4.53; 6.47, and 12.94 mmol L<sup>-1</sup> was evaluated separately. Talc was used in the preparation of the powdered mixtures with rooting regulators. The auxins were weighed separately, and the talc was added in small amounts until the desired concentration was achieved. This procedure enabled the homogenization of the powder.

Four hundred and eighty stem cuttings of the *R. grandiflora* were selected, consisting of only one apical article, their length ranging from 11.8 to 12.8 cm, and their diameter, from 3 to 5 mm. Before the powder was applied, about four cuts were made at the base of the stem cutting in order to stimulate root formation. The base of the stem cuttings was dipped into the mixture of talc and plant growth regulator, IBA or NAA, and then placed in polystyrene propagation flats containing forty-eight cells of 120 mL each. The substrate was a mixture of fir bark of *Pinus* sp. and fern fiber, in the proportion of 1 to 1, and each stem cutting was inserted up to 1/3 of its total length into individual cells. The flats were transferred to a greenhouse, with 50% shading, relative humidity at 60-90%, tempera-

ture ranging from 20 to 25°C, and kept there until the end of the experiment. The frequency of watering was established according to the variation in the substrate moisture content, which was maintained between 100 and 70%.

The experimental design was a completely randomized consisting of eight treatments, five sampling periods (0; 20; 50; 80, and 150 days), and twelve replicates. The mean tests were compared for each plant growth regulator and rate, as well as for each sampling period. During samplings, each plant was separated into roots, stem cutting and shoot bud differentiation. A ruler was used to measure the root length. After identification, the samples were dried at 80°C until constant weight. Data were analyzed by the Newman-Keuls test at 5%.

## RESULTS AND DISCUSSION

During the first 20 days, there was a reduction in the dry mass of the stem cuttings for all treatments (Figure 1, Table 1). From day 20 to day 80, stem cuttings of the control treatment, and those treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA had a larger accumulation of dry mass, reaching their maximum values at day 80, which corresponded to the dry mass observed at the beginning of the experiment. For these treatments, after that period and up to day 150, the amount

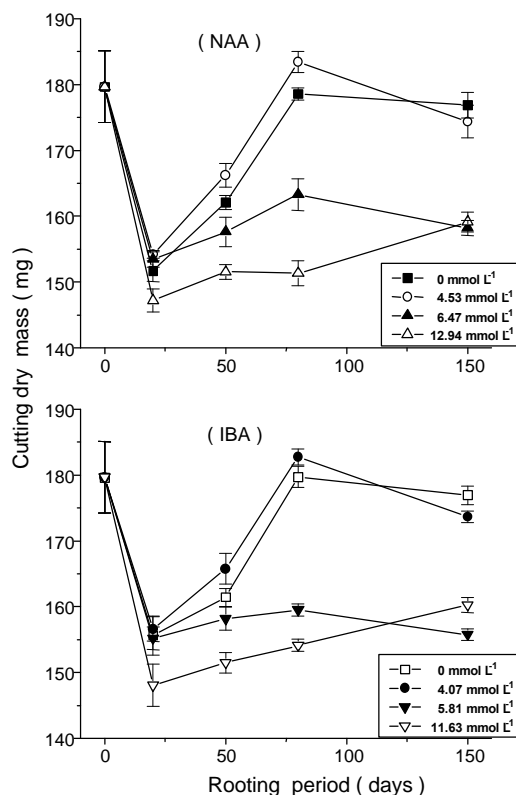


Figure 1 - Changes in the dry mass of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

of dry mass of the stem cuttings remained unchanged, or had a small decrease, and there was no difference between plant growth regulators. On the other hand, as for the first twenty days, stem cuttings treated with 5.81 mmol L<sup>-1</sup>, or 11.63 mmol L<sup>-1</sup> of IBA, and with 6.47, or 12.94 mmol L<sup>-1</sup> of NAA recovered partially their initial dry mass, reaching 85-90% at the end of the experiment, which corresponds to a difference in relation to stem cuttings of the other treatments (Table 1). Even though the curve shown in Figure 2 does not represent the biological process, it was obtained by linear regression and reveals the gain of dry mass achieved at the last sampling, thus indicating that stem cuttings of the control, and those treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA, resumed their growth.

For all treatments, the root formation process initiated twenty days after the beginning of the experiment, coinciding with the smallest dry mass of the stem cuttings (Figures 1 and 3, and Table 2). On day 50, the control stem cuttings and those treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA began to present a higher increase in root weight as compared to stem cuttings treated with 5.81 and 11.63 mmol L<sup>-1</sup> of IBA, or 6.47 and 12.94 mmol L<sup>-1</sup> of NAA. At the last sampling date, the dry mass of roots accumulated in the control stem cuttings was larger than in stem cuttings treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA (Table 2), which in turn was larger than that observed for stem cuttings treated with 11.63 mmol L<sup>-1</sup> of IBA, or 12.94 mmol L<sup>-1</sup> of NAA, and for stem cuttings treated with 5.81 mmol L<sup>-1</sup> of IBA, or

Table 1 - Mean values of dry mass of *Rhipsalis grandiflora* Haw. (Cactaceae) cutting, in mg, in those treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

Plant regulators/ concentration	Rooting period					Mean <sup>1</sup>
	0	20	50	80	150	
mmol L <sup>-1</sup>	----- days -----					
IBA 0	176.03 a <sup>2</sup>	150.38 fgh	162.18 bcd	178.42 a	176.56 a	168.71 A
IBA 4.07	176.03 a	149.96 fgh	165.18 bc	182.02 a	175.23 a	169.68 A
IBA 5.81	176.03 a	149.47 fgh	156.48 cdef	160.85 bcd	158.44 cdef	160.25 B
IBA 11.63	176.03 a	144.96 h	151.87 efgh	154.20 defg	160.59 bcd	157.53 C
NAA 0	176.03 a	150.78 efgh	162.82 bcd	179.05 a	178.57 a	169.45 A
NAA 4.53	176.03 a	151.34 efgh	166.06 b	182.57 a	174.44 a	170.09 A
NAA 6.47	176.03 a	150.75 efgh	157.05 cdef	161.32 bcd	158.05 bcdef	160.64 B
NAA 12.94	176.03 a	146.03 gh	151.82 efgh	151.18 efgh	159.43 bcde	156.90 C
Mean <sup>1</sup>	176.03 A	149.21 D	159.18 C	168.70 B	167.66 B	164.16

CV (%)= 4.0; s= 6.51

<sup>1</sup>Mean followed by the same upper case letter are not different by the Newman-Keuls test at 5%.

<sup>2</sup>Mean followed by the same lower case letter are not different by the Newman-Keuls test at 5%.

Table 2 - Mean values of the dry mass of roots, in mg, of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

Plant regulators/ concentration	Rooting period					Mean <sup>1</sup>
	0	20	50	80	150	
mmol L <sup>-1</sup>	----- days -----					
IBA 0	0.00 k <sup>2</sup>	11.97 j	21.13 g	28.69 de	63.52 a	25.06 A
IBA 4.07	0.00 k	11.73 j	21.21 g	26.83 f	60.89 b	24.13 B
IBA 5.81	0.00 k	11.62 j	14.37 i	17.89 h	29.31 d	14.64 C
IBA 11.63	0.00 k	11.49 j	12.02 j	18.17 h	31.24 c	14.58 C
NAA 0	0.00 k	11.72 j	21.19 g	28.56 e	63.34 a	24.96 A
NAA 4.53	0.00 k	11.78 j	21.23 g	26.61 f	60.88 b	24.10 B
NAA 6.47	0.00 k	11.59 j	14.27 i	17.67 h	29.17 de	14.54 C
NAA 12.94	0.00 k	11.47 j	12.08 j	18.03 h	30.78 c	14.47 C
Mean <sup>1</sup>	0.00 E	11.67 D	17.19 C	22.81 B	46.14 A	

CV (%)=3.4; s= 0.66

<sup>1</sup>Mean followed by the same upper case letter are not different by the Newman-Keuls test at 5%.

<sup>2</sup>Mean followed by the same lower case letter are not different by the Newman-Keuls test at 5%.

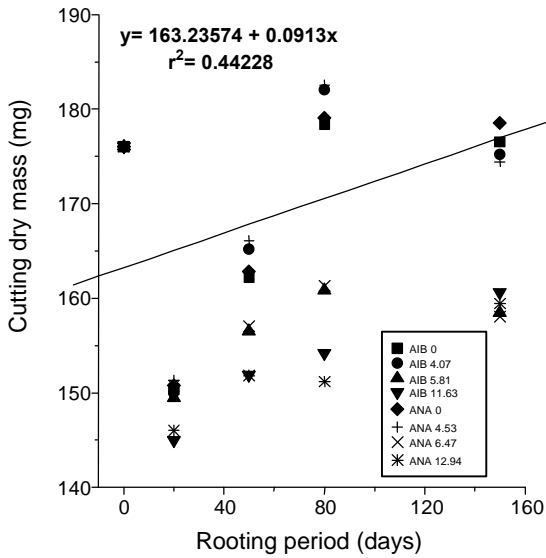


Figure 2 - Linear regression for *Rhipsalis grandiflora* Haw. (Cactaceae) dry mass of cuttings, in those treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

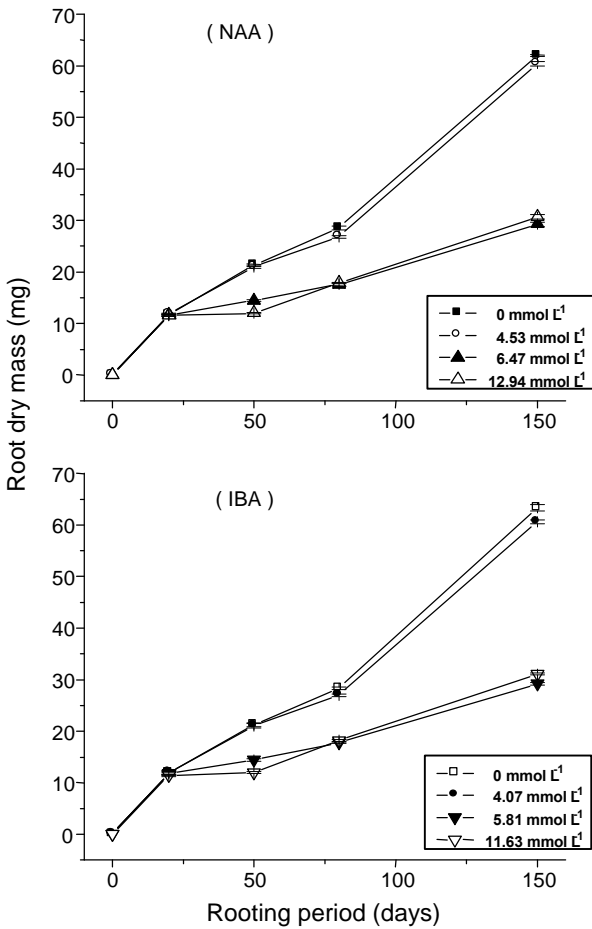


Figure 3 - Accumulation of dry mass of roots, of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

6.47 mmol L<sup>-1</sup> of NAA. Stem cuttings treated with 5.81 and 11.63 mmol L<sup>-1</sup> of IBA, or 6.47 and 12.94 mmol L<sup>-1</sup> of NAA, accumulated nearly 46-48% of dry mass of roots compared to those treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA, or in relation to the control stem cuttings, indicating that higher concentrations of auxins affected negatively the accumulation of root dry mass. For each concentration, there were no differences among the effects of the plant growth regulators, as the regression curve obtained from the dry mass values shows a constant root increase rate, which actually occurred for the control stem cuttings, 4.07 mmol L<sup>-1</sup> of IBA, and 4.53 mmol L<sup>-1</sup> of NAA (Figure 4).

Stem cuttings treated with 4.07 mmol L<sup>-1</sup> of IBA and 4.53 mmol L<sup>-1</sup> of NAA were the ones that had the largest accumulation of dry mass of shoot bud differentiation, followed by stem cuttings in the control treatment, having an accumulation of shoot bud differentiation about 40% smaller (Figure 5 and Table 3). The dry mass of shoot bud differentiation for stem cuttings treated with 5.81 and 11.63 mmol L<sup>-1</sup> IBA, or 6.47 and 12.94 mmol L<sup>-1</sup> NAA is, on the average, 38% of that treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA. Accumulation of dry mass in the shoot bud differentiation for stem cuttings treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA was more noticeable in the last period of the experiment, that is, from day 80 to day 150 (Figure 5 and Table 3). Regarding the dry mass of shoot bud differentiation, stem cuttings of the control and those treated with IBA 4.07 and NAA 4.53 (Figure 6), tend towards accumulation. Comparatively, stem cuttings of the other treatments, 5.81 and 11.63 mmol L<sup>-1</sup> of IBA, or 6.47 and 12.94 mmol L<sup>-1</sup> of NAA showed smaller accumulation of dry mass of shoot bud differentiation.

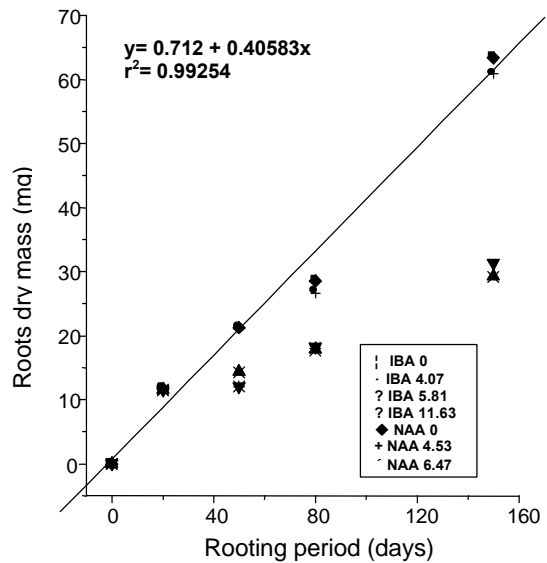


Figure 4 - Linear regression for dry mass of roots, in *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

Table 3 - Means values of shoot bud differentiation, in mg, of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

Plant regulators/ concentration	Rooting period					
	0	20	50	80	150	Mean <sup>1</sup>
mmol L <sup>-1</sup>	days					
IBA 0	0.00 t <sup>2</sup>	0.00 t	31.13 p	82.26 hi	135.93 c	49.86 C
IBA 4.07	0.00 t	0.00 t	36.07 o	121.00 e	229.92 a	77.40 A
IBA 5.81	0.00 t	0.00 t	25.35 r	58.07 l	84.64 g	33.61 E
IBA 11.63	0.00 t	0.00 t	20.97 s	52.66 n	80.82 ij	30.89 G
NAA 0	0.00 t	0.00 t	29.48 q	80.11 j	122.50 d	46.42 D
NAA 4.53	0.00 t	0.00 t	37.39 o	115.10 f	201.67 b	70.83 B
NAA 6.47	0.00 t	0.00 t	24.72 r	60.47 k	79.97 j	33.03 E
NAA 12.94	0.00 t	0.00 t	22.03 s	55.27 m	83.17 h	32.10 F
Mean <sup>1</sup>	0.00 D	0.00 D	28.39 C	78.12 B	127.33 A	

CV (%)= 3.8; s= 1.79

<sup>1</sup>Mean followed by the same upper case letter are not different by the Newman-Keuls test at 5%.

<sup>2</sup>Mean followed by the same lower case letter are not different by the Newman-Keuls test at 5%

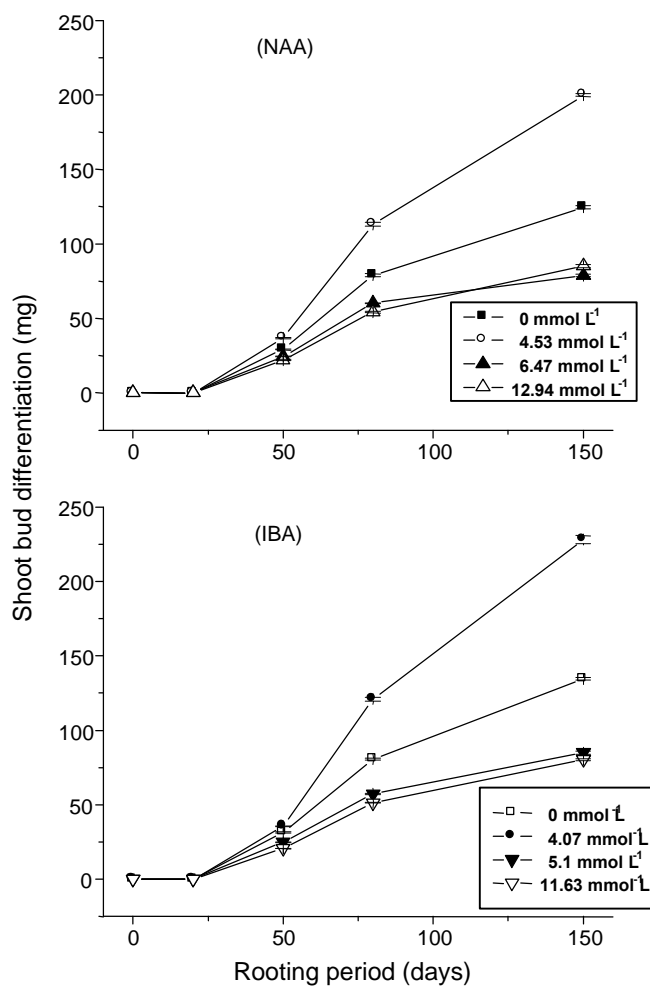


Figure 5 - Accumulation of shoot bud differentiation dry mass, of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup> and, NAA at 0, 4.53, 6.47 and 12.94 mmol L<sup>-1</sup>.

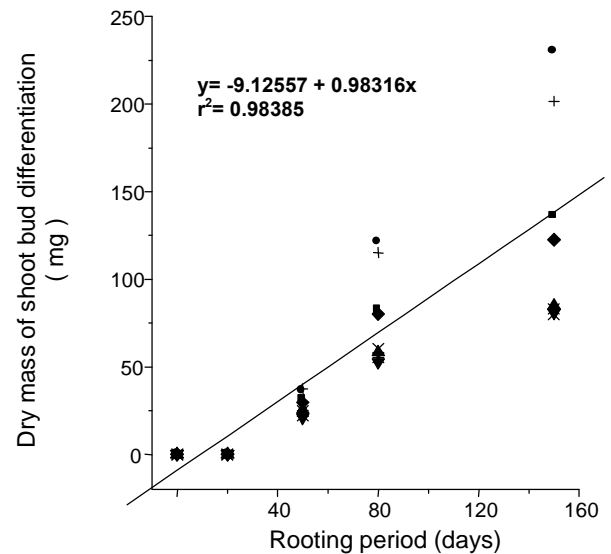


Figure 6 - Linear regression for dry mass of shoot bud differentiation, of *Rhipsalis grandiflora* Haw. (Cactaceae) cuttings treated with IBA at 0, 4.07, 5.81 and 11.63 mmol L<sup>-1</sup>.

Both plant growth regulators showed similar activities in promoting root system formation and shoot bud differentiation growth (Figures 1, 2, 3, 4, 5, and 6, and Tables 1, 2, and 3). Apparently, *R. grandiflora* stem cuttings have an adequate endogenous level of auxin for initiating root formation, since the stem cuttings that were not treated with the plant growth regulators developed satisfactorily.

In relation to metabolic changes that occurred in stem cuttings, formation of the root system was initiated only after they had a decrease in dry mass, for all treatments. Thus, once they were separated from the stock plant, and kept in proper growth conditions, the stem cuttings would

have part of their reserves hydrolysed, probably starch and/or other storage carbohydrates, to form roots. This is supported by studies carried out by Stuart (1938), Molnar & LaCroix (1972), and Breen & Muraoka (1974), which indicate that the hydrolysis of starch stored in the cutting is stimulated as root formation starts. Therefore, the hydrolysis of carbohydrates in the cutting would be important during the rooting process, in order to supply intermediate compounds as source of energy and carbon skeletons.

Opposite to the observations of Stuart (1938), Molnar & LaCroix (1972), Cuquel et al. (1992), Cuquel & Minami (1994), and Tofanelli et al. (2002), the exogenous application of auxin inhibited the accumulation of dry mass in the roots, as stem cuttings for control treatment presented a larger amount (Figure 3 and Table 2). Similar results were obtained by Prati et al. (1999), indicating that the application of IBA or NAA does not promote rooting in semi-hardwood cuttings of 'Tahiti' lime (*Citrus latifolia* Tanaka). On the other hand, application of 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA stimulated the accumulation of dry mass in shoot bud differentiation, which was probably the result of an increase in the metabolic activity in stem cuttings and greater flow of metabolites to the growing shoot bud differentiation (Figure 5 and Table 3), an information that agrees with Breen & Muraoka (1973) and Davis & Haissig (1990), who detected an increase in the sink activity of shoot bud differentiation and a higher hydrolysis rate for carbohydrates stored in cuttings treated with IBA.

Sprouting of the aerial part occurred only 50 days after the initial sampling (Figure 5), at the time when the incorporation of the dry mass in stem cuttings was initiated (Figure 1), and they already had roots (Figure 3). As for day 80, when stem cuttings of the control and those treated with 4.07 mmol L<sup>-1</sup> of IBA, or 4.53 mmol L<sup>-1</sup> of NAA recovered the dry mass of the beginning of the experiment, the accumulation rate of dry mass in root and shoot bud differentiation increased, yielding more roots and aerial part. Therefore, for the production of *R. grandiflora* from stem cuttings, the exogenous application of auxin, IBA at 4.07 mmol L<sup>-1</sup>, or NAA at 4.53 mmol L<sup>-1</sup> did not promote increase in roots directly, but the shoot bud differentiation, when compared to the production of plants without the application of auxins for rooting.

Application of auxin did not affect root length, its maximum value ranging from 14 to 15 cm in the concentrations used in our experiments. On the other hand, an opposite result was obtained by Mayer et al. (2001), who found that IBA stimulated the emission of roots and increased the length in the propagation of *Prunus mume* Sieb & Zucc. from herbaceous cuttings. Thus, for *R. grandiflora*, genetic component play a main role in rooting, acting independently of the applications of plant growth regulators. Plant production of *R. grandiflora* can be achieved from mature apical articles, which seem to have

the proper levels of auxins. Besides being expensive, high rates of plant growth regulators are unnecessary, and may even be harmful for the development of cuttings.

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