



## ***In vitro* Cultivation of Forage Palm CV. Giant with Different Concentrations of 1-Naphthaleneacetic Acid under Artificial and Natural Light**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors LCNL and CMAM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BAT and SSR managed the analyses of the study. Authors MMJ and SN managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The forage palm is cultivated in several arid and semi-arid regions of the world, characterized by water scarcity. In Brazil, the plant is a good option for forage production, meeting the food demand of animals, because it is a voluminous food, abundance in water and nutrients. The use of *in vitro* micropropagation techniques, is an alternative to increase its production, allowing to produce large amount of disease-free plants in a short time and at reduced costs. This practice requires usually the use of growth regulators. Among these, auxin like 1-Naphthaleneacetic acid (NAA), is capable of

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exert the functions on the expansion cellular and in the cellular stretching, and may also promote the cellular division in tissue culture. Therefore, the objective of this work was to verify the difference in the development of inoculated plants of forage palm cv. Giant with different concentrations of auxin under controlled light and natural light. The experiments for this study were performed in the Plant Biotechnology Laboratory of the Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG Norte, in Nova Porteirinha-Minas Gerais, between August 2015 and December 2015. It was conducted in a completely randomized design, in a plot subdivided 5x2 (NAA concentration x type of light). At 30 days of culture, shoot height and diameter, as well as the number of shoots and roots were evaluated. The best dose of NAA was established between 2-3 mg L<sup>-1</sup>, with better explant development in artificial light.

**Keywords:** Micropropagation; NAA; *Opuntia ficus-indica*; rhizogenesis; shoots and roots production.

## 1. INTRODUCTION

The arid and semi-arid regions have 55% of the world's territory, making more than 2/3 of the total area of 150 countries and encompassing almost one billion people. The Brazilian semi-arid region covers about 10% of the national territory and around 70% of the area of the Northeast region, the north of Minas Gerais [1]. Given the wide range of semi-arid areas, the search for alternatives that minimize the lack of the natural resource, like water, is essential, and forage palm is a great option to meet the demand during the dry season [2].

The forage palm (*Opuntia* and *Nopalea*) is an important food in the livestock activity, showing itself as an option for the arid and semi-arid regions of the Brazilian Northeast due to being a plant adapted to the climatic conditions of the region presenting special physiology in relation to the absorption, and loss of water, supporting long periods of drought, and being able to obtain productivity of up to 40 tons of dry matter per hectare [3].

In the recent years, there has been an increase in the number of researches focusing on alternative fodder feeds, adapted to the region, to meet the requirements of maintaining and producing animals, at low cost in critical periods of prolonged droughts [4].

The traditional propagation of the *Opuntia* occurs through the cutting of the cladodes. However, since large quantities of material are demanded by large plantations, this becomes a serious practical problem [5]. Thus, *in vitro* cultivation techniques are used to obtain an efficient multiplication system on a large scale and at reduced costs [5]. This technique can result in high multiplication rate, genetic uniformity, and better quality of the plants, compared to the conventional method [6].

Within this technique, culture media are used, which provide explants with essential nutrients. It is also usually necessary to supplement the media with phytohormones, a combination of an auxin with a cytokinin [7], in order to overcome possible deficiencies of the endogenous levels of hormones in the explants used to establish the culture [8,9]. Among them, auxin, 1-naphthaleneacetic acid (NAA) is widely used, but its concentration is one of the factors that influences the *in vitro* development process [10].

In micropropagation there are numerous studies related to the quality of light and the effects of the spectrum and the levels of irradiance on the development of explants grown is undeniable [11]. Light has a significant effect on the plant morphogenesis, also on the effectiveness of *in vitro* cultures [12].

The use of natural light may have advantages over the artificial lighting system, especially in relation to morphophysiological changes. In the previous studies, the growth of micropropagated shoots and the improvement of their physiological characteristics, due to the natural environment of the tissue culture, which facilitated the adaptation of the plants when transplanted to an *ex vitro* environment was observed [13].

The objective of the present study was to verify the best morphogenetic responses in the multiplication of the shoots introduced to MS [14] medium with different concentrations of auxin under controlled and natural light.

## 2. MATERIALS AND METHODS

### 2.1 *In vitro* Establishment

The experiment was performed in the Plant Biotechnology Laboratory of the Empresa de

Pesquisa Agropecuária de Minas Gerais - EPAMIG Norte, in Nova Porteirinha, MG.

The forage palm explants of the cultivar Gigante (*Opuntia ficus-indica* Mill.) withdrawn from the EPAMIG field were used as a source for *in vitro* explants. Those explants were established *in vitro* after 60 days of inoculation.

During the inoculation, the cladodes were cut to obtain explants (0.5 cm in length) for *in vitro* culture. The palm explants were subsequently submitted to the disinfection process, which consisted of immersion for one minute in 70% alcohol. Another immersion was made for ten minutes in sodium hypochlorite (2.0%), and then the three-fold wash of the explants was performed with distilled and autoclaved water.

The explants were inoculated into MS culture medium [14], supplemented with 0.9% sucrose; 0.1 mg L<sup>-1</sup> inositol; 8 g L<sup>-1</sup> of agar for 60 days until the shoot stage. In this period of initial establishment, no phytohormones were used for the development of the explant.

After this period, the shoots formed were used for the experiment, in which the shoots were subcultured to a pre-defined length of 0.5 cm and introduced into MS medium with 9% sucrose; 0.1 mg L<sup>-1</sup> inositol and at different concentrations of 1-Naphthaleneacetic acid (NAA) 0.0; 1.0; 2.0; 3.0 and 4.0 mg L<sup>-1</sup>. The culture media was solidified with 8 g L<sup>-1</sup> agar. The pH of the medium was adjusted to 5.8 ± 0.1 prior to autoclaving for 20 minutes. The aseptic *in vitro* plantlets were subcultured onto baby-food glass jars containing 50 ml of MS medium supplemented with 3% sucrose (pH 5.7) and solidified with 7 g L<sup>-1</sup> agar (Fisher®, Chicago, IL, USA). After explants introduction cultures were maintained under controlled environmental conditions; 27 ± 2°C; 52 μmol m<sup>-2</sup> s<sup>-1</sup>; 16/8 light/dark using Philips® LED top lighting and to the greenhouse under natural light, temperature around 34°C, and relative humidity above 75% [15]. At 30 days the explants were transferred to a new medium with the same composition.

In a laminar flow hood the palm was subcultured and established in culture medium with appropriate treatments. The explants were taken to the growth room with controlled artificial light and to the greenhouse under natural light. At 30 days the explants were transferred to a new medium with the same treatments in order to maintain the medium nutrients.

## 2.2 Statistical Analyses

The experiment was conducted in a completely randomized design, in a subdivided plot scheme with 5 concentrations of NAA and 2 types of light (artificial and natural). Thus, we had a total of 10 treatments, with 6 replicates, each replicate being constituted by four explants.

The results were submitted to analysis of variance through the SISVAR program [16], considering as sources of variation, light and doses of NAA. The scheme of subdivided plots was adopted, being the type of light in the plots and in the subplots doses of NAA.

The interaction was deployed, or not, according to significance. The effect of controlled light and natural light were compared by the F test and the NAA doses were evaluated by regression analysis using orthogonal polynomials by the decomposition of the squared sum of the interval into linear, quadratic and cubic effect. The coefficients of variation for the plot (CV a) and the subplot (CV b) were calculated. For all conclusions, α = 0.05 was considered.

The statistical model adopted for the analyzes was:

$$Y_{ijk} = \mu + \alpha_i + (\alpha\delta_{ik}) + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

em que:  $Y_{ijk}$  = dependent variables;  $\mu$  = Average population;  $\alpha_i$  = Plot effect,  $i = 1, 2$ ;  $(\alpha\delta)_{ij}$  = Experimental plot error;  $\beta_j$  = Effect of the subplot,  $j = 1, 2, 3, 4$ ;  $(\alpha\beta)_{ij}$  = Light effect and NAA  $j$ ; Random, normal and independent error, distributed with mean 0 and variance  $\sigma^2$ .

For the variable number of shoots the transformation was made, square root of  $Y + 0.5$ , due to many repetitions with values 0.

## 2.3 Evaluation of the Experiment

After 30 days after implantation, the number of shoots, number of roots, length and diameter of the regenerating explants were evaluated.

## 3. RESULTS AND DISCUSSION

There was no significant interaction ( $P = .05$ ) between the NAA doses and light in any of the analyzed variables (number of shoots, number of roots, length and diameter of the regenerating explants) during the 30 days of evaluation. Their

effects, however, act independently, so they were studied isolated.

Controlled light provided the highest average in comparison of natural light for the shoot height variable (Table 1). This may have occurred due to ambient conditions inside the growth room, which had a constant temperature and controlled luminosity from 16 hours in the light to 8 hours in the dark, and promoted the better height of the plants. The plants that went to the greenhouse were subject to temperature and luminosity oscillation. In some hours the temperature inside the greenhouse was 50°C and the peak of luminosity, occurred around 15 pm. The greenhouse, where the experiment was performed had no internal temperature control, which may be one of the reasons of the lower morphogenetic response of the *in vitro* plants.

The highest average number of roots was also obtained in controlled light compared to ambient light (Table 1). The production of roots is fundamental for the development of the plant in the acclimatization, because it is important to the plant fixation on substrate.

There were no significant differences ( $P = .05$ ) for the variables explant diameter and, number of shoots (Table 1).

In the present study, the number of shoots was very low during the evaluation. In contrast, working with two cultivars of forage palm (*Opuntia* spp.), Alves et al. [17] obtained different results, indicating a variability of responses between the palm genotypes and the BAP concentrations used for shoot growth. The authors observed in their work, that with the increase of BAP concentration, the shoots percentage was also increased to an optimum concentration. After this point there was a tendency for a decrease in the percentage of response to shoot induction. Similar results were described by García [18] and Mohamed [19] studying the species *O. ficus indica*.

For the number of roots, there was a significant difference ( $P = .05$ ) within the NAA doses, which is shown in Fig. 1. The effect of the NAA concentrations on the number of roots was quadratic. It is observed that the increase of the hormone concentration implies an increase in the number of roots (39.65 roots) at 30 days, decreasing after reaching an optimum concentration of 2.72 mg L<sup>-1</sup>.

Results obtained with *Cattleya bicolor* Lindl. (Orchidaceae) showed that the effect of NAA on

root length during the first 180 days was the 2 mg L<sup>-1</sup> concentration of this auxin significantly stimulated the growth of this organ [20].

The application of auxin to supra-optimal concentration causes an inhibitory effect on the growth of vegetative organs [21]. This affirmation was confirmed with the result obtained in the highest concentration used in this study.

Sometimes excessive amounts of auxin stimulate callus production [22]. Although NAA can induce root formation, in some species sometimes even better than IBA, it may also cause undesirable effects because it is more toxic to plant tissues [23].

In the present study, the absence of NAA was not significantly inhibitory to root development. A similar result was found by Pasqual [24], who affirmed that the rooting of *Mammillaria bocasana* (Cactaceae) occurs independently of NAA concentration. These authors observed a tendency to inhibit root development in higher concentrations of NAA, which was also observed for *Gymnocalycium buldiamur* L., another species of cactus. However, the absence of NAA during *in vitro* culture of *Pyrus communis* L. (Rosaceae) inhibits its root development. The results show that all treatments that contain this auxin stimulate the formation and elongation of root [25]. This result suggested that the amount of endogenous auxin in the plant is not enough to ensure rooting of the species. This is observed in the field, in which the forage palm cv. Giant plant ensures a lot of shoots, however the root system is weak and can not guarantee good plant support.

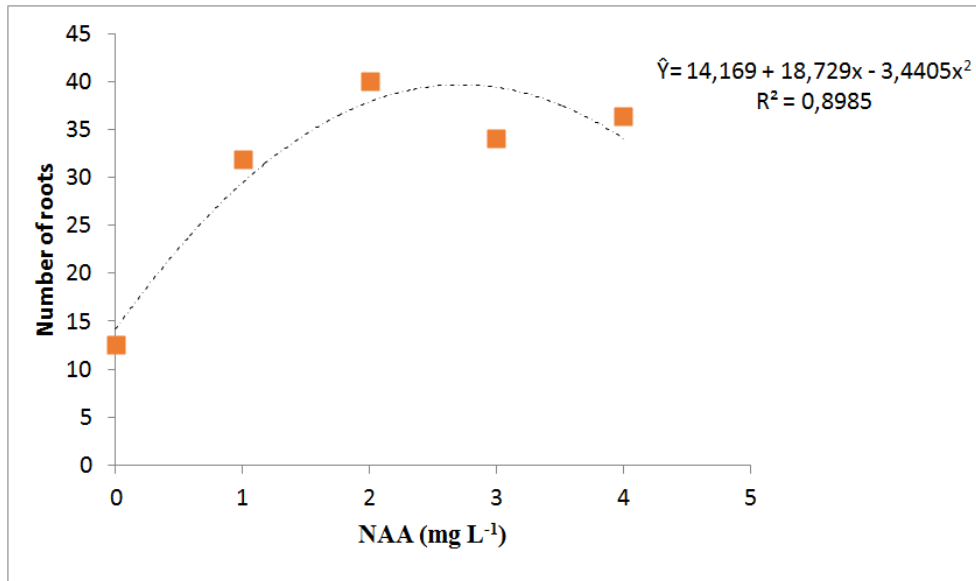
The effect of NAA on height of forage palm shoots presented quadratic behavior (Fig. 2). It is observed, that with the increase of the NAA concentration, the explant's height is increased by 17.38 mm, to an optimum concentration of 1.99 mg L<sup>-1</sup> at 30 days of culture. After this point there is a decrease in response.

The diameter of the *in vitro* cultured explants at 30 days (Fig. 3) also increased (3.55 mm) as the NAA concentration increased, having an optimum concentration of 2.79 mg L<sup>-1</sup>. After this point there is a decrease in response. Working with the same cultivar, we observed that concentrations higher than 2 mg L<sup>-1</sup> of NAA caused inhibitory effects on the development of these characteristics, as well as on the number of shoots [26].

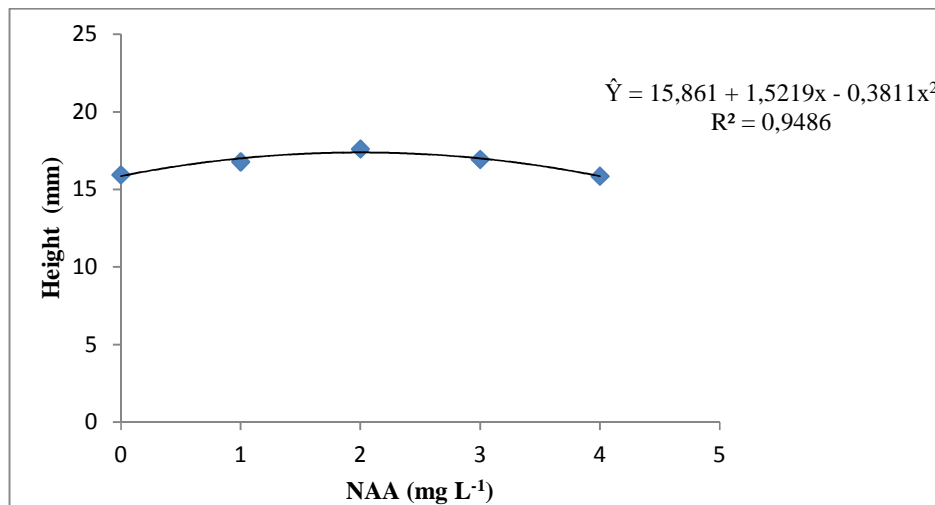
**Table 1. The height and diameter of regeneratin explants, as well as the number of shoots (SH) and roots (RO), observed for the explants submitted to the types of lights (L Amb and L Cont) at 30 days of evaluation**

	L Amb	L Cont	CVa (%)	CVb (%)
Height	15,09 b	18,14 a	10,71	8,69
Diameter	3,47 a	3,26 a	13,65	11,74
SH	0,74 a	0,85 a	38,13	34,61
RO	26,13 b	35,83 a	24,82	23,71

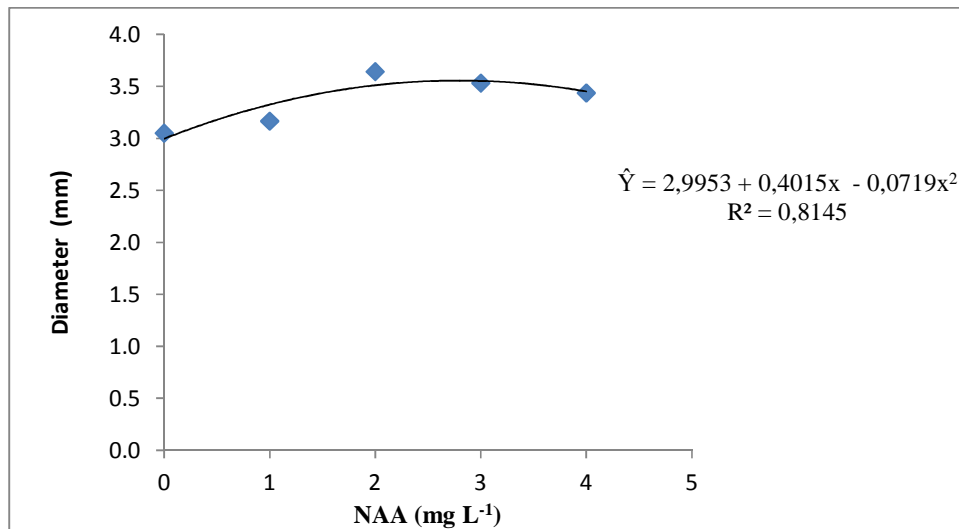
Means followed by the same letter in the rows do not differ from each other by the F test, at 5% probability. Data of the number of shoots were submitted to transformation of square root of Y + 0.5



**Fig. 1. Number of roots of *Opuntia ficus* Giant explants at 30 days of culture according to the different doses of 1-naphthaleneacetic acid (NAA)**



**Fig. 2. Height of the *Opuntia ficus* Giant shoots at 30 days of culture according to the different doses of 1-naphthaleneacetic acid (NAA)**



**Fig. 3. Diameter of the *Opuntia ficus* Giant explants at 30 days of culture according to the different doses of 1-naphthaleneacetic acid (NAA)**

The increase the development of *in vitro* explants will increase the photosynthetic capacity of the plant. Thus, it is suggested that these variables have importance for acclimatization process, because a large amount of stomata is required for the plant to develop under *ex vitro* conditions, so the greater surface of the tissue, the greater transpiration and water loss.

Further experiments should be performed with Giant palm, to determine the best combinations of auxins with cytokinins, to increase the development of *in vitro* explants.

#### 4. CONCLUSION

The best dose of 1-naphthaleneacetic acid (NAA) for *in vitro* development of the giant palm was 2 to 3 mg L<sup>-1</sup>.

For economy issues in the production system, it is recommended to use the concentration of 2 mg L<sup>-1</sup>.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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