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EFFECT OF BRANCH MATURITY ON THE EFFICIENCY OF VEGETATIVE PROPAGATION OF ORA-PRO-NÓBIS

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Abstract

The ora-pro-nóbis is an unconventional food plant, widely recognized for its high protein content, which makes it a highly relevant alternative for food security and the fight against hunger. It is predominantly propagated by cuttings. In this context, the aim of this study was to assess the efficiency of vegetative propagation of ora-pro-nóbis, taking into account the size and physiological maturity of the branches used. The experiment was carried out in the municipality of Santo Cristo-RS, in a completely causal design, with 6 treatments and 4 replications, totaling 24 experimental units, each containing 3 cuttings. The treatments consisted of (T1) a 5 cm basal cut, (T2) a 5 cm central cut, (T3) a 5 cm apical cut, (T4) a 10 cm basal cut, (T5) a 10cm central cut and (T6) a 10 cm apical cut each. The variables evaluated included rooting percentage, number of roots, length of the largest root, root volume, weighted root diameter, root area, green mass and dry mass of the roots, as well as sprouting percentage, number of sprouts, number of leaves per sprout, green mass and dry mass of the sprouts with leaves. The evaluation took place 60 days after planting. The results showed differences between the treatments for all the variables analyzed. The treatments with 10 cm basal and central cuttings stood out from the other treatments with 5 cm cuttings. Treatment 4 appears to be the best option for implementing a production system for this plant, as it shows the best results in terms of characteristics such as the number of leaves and the production of green and dry mass of the shoot, as well as the number of roots and the dry mass of the roots.

Keywords: *Pereskia aculeata*; mini-staking; physiological age; branch size; type of cuttings.

EFEITO DA MATURAÇÃO DOS RAMOS NA EFICIÊNCIA DA PROPAGAÇÃO VEGETATIVA DE ORA-PRO-NÓBIS

Resumo

A ora-pro-nóbis é uma planta alimentícia não convencional, amplamente reconhecida por seu elevado teor de proteínas, o que a torna uma alternativa de grande relevância para a segurança alimentar e o combate à fome. Sua propagação ocorre, predominantemente, por meio de estaquia. Neste contexto, o presente estudo teve como objetivo avaliar a eficiência da propagação vegetativa da ora-pro-nóbis, considerando o tamanho e a maturação fisiológica dos ramos utilizados. O experimento foi realizado no município de Santo Cristo-RS, em delineamento inteiramente causalizado, com 6 tratamentos e 4 repetições, totalizando 24 unidades experimentais, cada uma contendo 3 estacas. Os tratamentos foram formados por (T1) corte basal de 5 cm, (T2) corte central de 5 cm, (T3) corte apical de 5 cm, (T4) corte basal de 10 cm, (T5) corte central de 10 cm e (T6) corte apical de 10 cm cada. As variáveis avaliadas incluem a porcentagem de enraizamento, o número de raízes, o comprimento da maior raiz, o volume das raízes, o diâmetro ponderado das raízes, a área das raízes, a massa verde e a massa seca das raízes, assim como a porcentagem de brotação, o número de brotações, o número de folhas por brotação, a massa verde e a massa seca das brotações com folhas. A avaliação ocorreu aos 60 dias após o plantio. Os resultados mostraram diferenças entre os tratamentos avaliados para todas as variáveis analisadas. Os trataremos com estacas basais e centrais com 10 cm se destacaram dentre os demais tratamentos com estacas de 5 cm. O tratamento 4 se apresenta como a melhor opção na implementação de um sistema de produção desta planta, já que apresenta melhores resultados nas características como número de folhas e produção de massa verde e seca da brotação, assim como número de raízes e massa seca de raízes.

Palavras - chave: *Pereskia aculeata*; miniestaquia; idade fisiológica; tamanho do ramo; tipo de estaca.

Introduction

Ora-pro-nóbis (*Pereskia aculeata* Miller) is a shrub plant belonging to the Cactaceae family and is native to the Americas. Its structure is composed of long branches with the presence of spines and lanceolate leaves, typical of succulent species. The flowers are white in color (Puiatti, 2019), and the fruits are classified as yellow berries. It is recognized as a non-conventional vegetable, as there is no established production chain for this species within the food production systems (Brasil, 2010; Maciel *et al.*, 2021). Its exploitation, as is the case with most native medicinal species in Brazil, is carried out through wild harvesting. For this reason, ora-pro-nóbis is listed in the red list of threatened species (IUCN, 2017), making it imperative to implement actions for the protection and conservation of this species.

Non-conventional vegetables are part of the non-conventional edible plants (PANCs). PANCs have great nutritional and dietary potential (Cavalcante *et al.*, 2023). However, they are often considered weeds, which can be native or exotic species (Cavalcante *et al.*, 2023; Kinupp, 2007). *P. aculeata* has significant pharmacological and nutritional potential, standing out as a plant of interest for both health and sustainable food (Jardim *et al*., 2021).

In Latin America and the Caribbean, approximately 267 million people suffer from food insecurity (FAO, 2021). In this context, it is affirmed that PANCs could play a role in the future of human nutrition in Brazil (Valente Neto *et al*., 2022). Ora-pro-nóbis is an alternative to address nutritional deficiencies in people facing social vulnerability (Dias; Durigan; Guimarães, 2018).

The amount of biomass produced by PANCs can vary according to soil and climate conditions (Kinupp, 2007). In this regard, ora-pro-nóbis stands out for its low soil fertility requirements and high drought resistance (Brasil, 2010). Another important factor influencing the development and yield of PANCs is the type of propagation used for these species. For ora-pro-nóbis, vegetative propagation is mainly done through stem cuttings, which involves rooting parts of the plant, such as branches, roots, and leaves. The results are highly dependent on the genotype, plant age, and type of cutting (Silva *et al*., 2015; Wendling, 2004).

Typically, the size of the cuttings used for vegetative propagation is 20 cm, as recommended by Brasil (2010). However, some authors use mini-cuttings, which are considered a less costly practice (Tofanelli; Mógor, 2021). Larger cuttings have more energy reserves compared to smaller ones (Silva *et al*., 2015). Similarly, basal cuttings have higher carbohydrate, hormone, and other compound reserves that aid in sprouting and rooting (Dutra; Kersten; Fachinello, 2002; Rocha *et al*., 2021).

According to the general pattern of national ora-pro-nóbis production, it is limited to small gardens through wild harvesting from natural formations, with no record of commercial production. Therefore, seedling production can facilitate the cultivation of this species in a technical manner, ensuring the genetic quality of the seedlings (Souza *et al*., 2023). In this context, the objective of this study was to evaluate the rooting of ora-pro-nóbis mini-cuttings, based on the size and physiological maturity of the branch, aiming to identify the most effective treatment for the propagation of this species.

Material and Methods

Study location, plant material used, and experimental design

The experiment was conducted on a private property located in the municipality of Santo Cristo, Rio Grande do Sul, with geographical coordinates of 27°49'56" S and 54°39'02" W. The region has a humid subtropical climate (Cfa), characterized by hot summers and mild winters, with an average annual temperature of around 18°C and an average precipitation of 1,700 mm per year. These climatic conditions are suitable for the production of various perennial species, including ora-pro-nóbis (*P. aculeata* Mill.), a hardy plant widely used in agroforestry systems.

The collection of plant material took place in July 2022, using ora-pro-nóbis mother plants with an average age of four years, which were cultivated in the open field. The branches intended for propagation were randomly collected from the plants and, after collection, divided into three parts: basal, central, and apical. The division was done equally with the aid of pruning shears previously disinfected with a sodium hypochlorite solution in a 1:3 ratio to ensure the health of the material. From the collected branches, cuttings of two different lengths were prepared: 5 and 10 centimeters.

The experimental design adopted was completely randomized, consisting of six treatments that varied according to the size and position of the cut on the branch. The treatments were: basal cut of 5 cm (T1), central cut of 5 cm (T2), apical cut of 5 cm (T3), basal cut of 10 cm (T4), central cut of 10 cm (T5), and apical cut of 10 cm (T6). The objective of these treatments was to evaluate the influence of the size and location of the cutting on the rooting and development of the plants.

Four repetitions were conducted for each treatment, totaling 24 experimental units. Each experimental unit was composed of three cuttings, resulting in a total of 72 cuttings for evaluation. The selection of the cuttings did not follow a growth pattern, being collected randomly. The analyzed variables included the percentage of rooting, root development (green and dry mass), bud formation, number of leaves, and green and dry mass of the buds, aiming to identify the best treatment for the vegetative propagation of ora-pro-nóbis.

Management of the Experiment

The cuttings were placed in plastic trays with 72 cells, each 5 cm deep, containing the commercial substrate Garden Plus®, with a planting depth of three centimeters. Subsequently, they were randomly distributed on wooden benches located one meter above the ground and covered with 50% shading net at a height of 60 cm above the cuttings. The trays were randomly rearranged every three days to ensure uniform exposure to sunlight for all treatments. The plants were watered with a manual watering can every two days, always in the morning.

Experiment Evaluation

After 60 days from the start of the experiment, the evaluation of the cuttings began. The cuttings were carefully removed from the substrate and thoroughly washed to eliminate all material adhered to the roots. Then, the number of roots, shoots, and leaves on each cutting were counted through direct observation. Root length was measured using a graduated ruler.

To evaluate root volume, weighted diameter, rooting percentage, and root area, the Safira software. This process involved taking photographic images of the roots on a white background. The images were uploaded to a computer where the software allowed root analysis. It was necessary to indicate a reference scale, measure one root, and manually adjust the threshold, which varied between 80 and 100 cm. After this procedure, the data obtained were exported for further processing and analysis.

The fresh weight measurements of the roots and shoots were taken using an analytical balance after separating these components from the cuttings. Then, the roots and shoots were placed in labeled paper bags and dried in a forced-air circulation oven at 60°C until reaching a constant weight. After the drying process, the dry mass of the roots and shoots was determined, allowing for comparison between the applied treatments.

Statistical Analyses

The data obtained were initially tested for homogeneity of variances using the Cochran and Bartlett Test (Hartley, 1950). Once the assumptions of homogeneity were met, the results were subjected to analysis of variance (ANOVA) using the F-test ($p<0.05$). Results that were significant in the F-test were further analyzed using the Scott-Knott means test ($p<0.05$). All statistical analyses were conducted using the Assistat software, version 7.7, according to Silva and Azevedo (2009).

Results and Discussion

Root System Evaluation

The results obtained for the rooting percentage (Table 1) showed significant differences between the treatments. It was observed that treatments T2, T4, T5 and T6 achieved 100% rooting, while the others showed lower values, but still higher than 70%. These results highlight the high rooting capacity of ora-pro-nóbis, regardless of the type of cutting or the length of the cuttings. This behavior can be attributed to the plant's hardiness, as observed by Brasil (2010), and the ease of rooting reported by Zem, Zuffellato-Ribas and Koehler (2016) and Dutra, Kersten and Fachinello (2002). These authors, when evaluating growth parameters in ora-pro-nobis cuttings, also reported high rooting rates, ranging from 87.5% to 100%.

With regard to the green and dry mass of the roots, the treatments showed significant differences. Treatment T4 (10 cm basal cut) stood out as the best performer, with a green mass of 0.2233 g and a dry mass of 0.1600 g. It did not differ significantly from T5 in terms of green mass. These results can be explained by the fact that basal and longer cuttings have greater energy reserves and higher concentrations of auxin hormones, which stimulate root development (Pontes Filho *et al.*, 2014). In studies by Souza *et al.* (2023), basal cuttings also showed greater rooting capacity and accumulation of root biomass, corroborating the findings of the present study.

On the other hand, treatment T3 (5 cm apical cut) showed the lowest green and dry mass values, differing from T6 only in green mass. This result can be attributed to the lower lignification and lower accumulation of reserve substances in the apical cuttings, as discussed by Faivre-Rampant *et al*. (2002). These factors limit the ability of these cuttings to form robust roots and accumulate root biomass. Similar studies in other species, such as pitaya, also report that the length and location of the cutting significantly influence root biomass, as observed by Pontes Filho *et al*. (2014).

Table 1. Rooting percentage of cuttings and mean green mass and dry mass of ora-pro-nóbis roots, after 60 days. 2022. Santo Cristo, RS.

Treatments	Rooting (%)	Green mass (g)	Dry mass (g)
T1	75,00 c	0,1816 b	0,0420 c
T2	100,00 a	0,0595 b	0,0500 c
T3	83,34 b	0,0123 c	0,006 d
T4	100,00 a	0,2233 a	0,1600 a
T5	100,00 a	0,2303 a	0,1110 b
T6	100,00 a	0,0413 c	0,0532 c
CV (%)	16,35	22,86	17,87

T1: basal cut of 5 centimeters; **T2**: central cut of 5 centimeters; **T3**: apical cut of 5 centimeters; **T4**: basal cut of 10 centimeters; **T5**: central cut of 10 centimeters; and **T6**: apical cut of 10 centimeters. Means followed by the same letter in the column do not differ according to the Scott-Knott test at a 5% probability level.

According to studies evaluating vegetative propagation in other plant species, such as pitaya, the size of the propagation material affects the dry mass of the roots (Pontes Filho *et al*., 2014). This can be explained by the lower lignification and accumulation of reserve substances in smaller cuttings (Faivre-Rampant *et al*., 2002). Regarding the variables of root area and diameter, the treatments also showed significant differences, with T2 and T6 standing out for presenting the lowest values for these variables (Table 2). For root diameter, treatment T3 also showed lower values, matching those of T2 and T6. These results are consistent with the lower dry mass values observed in these treatments (Table 1).

Table 2. Mean root area and weighted diameter of ora-pro-nóbis cuttings, after 60 days. 2022. Santo Cristo, RS.

Treatments	Area (mm^2)	Diameter (mm)
T1	184,39 a	0,87 a
T2	105,42 b	0,64 b
T3	175,87 a	0,62 b
T4	173,08 a	0,82 a
T5	148,48 a	0,86 a
T6	102,29 b	0,68 b
CV (%)	23,82	21,14

T1: basal cut of 5 centimeters; **T2**: central cut of 5 centimeters; **T3**: apical cut of 5 centimeters; **T4**: basal cut of 10 centimeters; **T5**: central cut of 10 centimeters; and **T6**: apical cut of 10 centimeters. Means followed by the same letter in the column do not differ according to the Scott-Knott test at a 5% probability level.

Treatments T4 and T5 had the highest numbers and lengths of roots (Table 3), while T3 had the lowest values. The superior performance of T4 and T5 can be explained by the physiological condition of the longer basal and central cuttings, which have greater lignification and hormonal and nutritional reserves, as described by Fachinello *et al.* (2005). These factors are crucial for the rooting process, as they provide the energy and hormones needed for root development. Recent studies by Karas (2017) reinforce that longer cuttings have greater energy reserves, which contributes to more vigorous root development.

On the other hand, less lignified cuttings, such as those from T3, had the lowest number of roots, possibly due to the higher concentration of phenolic compounds, which inhibit rooting (Faivre-Rampant *et al.*, 2002). This highlights the importance of properly selecting the cutting site and the length of the cuttings in order to optimize rooting. In addition, the greater length of the roots in T5 and T4 may be associated with the greater amount of energy reserves in these cuttings, as suggested by Souza *et al.* (2023).

Table 3. Number of roots and average length of the longest root of ora-pro-nóbis cuttings, at 60 days. 2022. Santo Cristo, RS.

Treatments	Number of roots	Length (cm)
T1	4,06 c	5,48 c
T2	4,96 c	4,99 c
T3	2,00 d	1,82 d
T4	10,76 a	7,29 b
T5	8,33 b	8,93 a
T6	3,80 c	5,79 c
CV (%)	17,88	17,80

T1: basal cut of 5 centimeters; **T2**: central cut of 5 centimeters; **T3**: apical cut of 5 centimeters; **T4**: basal cut of 10 centimeters; **T5**: central cut of 10 centimeters; and **T6**: apical cut of 10 centimeters. Means followed by the same letter in the column do not differ according to the Scott-Knott test at a 5% probability level.

Less lignified cuttings, such as those from treatment T3, exhibited the lowest number of roots, possibly due to a higher concentration of phenolic compounds, which inhibit rooting (Faivre-Rampant *et al.*, 2002). This emphasizes the importance of selecting the cutting location and size of the cuttings to optimize rooting. The greater root length observed in T5 and T4, as noted by Souza *et al.* (2023), may be associated with the higher amount of energy reserves in these larger cuttings, as suggested by Karas (2017).

Regarding the number of shoots, only T1 and T3 differed from the other treatments, showing the lowest values. This suggests that the length of the cuttings influences the number of shoots due to the greater accumulation of carbohydrate reserves and hormones (Kluge; Tezotto-Uliana; Silva *et al*., 2015).

In terms of the green and dry mass of the shoots, treatment T4 (basal cut with 10 centimeters) showed the highest values among all treatments (Table 4). This suggests that the length of the cutting positively influences the number of shoots, probably due to the greater reserves of carbohydrates and hormones that assist in the emission of new vegetative structures (Kluge; Tezotto-Uliana; Silva *et al.*, 2015). Additionally, the higher dry mass observed in T4 can be explained by the more vigorous development of the root system (Table 3 – greater number of roots in T4), which facilitates the absorption of water and nutrients, promoting more robust growth of the shoots.

Table 4. Percentage of sprouting, average green mass, and dry mass of the shoots in ora-pro-nóbis cuttings, after 60 days. 2022. Santo Cristo, RS.

Treatments	Sprouting (%)	Green Mass (g)	Dry Mass (g)
T1	77,78 b	3,80 b	0,49 b
T2	100,00 a	1,59 c	0,16 c
T3	77,78 b	0,93 c	0,16 c
T4	100,00 a	6,42 a	0,74 a
T5	100,00 a	3,92 b	0,43 b
T6	100,00 a	3,32 b	0,33 b
CV (%)	9,79	18,34	29,74

T1: basal cut of 5 centimeters; **T2**: central cut of 5 centimeters; **T3**: apical cut of 5 centimeters; **T4**: basal cut of 10 centimeters; **T5**: central cut of 10 centimeters; and **T6**: apical cut of 10 centimeters. Means followed by the same letter in the column do not differ according to the Scott-Knott test at a 5% probability level.

The highest values of green and dry mass of the shoots in treatment T4 suggest that longer cuttings from the basal part are more efficient in biomass production. This behavior may be related to the greater accumulation of energy in the lower parts of the plant, where there is more lignification and nutritional reserves, essential factors for the initial growth of the shoots (Souza *et al*., 2023). The fact that basal cuttings show greater efficiency in producing leaves and shoots may also be linked to the better development of the root system, which, as suggested by Kluge, Tezotto-Uliana and Silva (2015), facilitates nutrient absorption, reflecting in greater biomass production.

The results found for T4 corroborate previous studies suggesting better performance of basal cuttings compared to apical ones (Souza *et al*., 2023). The greater lignification of basal cuttings may have played a key role in favoring water retention, which in turn drives the development of the shoots and biomass production (Souza *et al*., 2023). This greater capacity for water retention may have directly contributed to the higher values of green and dry mass observed in treatment T4.

For the number of leaves variable, treatment T4 again stood out, presenting the highest value, followed by T5. For the number of shoots, treatments T5, T4, and T6 showed the highest values (Table 5). The presence of a greater number of leaves reflects the ability of the cuttings to perform photosynthesis more efficiently, accumulating a larger amount of biomass. The better performance of basal cuttings may be related to the fact that these parts of the plants have a greater amount of energy and nutrient reserves, favoring greater leaf development, as reported by Souza *et al.* (2023). The larger number of leaves allows for greater carbohydrate production, essential for the growth and overall development of the cuttings.

Table 5. Average number of leaves and number of shoots of ora-pro-nóbis cuttings, at 60 days. 2022. Santo Cristo, RS.

Treatments	Number of leaves	Number of shoots
T1	12,07 c	1,55 c
T2	6,00 d	1,72 c
T3	5,00 d	1,27 c
T4	24,10 a	2,88 b
T5	17,22 b	3,33 a
T6	11,87 c	2,66 b
CV (%)	11,59	13,67

T1: basal cut of 5 centimeters; **T2**: central cut of 5 centimeters; **T3**: apical cut of 5 centimeters; **T4**: basal cut of 10 centimeters; **T5**: central cut of 10 centimeters; and **T6**: apical cut of 10 centimeters. Means followed by the same letter in the column do not differ according to the Scott-Knott test at a 5% probability level.

Finally, the variability observed in the responses among the different treatments highlights the importance of adequately selecting the cutting location and length of the cuttings to optimize the vegetative propagation of ora-pro-nóbis. The treatments with 10 cm cuttings, particularly the basal ones with 10 cm (T4), proved to be more effective in promoting the number of leaves and the production of green and dry biomass of the shoots, as well as the number of roots and root dry mass, demonstrating the relevance of the balance between lignification, energy reserves, and rooting potential for the success of propagation. Therefore, these results reinforce the importance of choosing the appropriate plant material to improve cultivation performance, aligning with previous studies on the topic (Kluge; Tezotto-Uliana; Silva *et al.*, 2015; Souza *et al.*, 2023).

The results of this study largely corroborate the existing literature, which points to the superiority of basal and longer cuttings over apical and shorter ones in the vegetative propagation process. Classic studies, such as those by Dutra, Kersten and Fachinello (2002), already indicated that basal cuttings have greater reserves of carbohydrates and hormones, favoring rooting and initial growth. Similarly, Souza *et al.* (2023) highlighted the importance of the length of the cuttings in the production of biomass in ora-pro-nóbis, reinforcing the findings of the present study.

In addition, the results obtained are also in line with studies performed on other vegetative species, such as pitaya and rosemary, which have shown that the length of cuttings directly affects the dry mass and number of roots (Pontes Filho *et al.*, 2014; Silva *et al.*, 2015). This alignment with existing literature strengthens the scientific relevance of this study and highlights its contribution to advancing knowledge about the vegetative propagation of ora-pro-nóbis.

CONCLUSIONS

The results of this study revealed that treatment T4 (10 cm basal cuttings) stood out in several variables evaluated, highlighting its efficiency for the vegetative propagation of ora-pro-nobis. This treatment had the highest values for number of leaves, green and dry biomass of the shoots, number of roots and dry mass of the roots, outperforming the 5 cm treatments and the apical cuttings. These characteristics are directly related to the greater lignification and energy reserves of the basic seasons, which enhance the initial development of the plant.

An analysis of the root system revealed that the longer basal cuttings had interesting advantages in terms of rooting, number and biomass of roots. These observations corroborate the possibility that the location and length of the cuttings influence the physiological processes of rooting and biomass accumulation. While the treatments with apical cuttings showed inferior performance, probably due to lower lignification and nutritional reserves, T4 showed greater capacity to sustain initial growth and promote nutrient absorption.

In addition, the superior performance of T4 cuttings in terms of shoot biomass and number of leaves highlights the importance of selecting propagative material with greater photosynthetic potential. The greater number of leaves in this treatment suggests a higher capacity for photosynthesis, which contributes to growth and biomass accumulation. Thus, the interaction between a robust root system and leaf expansion capacity makes T4 a strategic choice for the ora-pro-nóbis technical strategy.

In this way, this study reaffirms the role of the physiological and structural characteristics of the stations in the vegetative propagation of ora-pro-nóbis. The choice of cuttings based on 10 cm not only improved the efficiency of the propagation process, but can also help to build more sustainable and economically viable production systems. It is recommended that future research evaluate the interaction of different substrates and environmental conditions with these results, further optimizing the technical management of the species.

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