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Methods for seedling production and establishment of forage peanut in an intercropping with BRS Tamani grass

Métodos para produção de mudas e estabelecimento do amendoim forrageiro em consórcio com capim BRS Tamani

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ABSTRACT The objective of this study was to explore methods for producing forage peanut (*Arachis pintoi*) cv. Belomonte seedlings and their introduction into an established pasture with Tamani grass. The experiment was conducted in two phases. In the first phase, seedlings were generated from stolons with three treatments: stolons produced in water; stolons produced in nutrient solution; and stolons produced with commercial substrate. The treatment with commercial substrate did not produce sufficient seedlings for the second phase of the experiment and was, therefore, discontinued. In the second phase, the seedlings were planted in an established pasture of BRS Tamani grass. The experiment was laid out in a completely randomized design with three treatments: peanut seedlings produced in water, peanut seedlings produced in nutrient solution, and raw stolon. The variables evaluated included seedling survival rate and the survival and development of the different types of rooted forage peanut seedlings at 30, 60, 90, and 120 days after planting. Stolon survival rate exceeded 70% for the treatments using pure water and nutrient solution. The average stolon length and the number of leaves per plant were greater for the treatment using the nutrient solution. At the end of the experiment, there was no significant difference in the number of live plants. Forage peanut seedlings can be produced using a nutrient solution for broadleaf plants or simply with water, indicating easy production. Pre-rooting the seedlings in a nutrient solution for broadleaf plants favors the growth of stolons and leaves.

Keywords *Arachis pintoi*, *Megathyrsus maximus*, pasture diversification, sustainability

RESUMO *O objetivo do trabalho foi estudar métodos de produção de mudas de amendoim forrageiro (Arachis pintoi) cv. Belomonte e sua introdução em pasto já estabelecido com capim Tamani. O experimento foi realizado em duas fases. Na primeira fase foi realizada a produção de mudas a partir dos estolões com três tratamentos: estolões produzidos em água; estolões produzidos em solução nutritiva e estolões produzidos com substrato comercial. Para o tratamento com substrato comercial, este não obteve mudas suficientes para a segunda fase do experimento, sendo descartado. A segunda fase consistiu no plantio das mudas em pastagem já estabelecida de capim BRS Tamani. O delineamento foi inteiramente casualizado com três tratamentos: mudas de amendoim produzidas em água, mudas de amendoim produzidas em solução nutritiva e estolão bruto. As variáveis avaliadas foram o índice de sobrevivência de mudas e a sobrevivência e desenvolvimento dos diferentes tipos de mudas enraizadas de amendoim forrageiro aos 30, 60, 90 e 120 dias após plantio. O índice de sobrevivência dos estolões foi superior a 70% para os tratamentos utilizando água pura e solução nutritiva. O comprimento médio dos estolões e o número de folhas por planta, foram superiores para o tratamento utilizando solução nutritiva. No final do experimento, o número de plantas vivas não foi diferente. A produção de mudas enraizadas de amendoim forrageiro pode ser realizada utilizando solução nutritiva pra folhosas ou apenas com água, indicando facilidade na produção. O enraizamento prévio das mudas em solução nutritiva para folhosas favorece o crescimento de estolões e folhas.*

Palavras-chave *Arachis pintoi, Megathyrsus maximus, diversificação de pastagens, sustentabilidade*

1. Introduction

Forage peanut (*Arachis pintoi*) is a legume noted for its robust forage production, high nutritional value, shade tolerance, and excellent ground cover capability (Miranda, 2008). However, one challenge in establishing pastures intercropped with forage peanuts arises during the establishment phase. The legume can be propagated through seeds or seedlings (stolons). Due to

its characteristic of forming seeds below the ground, seed harvesting presents challenges (Perez, 2008), and seedlings are scarce and costly to obtain. Thus, strategies aimed at optimizing the production of seedlings with greater viability should be explored.

The BRS Tamani cultivar, belonging to the *Megathyrsus maximus* species, requires fertile soils compared to forage plants of the genus Urochloa and is compact, forming clumps. Therefore, intercropping with forage peanut offers potential advantages, given the legume's ability to fix nitrogen and its characteristic of covering the ground between grass clumps. This arrangement enhances land use efficiency and pasture resilience and reduces weed incidence (Andrade et al., 2023).

The intercropping of *M. maximus* species with legumes has been practiced in various regions of Brazil (Tamele, 2016; Assis et al., 2018). Despite its prevalence, there is a lack of research on the specific techniques for planting seedlings and effectively establishing forage peanut within established pasture systems.

Thus, the aim of this study was to investigate different methods of producing forage peanut seedlings of the Belomonte cultivar and the introduction of the legume into an existing pasture with BRS Tamani grass.

2. Material and methods

The experiment was divided into two phases: the first in a greenhouse, where the forage peanut seedlings were produced, and the second in the field, where various types of forage peanut seedlings were planted in an already established pasture of BRS Tamani grass. Both phases of the experiment were conducted at the Tancredo Neves Campus of the Federal University of São João del Rei, located in São João del Rei, Minas Gerais, Brazil.

The first phase was conducted from December 21, 2022, to January 12, 2023. The experimental design was completely randomized, consisting of three treatments and five replicates. The treatments included: 1) Pure Water: forage peanut stolons immersed in pure water; 2) Nutrient Solution: forage peanut stolons immersed in a commercial nutrient solution; and 3) Commercial Substrate: forage peanut stolons planted in seedling trays with commercial substrate "Terra preta" (peat, charcoal and pine bark). The variable evaluated was the stolon survival index (SSI), observing the number of live stolons in each treatment after the experimental period (23 days).

To prepare the stolons, they were harvested from an area already established with forage peanut cv. Belomonte. The mature stolons were collected and washed to remove excess soil. They were then standardized to a length of approximately 15 cm, each with at least three nodes, and all leaves were removed. The cleaned stolons underwent disinfection by being immersed in a 2.5% sodium hypochlorite solution for 5 min and then rinsed in pure water to remove any residual solution.

The treatments using pure water and nutrient solution were conducted in plastic pots with a capacity of 500 mL. For the treatment in trays with commercial substrate, trays measuring 27 cm × 27 cm and 36 cells, each measuring 3.7 cm × 3.7 cm × 3 cm, were utilized.

For the pure water treatment, stolons were immersed in water. In the nutrient solution treatment, the commercial solution was prepared according to the package instructions. This nutrient solution is formulated explicitly with both macro- and micronutrients suitable for the production of vegetables and leafy greens in general, providing nitrogen 10%, calcium 15%, magnesium 2%, phosphorus 8%, potassium 30%, sulfur 3%, iron 0.14%, boron 0.04%, manganese 0.04%, copper 0.03%, zinc 0.019%, molybdenum 0.009%, nickel 0.006%, and cobalt 0.002%. Accordingly, 4.29 g of the formula was dissolved in 5 L of water to prepare the solution.

A commercial substrate was employed for the treatment using trays. Each cell was planted with one stolon. The cells were 3 centimeters high, and only one node of the stolon was covered with the substrate. The stolons were manually irrigated daily using gardening watering cans.

Analysis of variance was conducted using R Studio software, adopting a 5% error probability level for the analysis. Treatment means were compared using the Duncan test at a 5% significance level.

Additionally, in the first phase of the experiment, which involved producing seedlings from commercial substrate in trays, insufficient seedlings were generated for field planting, leading to the exclusion of this treatment from the trial. Therefore, the second phase commenced immediately following the conclusion of the first phase. The experimental design was structured in randomized blocks with three treatments: 1) Pure Water: seedlings from forage peanut stolons produced in pure water; 2) Nutrient Solution: seedlings from forage peanut stolons produced in nutrient solution; and 3) Raw Stolon: forage peanut stolons collected on the day of planting. This setup included four replicates (blocks), totaling 12 experimental units.

The seedlings grown in pure water and nutrient solution originated from the first phase of the experiment, and the raw stolons collected on the day of planting underwent the same disinfection process as in the first phase.

The established BRS Tamani grass pasture was trimmed to a height of approximately 20 cm. The pre-rooted seedlings were planted between the rows of BRS Tamani grass on January 12. The plots measured 3 × 3 m (9 m²) and included four rows of BRS Tamani grass interspersed with three rows of the legume. Using an earthworm digger, 10 holes were made, each 10 cm deep and 30 cm apart. Fertilizer was applied to these holes, followed by planting one rooted stolon per hole at a depth of 10 cm, positioned vertically, corresponding to two-thirds of the stolon's burial depth.

Figure 1 illustrates the regional climate data relevant to the planting period and subsequent evaluations.

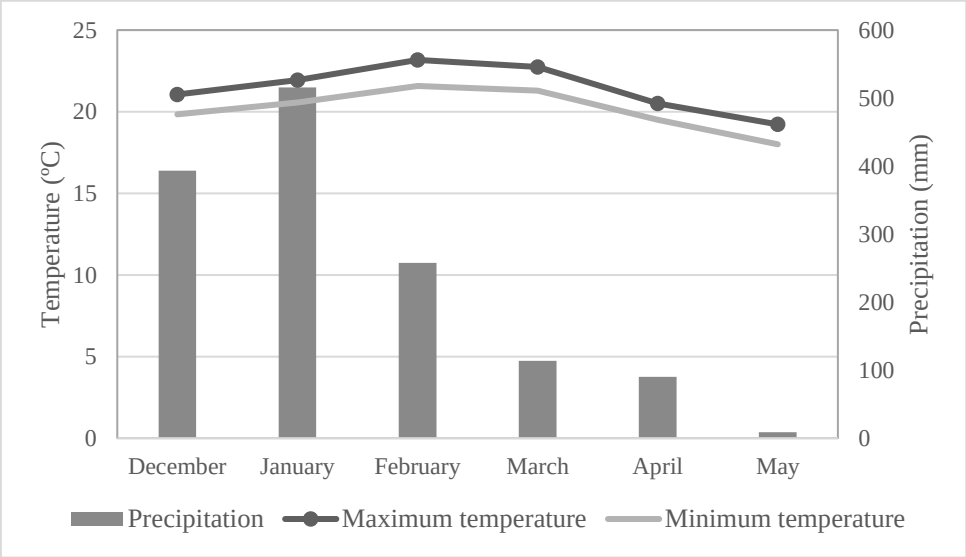

Figure 1 – Climate data during the experimental period between December 2022 and May 2023.

Weeding was carried out manually at 26 and 42 days to remove weeds and invasive plants.

To prevent shading of forage peanut, the BRS Tamani grass was cut to a residual height of approximately 20 cm at 30, 60, 90, and 120 days after planting the legume. No irrigation was employed in the field during the experiment.

Agronomic evaluations of the forage peanut and forage mass production of BRS Tamani grass were conducted at 30, 60, 90, and 120 days. In each plot, five plants were randomly selected and tagged for morphogenetic assessments. The evaluated traits and their descriptions are as follows: 1) Number of live plants per m² (NLP): All planted stolons were counted, and the calculation was based on the number of live plants relative to the plot area; 2) Seedling survival rate in the field (SSR): After counting all planted stolons, the survival rate (in percentage terms) was calculated based on the number of live plants in each plot relative to the number of planted stolons; 3) Number of stolons per plant (NSP): All stolons on the identified plants were counted, and the average number of stolons per forage peanut plant was calculated; 4) Average stolon length (SL): The total length of stolons per identified plant was measured using a graduated ruler, and the average was calculated; 5) Height of forage peanut plants (PH): The height from the base to the top of each identified plant was measured, and the average height was calculated; and 6)Number of leaves per plant (NLP): The number of fully expanded green leaves on each identified plant was counted.

Analysis of variance was performed using R Studio software. The error probability level set for the analysis of variance was 5%. The comparison of the treatment means was estimated using the Duncan test, also at a 5% significance level.

3. Results and Discussion

For the stolon survival rate variable, the treatment using commercial substrate had the lowest percentage of live stolons, at 39%. The treatments using the nutrient solution and pure water showed no significant difference, with 77% and 84% survival rates, respectively (Table 1). The method of cleaning and disinfecting the stolons was adopted because, among the various factors that can determine the success of planting forage peanuts, cultivation practices for controlling pests and diseases are extremely important. Thus, disinfecting seedlings is a beneficial practice that is not only low-cost and labor-efficient but also reduces the presence of disease-causing agents in the seedlings. This underlines the ease and simplicity of vegetative propagation of forage peanut, potentially enabling the use of this legume by small-scale producers with limited resources and access to technical guidance.

Table 1 – Stolon survival index (SSI) of forage peanut cultivar Belomonte.

Treatments	SSI (%)
Nutrient solution	77[a]
Pure water	84[a]
Commercial substrate	39[b]
CV (%)	14.35

Means followed by different letters in the same column differ statistically at the 5% level.

One of the reasons for the failure in seedling production in trays with commercial substrate may be related to the size of the tray cells, which had a capacity of 42.87 cm³ and were approximately 3 cm high. With an average stolon length of 15 cm, the seedlings had minimal contact with the substrate; some seedlings had no contact between the buds and the substrate, limiting the absorption of water and nutrients, as well as restricting root development, thereby impairing their growth.

Miranda (2008) conducted an experiment on the production of forage peanut cv. Belomonte seedlings from stolons with at least three nodes, using Styrofoam trays with a capacity of 100 cm³ for planting, ensuring that at least one node of the stolon was buried in the substrate. The control

treatment without the inoculation of mycorrhizal fungi achieved a 52% survival rate for the stolons, indicating that more than half of the planted stolons managed to develop even without the addition of growth-promoting fungi. This supports the notion that the failure in seedling production may be attributed to the use of trays with small cells and insufficient substrate contact at the reproduction points (buds).

For the number of live plants per m² (NLP), at 30 days of evaluation, there was no significant difference between treatments (Table 2). By 60 days, a statistical difference was observed, with the treatment using raw stolon outperforming the treatment with seedlings produced in the nutrient solution. However, in the first half of the experiment (at 90 and 120 days after planting), no differences were observed between treatments for this variable (Table 2). Although the initial NLP was modest, there was no sharp decline in these values throughout the experiment. One of the determining factors for the successful establishment of forage peanut seedlings is maintaining soil moisture, as stolons are sensitive to dehydration. According to Valentim et al. (2000), the ideal planting period for forage peanut is at the onset of the rainy season, which provides favorable soil moisture conditions and helps avoid dry spells that can compromise the survival and development of the newly planted seedlings. Figure 1 illustrates that the rainfall index was conducive for planting forage peanut, with rainfall exceeding 400 mm between December and January and 250 mm in February, the month following planting. The average NLP among the treatments was 2 plants/m² at 30 days and declined slightly to 1.7 plants/m² by the end of the experimental period. The establishment of forage peanut plants is typically slow, so the death of some of the planted seedlings was expected. A significant factor influencing the rate of legume establishment is the lateral growth of the stolons (Valentim et al., 2003). Hence, even though the NLP decreased over time, the remaining plants have the capability to extend their stolons, effectively colonizing new areas and thereby achieving substantial ground coverage.

Table 2 – Average number of live plants per m² at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	1.86[a]	1.80[b]	1.72[a]	1.67[a]
Pure water	2.25[a]	1.89[ab]	1.75[a]	1.72[a]
Raw stolon	2.39[a]	2.11[a]	1.89[a]	1.72[a]
CV (%)	13.77	6.83	9.41	12.86

Means followed by different letters in the same column differ statistically at the 5% level.

A difference in seedling survival rate in the field (SSR) was only observed after 60 days. The treatment using raw stolons outperformed the treatment with seedlings produced in nutrient solution but showed no significant difference compared to the treatment using pure water (Table 3). By the end of the experiment, the SSR indicated that at least half of the planted stolons were successfully established and developed, irrespective of the treatment method. Valentim et al. (2001) suggest using six stolons per hole for planting forage peanut, with three stolons on each side of the hole. In this experiment, however, the recommended quantity could not be achieved due to a shortage of available stolons. Therefore, achieving an SSR of approximately 50%, regardless of the treatment used, was considered satisfactory, given that only one stolon was planted per hole. This outcome demonstrates that it is feasible to significantly reduce the demand for seedlings and, consequently, the costs associated with producing or acquiring these inputs for the introduction of forage peanut into established pasture areas.

Table 3 – Seedling survival index in the field at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	55.83[a]	54.17[b]	51.66[a]	50.00[a]
Pure water	67.50[a]	56.66[ab]	52.50[a]	51.67[a]
Raw stolon	71.66[a]	63.33[a]	56.66[a]	51.67[a]
CV (%)	13.77	6.83	9.33	12.86

Means followed by different letters in the same column differ statistically at the 5% level.

The number of stolons per plant only differed significantly at 30 days after planting (Table 4). The treatment using nutrient solution was superior to the other treatments, suggesting that prior rooting may have accelerated stolon emission. The ability to form new stolons is crucial for ensuring adequate ground coverage and is also important for producing new seedlings through vegetative propagation. After the 30-day evaluation, the treatments exhibited similar values for this trait, indicating that the production of new stolons was comparable across different treatments.

Table 4 – Number of stolons per plant of forage peanut at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	3.20[a]	3.65[a]	6.97[a]	9.21[a]
Pure water	2.60[ab]	2.90[a]	4.07[a]	6.30[a]
Raw stolon	1.85[b]	2.42[a]	3.64[a]	4.27[a]
CV (%)	22.15	33.38	53.98	55.70

Means followed by different letters in the same column differ statistically at the 5% level.

Significant differences in average stolon length (SL) were observed between treatments at 90 and 120 days, with the nutrient solution treatment showing superior results (16.32 cm and 18.37 cm, respectively) (Table 5). There was a growth increment of 2.05 cm in the 30-day interval. The growth of forage peanut stolons plays a vital role in the establishment of the legume, as it ensures the plants' ability to colonize new areas (Valentim et al., 2003). The superior performance when using the nutrient solution for the seedlings may be attributed to the solution providing essential nutrients that accelerate rooting, thereby enhancing nutrient absorption from the soil and increasing stolon length.

Table 5 – Average stolon length (expressed in cm) of forage peanut at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	6.87[a]	13.37[a]	16.32[a]	18.37[a]
Pure water	5.98[a]	9.70[a]	10.58[b]	11.37[b]
Raw stolon	5.41[a]	10.47[a]	9.34[b]	10.39[b]
CV (%)	13.14	29.77	21.42	28.49

Means followed by different letters in the same column differ statistically at the 5% level.

No statistical differences (P > 0.05) were observed in plant height between treatments during the experimental period (Table 6). However, a reduction in height was noted due to the creeping growth habit of forage peanut. Initially, the stolons were planted vertically, but as they developed, they grew parallel to the soil surface, forming a dense layer with regrowth points protected from animal grazing. Nonetheless, within the pasture canopy of the BRS Tamani cultivar, more shaded areas were observed where the growth of stolons and leaves reached the grass leaf area, making them more susceptible to animal grazing. This behavior of stolons, petioles, and leaves rising when intercropped with other grasses was also noted by Argel and Pizarro (1992).

Table 6 – Plant height of forage peanut at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	9.85[a]	8.86[a]	6.21[a]	5.84[a]
Pure water	10.22[a]	10.82[a]	8.92[a]	6.14[a]
Raw stolon	8.82[a]	8.73[a]	7.21[a]	5.33[a]
CV (%)	21.12	14.58	23.33	14.02

Means followed by different letters in the same column differ statistically at the 5% level.

A trait that enhances drought resistance in forage peanut is the closing and thickening of leaves and deep root systems (Valentim et al., 2001). Regarding the variable number of leaves, the average count across treatments was similar at 60 days, but at 30, 90, and 120 days, the treatment using nutrient solution yielded superior results (Table 7).

Table 7 – Number of leaves per plant of forage peanut at 30, 60, 90, and 120 days after planting.

Treatment	Days after planting			
	30 days	60 days	90 days	120 days
Nutrient solution	9.85[a]	8.86[a]	6.21[a]	5.84[a]
Pure water	10.22[a]	10.82[a]	8.92[a]	6.14[a]
Raw stolon	8.82[a]	8.73[a]	7.21[a]	5.33[a]
CV (%)	21.12	14.58	23.33	14.02

Means followed by different letters in the same column differ statistically at the 5% level.

As demonstrated in this study, it is feasible to produce forage peanut seedlings from an already established area of the legume, potentially reducing production costs. Aligning the planting season with periods favorable for rainfall is crucial as it ensures soil moisture and supports plant development. It is important to highlight that, regardless of the planting method for forage peanut, its growth and establishment are slow; hence, thorough preparation of the area and ensuring seedling viability are important to achieve successful ground coverage.

4. Conclusions

Rooted seedlings of forage peanut cv. Belomonte can be successfully produced using either a commercial nutrient solution for leafy vegetables or pure water, with both methods yielding similar

results in terms of seedling survival rates. This finding suggests that seedling production is more easily accessible for producers. Pre-rooting forage peanut stolons in a nutrient solution enhances the initial growth of seedlings in the field compared to using freshly harvested stolons.

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Declaration of Conflict of Interest

The authors declared no conflicts of interest.

CRediT authorship contribution statement

Marcelle Patrício da Costa: Investigation, Writing, Data curation, Project administration, Methodology; **Daniel de Noronha Figueiredo Vieira da Cunha:** Data curation, Formal analysis; **Isadora Menezes Costa Tarôco:** Investigation; **Rodolfo Henrique Silva Pereira:** Investigation; **Lucas Sodré Granjeiro:** Investigation; **Carlos Mauricio Soares de Andrade:** Writing – review & editing; **Judson Ferreira Valentim**: Conceptualization, Methodology, Writing – original draft, Writing – review & editing; **Janaina Azevedo Martuscello:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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