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Article

Seed priming in *Clitoria ternatea* L. with multi-walled carbon nanotubes: A physicochemical and morphological approach

Condicionamento fisiológico de sementes em *Clitoria ternatea* L. com nanotubos de carbono de paredes múltiplas: Uma abordagem físico-química e morfológica

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Abstract: *Clitoria ternatea* L. is valued for its ornamental characteristics, medicinal properties, and culinary uses. However, efficient production of this species is constrained by seed coat dormancy, which impedes germination and seedling production. This study aimed to explore the physicochemical and morphological aspects of *C. ternatea* seeds, focusing on overcoming dormancy by applying multi-walled carbon nanotubes (MWCNTs) functionalized with carboxylic acid. The seeds were characterized by their physical, chemical, and mineral composition. Characterization included color dominance, geometry, thousand seed weight, and moisture content analyses. The seeds were treated with different concentrations (0, 100, 200, 400, 800 mg L^{-1}) of multi-walled carbon nanotubes (MWCNT) during 24 hours of soaking. Then the germination rates were evaluated and morphological analysis was performed using scanning electron microscopy. Regarding the morphometry of the seeds, they were characterized by an oblong shape, a predominance of black tegument coloration, high levels of proteins, carbohydrates, lipids, fibers, and the presence of minerals such as potassium, magnesium, iron, and calcium. Seeds soaked in MWCNTs at a concentration of 200 mg L^{-1} exhibited an increase in germination percentage and the formation of normal seedlings compared to the control group (seeds soaked in water). The results suggest that MWCNTs can help to overcome seed dormancy and improve the quality of the resulting seedlings. Image analysis and scanning electron microscopy were crucial in understanding the physicochemical characteristics of the seeds and their changes upon exposure to MWCNTs.
Keywords: edible flowers, MWCNTs, nanotechnology, propagation.

Resumo: *Clitoria ternatea* L. é valorizada por suas características ornamentais, propriedades medicinais e usos culinários. No entanto, a produção eficiente desta espécie é limitada pela dormência do tegumento, que impede a germinação e a produção de mudas. Este estudo teve como objetivo explorar os aspectos físico-químicos e morfológicos de sementes de *C. ternatea*, com foco na superação da dormência através da aplicação de nanotubos de carbono de paredes múltiplas (MWCNTs) funcionalizados com ácido carboxílico. As sementes foram caracterizadas quanto à sua composição física, química e mineral. A caracterização incluiu análises de dominância de cor, geometria, peso de mil sementes e teor de umidade. As sementes foram tratadas com diferentes concentrações (0, 100, 200, 400, 800 mg L^{-1}) de nanotubos de carbono de paredes múltiplas (MWCNT) 24 horas de embebição. Em seguida foram avaliadas as taxas de germinação e realizada análise morfológica por microscopia eletrônica de varredura. Quanto à morfometria das sementes, elas foram caracterizadas pelo formato oblongo, predomínio da coloração preta do tegumento, altos teores de proteínas, carboidratos, lipídios, fibras e presença de minerais como potássio, magnésio, ferro e cálcio. Sementes embebidas em MWCNTs na concentração de 200 mg L^{-1} apresentaram aumento na porcentagem de germinação e formação de plântulas normais em comparação ao grupo controle (sementes embebidas em água). Os resultados sugerem que os MWCNTs podem ajudar a superar a dormência das sementes e melhorar a qualidade das mudas resultantes. A análise de imagens e a microscopia eletrônica de varredura foram cruciais para a compreensão das características físico-químicas das sementes e suas alterações após a exposição aos MWCNTs.
Palavras-chave: flores comestíveis, MWCNTs, nanotecnologia, propagação.

Introduction

Clitoria ternatea L., has been widely recognized for its medicinal and nutritional applications. However, the species faces challenges stemming from the morphological characteristics of its seeds. The presence of a hard seed coat results in low germinative potential, leading to heterogeneous seedlings and difficulties in direct seeding (Avalos et al., 2004). Seed dormancy is common in many legume plants, including *C. ternatea*, and can be caused by endogenous and exogenous factors. In this species, dormancy is due to a hyaloplasm layer in the seed coat, which prevents water absorption and consequently the start of germination (Verma and Singh, 2017).

Traditionally, seed treatment with chemical and mechanical scarification has been used to overcome the dormancy of *C. ternatea* seeds. This includes the removal of the hyaloplasm layer through immersion in acid solutions or mechanical scarification (Verma and Singh, 2017). However, both methodologies need help with their processes. Mechanical scarification is manually performed, being meticulous and time-consuming, and using sulfuric acid treatment can cause abnormal seedling formation (Shobharani and Sundareswaran, 2018).

Given the challenge of overcoming seed dormancy, the use of nanotechnologies in germination has been gaining prominence over the years (Younis et al., 2021; El-Attar and Sakr, 2022). Nanoparticles are specialized particles that have a size range between 1 and 100 nm and possess different compositions and characteristics (High surface-to-volume ratio, optical properties, mechanical properties, magnetic properties, self-assembly capability, quantum effects, catalytic properties, biocompatibility and functionalization), which have been explored to improve plant cultivation conditions (Timóteo et al., 2019a; Pereira et al., 2021; Paiva et al., 2023). Nanoparticles have various applications in agriculture, especially in creating products such as pesticides, herbicides, fertilizers, and micronutrients. They are also noted for their more straightforward, economical, and innovative synthesis process (Timóteo et al., 2019b; Silva et al., 2020; Younis et al., 2021; Brandão et al., 2022).

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Among the nanotechnologies employed in agriculture, multi-walled carbon nanotubes (MWCNT) have been highlighted for promoting benefits in germination, growth, plant yield, and resistance to different stresses. MWCNTs trigger a series of beneficial physiological effects, such as the formation of nanopores in the aerial part of plants, which facilitate water absorption (Nile et al., 2022). Furthermore, they stimulate the expression of aquaporin genes, which regulate water absorption and the dispersion of reactive oxygen species in cell membranes. This process also promotes the rapid hydrolysis of starch, breaking seed dormancy and stimulating germination (Joshi et al., 2018; Ali et al., 2020).

The use of MWCNTs functionalized with carboxylic acids was evaluated on wheat seeds (*Triticum aestivum* L.), where early germination, rapid growth, and development of the root system were observed, significantly improving the grain yield per plant from 1.53 to 2.5 g, an increase of 63% (Joshi et al., 2018). Another study involving tree species (*Alnus viridis* L. and *Shepherdia canadensis* L.), the application MWCNTs at the concentration of 0.02 and 0.04 mg L^{-1}, facilitated seed dormancy breaking, resulting in 90% germination compared to 60% in the control at both concentrations used (Ali et al., 2020).

To understand the physical and morphological characteristics of seeds, and their potential changes when exposed to nanotechnologies, image analysis has proven to be a promising technique. This method optimizes the collection of both qualitative and quantitative information about seed structure. This technique contributes to seed quality evaluations, as it identifies anomalies and imperfections that may affect germination (Luz et al., 2021). Among the computational systems used for image analysis, GroundEye® has been indicated for its potential. This national equipment, available on the market, extracts various characteristics of seeds and seedlings, quickly and accurately processing data in graphs, tables, and histograms. Like scanning electron microscopy, which provides high-resolution images of seeds micro and nanometric structures, image analysis emerges as a promising technique for understanding their morphological characteristics and potential changes induced by nanotechnologies (Trujillo et al., 2019).

Given this context, the need arises for an innovative approach that not only overcomes dormancy but also profoundly understands the physical-chemical-morphological characteristics of *Clitoria ternatea* L. seeds subjected to carbon nanotubes, as well as their germination and initial seedling development.

Materials and Methods

Seed collection

The seeds of *Clitoria ternatea* L. were harvested from parent plants located on the Federal University of Lavras campus, in the Ornamental Plants Sector – Botanical Garden. The seeds were collected from plants in the reproductive phase that exhibited buds, flowers, and legumes in the same phenological period. A total of 5 parent plants were used to collect mature legumes (Fig. 1).

Fig. 1. Collection of parent plants: Healthy parent plants selected for their specific physical and morphological characteristics, used as the primary source for seed analysis.

Physical and morphological characterization of the seeds

The experiments were conducted at the Central Seed Research Laboratory at the Federal University of Lavras, where the seed batch image was captured using the GroundEye® equipment (2016). In the equipment tray, a sample of 100 seeds was arranged in a manner that they were separated from each other to capture the image to obtain the parameters: Color dominance, geometry, diameter, and perimeter.

To determine the thousand seed weight, seeds were manually counted, with eight repetitions of 100 seeds each, to determine the average weight. Subsequently, the following equation 1 was used:

(1) Thousand Seed Weight: (Sample Weight ×1000) / (Number of Seeds).

Source: Brasil (2009).

For the moisture test, five samples of 20 seeds each were placed in metal crucibles, weighed, and then placed in an oven at 105 °C for 24 hours. Subsequently, they were removed and placed in a desiccator with silica gel. After 10 minutes, the samples were weighed again (Brasil, 2009).

Determination of chemical composition

The seeds of *C. ternatea* were placed in an oven at 105 °C until a constant weight was reached to determine the moisture content of the batch. Afterwards, they were ground in a knife mill for approximately 20 minutes until a fine powder was obtained.

The determination of protein content was conducted using nitrogen determination equipment by combustion (LECO). The total fiber content was determined according to the methodology proposed by Van Soest & Wine, as cited by Silva (1990). The ether extract content (fat) was determined by extracting the flours with ethyl ether using a Soxhlet apparatus (AOAC, 1990). The ash content was determined by the gravimetric method based on the weight loss of the material subjected to heating at 550 °C in a muffle furnace (AOAC, 1990). The determination of carbohydrates was made by the difference between the contents of fiber, fat, ash, and protein.

Determination of mineral composition

The determination of minerals was carried out in the Soil Analysis Laboratory of UFLA, where the macronutrients: N, P, and K, and the micronutrients: Ca, Mg, Cu, Fe, Mn, Na, B, and Zn were quantified.

The sample of *C. ternatea* seeds was homogenized and triplicates were prepared for each mineral determination. The extraction method used was through Method 3051A (United States Environmental Protection Agency - USEPA 1998). This method is recommended by the National Environmental Council (CONAMA, 2009). It involves digestion in a hermetically sealed flask, using equipment with controlled pressure and temperature to dissolve the samples.

For this procedure, 1 g of the sample was digested with 10 mL of concentrated HNO_3 in PTFE Teflon® tubes for 10 minutes. After removing the tubes, they were allowed to reach room temperature before being opened. Subsequently, the extracts were filtered, and 10 mL of distilled water was added. The aliquots for reading were stored in polypropylene bottles until the time of analysis. All reagents used are of high purity grade (Sigma-Aldrich® or Merck®), with the concentrated HNO_3 having been previously distilled.

The elements were determined in the acid solution through flame atomic absorption spectrometry (FAAS) or graphite furnace atomic absorption spectrometry (GFAAS), depending on the different contents found and the detection limit of the equipment.

The validation of the method's precision and accuracy (QA/QC protocols) was conducted by comparison with a standard reference material sample from the Institute for Reference Materials and Measurements (Certified Reference Material BCR® - 320R – Channel Sediment) as a reference for the contents of N, P, and K and the micronutrients: Ca, Mg, Cu, Fe, Mn, Na, B, and Zn, in addition to a blank sample in each batch for calculating the detection limit of the equipment. The data were analyzed by Principal Component Analysis (PCA) (multivariate analysis), which allows extracting relevant information for its interpretation from a given data set (Matos et al., 2003).

Carbon nanotubes in seed germination

Multi-walled carbon nanotubes (MWCNT) functionalized with carboxylic acid, used in this study, were donated by CTNano-UFMG (Center for Technology in Nanomaterials and Graphene at the Universidade Federal de Minas Gerais). The stock solution was prepared at 1000 mg L^{-1} of MWCNT.

The solution was subjected to sonication (Branson – Digital Sonifier®, models 250 & 450) at 50% amplitude for 15 minutes, three times. The pH was measured after homogenizing the stock solution in the sonicator, revealing a pH of 5.5. The solution was placed in an amber bottle, protected with aluminum foil, and refrigerated until use. The MWCNT solutions at different concentrations (0,0 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1}, 400 mg L^{-1}, 800 mg L^{-1} were transferred to an Erlenmeyer flask, shielded from light with the aid of aluminum foil, along with the seeds designated for each treatment. The seeds were kept intact, that is, without undergoing a scarification process. Subsequently, they were immersed in the MWCNT solutions and distilled water as a control in a B.O.D. incubator for physiological conditioning, under constant aeration, at a controlled temperature of 25 °C for a period of 24 hours (Campbell, 2020).

After the 24-hour soaking period, the seeds were removed from the Erlenmeyer flask and lightly washed to remove excess MWCNT, then placed on paper to absorb any remaining excess moisture. For the germination test, the experiment was set up in a completely randomized design, with a total of 6 treatments, which were: Seed without soaking; Soaking in distilled water; Soaking in MWCNT 100 mg L^{-1}; Soaking in MWCNT 200 mg L^{-1}; Soaking in MWCNT 400 mg L^{-1}; Soaking in MWCNT 800 mg L^{-1}. All soaking treatments were conducted for 24 hours.

The germination test was conducted on paper, using Germitest paper. The paper was weighed and received 2.5 times its weight in distilled water. Subsequently, the tests were set up on paper, with four replicates of 50 seeds per treatment, in the form of rolls for each replicate and identified according to the treatment, maintained at a constant 25 °C.

The methodology was carried out following the Rules for Seed Analysis (Brasil, 2009), with specifications regarding the species and adaptations for the first and second counts, which were conducted 5 and 10 days after sowing, respectively, and the final count on the 15th day, where the number of normal and abnormal seedlings was stipulated. The germination speed index (GSI) was calculated according to equation 2:

$$(2)\ IVG = (N1/1) + (N2/2) + \cdots + Nn/n$$

Where: Nn = total number of germinated seeds; n = time interval. Source: Maguire (1962).

Analysis of seed morphology

The preparation and observation of samples under a scanning electron microscope were conducted at the Laboratory of Electron Microscopy and Ultrastructural Analysis (LME), in the Department of Phytopathology/UFLA. After the 24-hour soaking period (one with the seed soaked in distilled water (control) and another with MWCNT (200 mg L^{-1}), turgid (soaked) seeds were selected, then longitudinally cut for sample preparation, and subsequently immersed in a fixative solution (modified Karnovsky), pH 7.2, and stored in a cold chamber until the analyses were performed. Subsequently, the samples were dehydrated in a series of acetone (25%, 50%, 75%, 90%, and 100%, three times) and then taken to the critical point dryer where they were placed in individual cages, adequately identified with a piece of pencil-written paper.

The samples were mounted on aluminum "stubs" using carbon tape placed over a film of aluminum foil, coated with gold. After this process, the stubs were visualized in the Tescan Clara scanning electron microscope, where their images were captured and analyzed.

Experimental design and statistical analyses

The experiment was conducted in a completely randomized design (CRD) with 6 treatments and Scott-Knott test for mean comparison at a 5% significance level using the statistical software SISVAR (Ferreira, 2019).

Results and Discussion

Physical-chemical-morphological characterization of *Clitoria ternatea* L. seeds

In this study, seeds were considered mature when the legumes attained a light brown color. The harvested seeds had a thousand seed weight of 44 grams and a moisture content of 5.8%. Observing the legumes from the seed batch used in this research, it can be stated that they have similar length parameters and on average the same number of seeds per legume as found in the literature.

Observing the legumes from the seed batch used in this research, it can be stated that they have similar length parameters and on average the same number of seeds per legume as found in the literature, these values are within the expected range, with legumes being flat and linear oblong in shape, ranging from 4 to 13 cm in length and 0.9 to 1.2 cm in width, each legume containing between 6-10 seeds (Mukherjee et al., 2008). Through morphometric analysis, it was possible to observe differences in the size and coloration of the seeds, which may characterize heterogeneity in the morphology of the batch (Fig. 2).

Fig. 2. Mature legume and visible seeds of *Clitoria ternatea* L., highlighting the structure and morphological characteristics of the seeds.

To more accurately assess the physical characteristics of *Clitoria ternatea* L. seeds, data were extracted using the image analysis technique with the assistance of the GroundEye® equipment. The results of the evaluated parameters are presented in Table 1 and Fig. 3.

Table 1. Morphometric characteristics of *Clitoria ternatea* L. seeds

Characteristics	Medium values
Total size (mm²)	19.0
Sphericity of shape	16.23
Length (mm)	5.8
Width (mm)	3.6
Perimeter (mm)	17.5

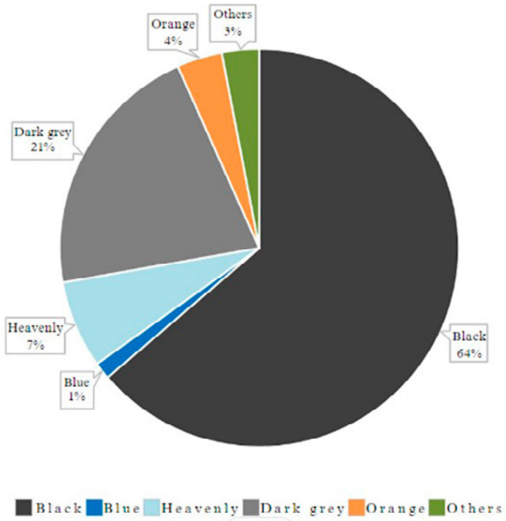

Fig. 3. Color dominance of *Clitoria ternatea* L. seeds.

These characteristics provided by the GroundEye® equipment allowed us to determine that the sphericity of the shape suggests that the seed has an oblong form. This is confirmed when the length is greater than the width, which in the case of this batch, the seeds had an average length of 5.8 mm and a width of 3.6 mm. In the literature, these data are sparse and inconsistent, but it is possible to state that the seeds range from 5-7 mm in length and 3-4 mm in width (Jamil et al., 2018), which confirms the results obtained by image analysis.

The seeds of *C. ternatea* have a predominance of black coloration followed by dark gray, demonstrating that more than 80% of the seeds possess a dark seed coat (Fig. 2 and 3). *C. ternatea* seeds exhibit variations in the coloration of the seed coat, and this heteromorphy is commonly observed in genera of the Fabaceae family. This characteristic is associated with the physiological quality of the seeds, where the difference in coloration can indicate the stage of maturation. For this reason, the visual morphological index can provide valuable information about the physiological maturity of the seeds (Silva et al., 2022).

In the seeds of *Crotalaria ochroleuca* L., belonging to the Fabaceae family, there is variability in the coloration of the seeds. Seeds with red coloration show germination and a low Germination Speed Index (GSI). In parallel, seeds with gray coloration have high values in the same parameters, indicating that the color of the seed coat influences the physiological quality of the seed and its germinative power (Silva et al., 2016).

To understand the composition of *C. ternatea* seeds beyond their physical characteristics, chemical analyses were conducted to obtain the profile of the seeds' centesimal composition (Table 2) and the determination of the mineral composition, highlighting the main minerals found in seeds in general (Table 3).

Table 2. Centesimal composition profile of *Clitoria ternatea* L. seeds.

Centesimal analysis	g 100 g^{-1}
Dry matter	60.00
Ash content	6.66
Crude protein	37.20
Ethereal extract - Lipids	15.00
Cudre Fibers	35.75
Carbohydrates	5.39

In this analysis, a total of ash, protein, lipids, fibers, and carbohydrates were found in 100 g of seeds. Comparing with a study that involved different stages of development of the *C. ternatea* legume, where values close to those of this study were found for ash, lipids, fibers, and carbohydrates, in 100 g of seeds at the mature legume stage, the recorded values were 3.15; 11.50; 31.02; 8.28 g, with the exception of protein, which was 7.83 g 100 g^{-1} (Padmanabhan et al., 2024), a comparatively low value in relation to the protein found in the seeds of the current study.

Studies detail the presence of anthocyanins and other bioactive compounds in *C. ternatea*. This suggests that variations in extraction and analysis methods can influence the protein content and other nutrients. The presence of anthocyanins and other bioactive compounds in *C. ternatea* may be related to variations in protein levels. Considering the complexity of interactions among different compounds, these can influence the bioavailability and analysis of proteins (Jeyaraj et al., 2022).

Comparing *C. ternatea* with the pine nut (*Araucaria angustifolia*), a species widely used in the cuisine of southern Brazil, it is noted that both have similar ash values (3.57 g in pine nut). However, *C. ternatea* stands out for a more balanced centesimal composition, with higher levels of protein (6.18 g in pine nut), fibers (6.62 g in pine nut), and lipids (2.30 g in pine nut). In contrast, the pine nut exhibits lower values in these components, especially in terms of protein. These nutritional differences highlight *C. ternatea* as a more nutritionally enriching alternative (Costa et al., 2023). A more detailed analysis of the mineral richness of *C. ternatea* will be presented in Table 3, offering a comprehensive view of its diverse nutritional contribution.

Table 3. Mineral profile of *Clitoria ternatea* L. seeds.

Mineral composition	mg 100 g^{-1}
Nitrogen	60.0
Phosphor	-
Potassium	1,221.46
Calcium	84.0
Magnesium	245.69
Copper	1.29
Iron	17.62
Manganese	-
Sodium	-
Boron	-
Zinc	5.49

Comparing the mineral composition of *C. ternatea* seeds with the study by Padmanabhan et al. (2024), variations in mineral contents are noted. In the present study, results indicate an increase of approximately 2% in iron content (17.62 mg 100 g^{-1}) compared to a previous study that analyzed mature seeds of the same species (17.15 mg 100 g^{-1}). However, regarding zinc, there is a significant reduction of about 39% in the seeds of the current study (5.49 mg 100 g^{-1}) compared with the seeds from the previous study (8.97 mg 100 g^{-1}). Magnesium showed a minimal variation, indicating stability in contents across different development phases. As for potassium, there was an increase of approximately 20% in the current seeds (1,221.46 mg 100 g^{-1}) compared to the previous study (1,016.70 mg 100 g^{-1}). However, calcium demonstrated a reduction of about 11% in the contents in the current seeds (84.0 mg 100 g^{-1}) compared to the mature seeds from the previous study (94.33 mg 100 g^{-1}).

These discrepancies can be attributed to different stages of development, seasonal factors, genetic variations, or specific analytical methods used in each research. Nonetheless, both investigations highlight the consistency in the mineral composition of *C. ternatea* seeds, underscoring their richness in essential nutrients and micronutrients (Padmanabhan et al., 2024). In comparison, the present study emphasizes a modest increase in iron, a significant reduction in zinc, and a substantial increase in potassium, while calcium shows a decrease, demonstrating the complexity of the mineral composition of this species in diverse contexts.

Compared with another species, the pinto bean (*Phaseolus vulgaris* L.), which has an iron content of 7.7 mg 100 g^{-1}, potassium of 1389.48 mg 100 g^{-1}, magnesium of 210.81 mg 100 g^{-1}, and sodium of 1.02 mg 100 g^{-1}, it can be said that *C. ternatea* is superior in iron and magnesium, while potassium is slightly lower in terms of mineral content (Lovato et al., 2017).

Carbon nanotubes in the germination of *Clitoria ternatea* L.

After soaking, the *C. ternatea* seeds were subjected to a germination test, and it was observed that seeds treated with 200 mg L^{-1} MWCNT had a higher germination rate compared to other treatments (Table 4). The treatments that induced the lowest germination percentages at 5 and 10 days were the seeds without soaking, soaked in water, and with 800 mg L^{-1} MWCNT. For all evaluated parameters (GSI and percentage of normal seedlings), the seeds treated with 200 mg L^{-1} MWCNT showed the best results.

Table 4. Germinative parameters and normal seedlings. Treatments with means followed by the same letter do not differ from each other by the Scott-Knott test, at 5% significance for the germination of *Clitoria ternatea* L.

Treatments	Germination at 5 days (%)	Germination at 10 days (%)	GSI	Normal Seedlings (%)
No soaking	2 ± 0.00 C	7 ± 0.25 D	0.52 ± 0.03 C	7 ± 0.48 D
Soaking in water	4 ± 0.48 C	7 ± 0.48 D	0.67 ± 0.14 C	8 ± 0.41 D
100 mg L^{-1} MWCNT	6 ± 0.00 B	13 ± 0.48 B	1.22± 0.05 B	12 ± 0.48 B
200 mg L^{-1} MWCNT	17 ± 0.65 A	28 ± 0.71 A	3.1 ± 0.20 A	22 ± 0.48 A
400 mg L^{-1} MWCNT	8 ± 0.48 B	10 ± 0.48 C	1.22 ± 0.10 B	9 ± 0.29 C
800 mg L^{-1} MWCNT	5 ± 0.48 C	6 ± 0.41 D	0.75 ± 0.12 C	6 ± 0.00 D

Various factors, such as water availability, access to oxygen, appropriate temperature, and light intensity influence seed germination. The use of MWCNT can result in an increase in germination due to their ability to improve water absorption (Shelar et al., 2023). From the data expressed in the table, it can be concluded that the concentration of 200 mg L^{-1} MWCNT had the most significant positive impact on germination (28%), GSI (3.1), and percentage of normal seedlings (22%).

In a study with barley, soybean, and corn seeds treated with MWCNT, it was shown that the improvement in germination is associated with an increase in the expression of aquaporin genes, which are responsible for the selective transport of water molecules while preventing the passage of ions and other solutes (Shelar et al., 2023). However, the seed coat dormancy present in *C. ternatea* seeds was not significantly broken by MWCNT, as germination was low. In this regard, it is understood that there are indications of improvement in the germination of seeds treated with 200 mg L^{-1} MWCNT, but not enough to overcome dormancy in the way they were exposed significantly.

In a study that used carboxylic acid-functionalized MWCNT, raw MWCNT, and graphene oxide to alleviate seed dormancy and improve seed germination in two species of boreal peatlands (*Betula pumila* L. and *Rhododendron groenlandicum* L.), it was demonstrated that *B. pumila* seeds treated with 0.02 or 0.04 mg L^{-1} of carboxylic acid-functionalized MWCNT showed better germination (75%) compared to the control (40%). In the case of *R. groenlandicum* seeds, treated with all types of MWCNT and graphene oxide evaluated, more than 90% germination was observed, surpassing the untreated seeds (control - 80% germination) (Ali et al., 2020).

A study on the species *Sorbus luristanica* Bornm. showed that the technique of soaking for 24 hours with MWCNT at a concentration of 350 mg L^{-1} assisted in breaking the dormancy present in the seeds of this species, in addition to improving germination parameters and seedlings (Sayedena et al., 2018).

It is worth noting that the results in this work indicate that the presence of MWCNT at the evaluated concentration (800 mg L^{-1}) does not interfere with the germination process, since the germination percentage is equal to the treatment without the addition of MWCNT.

Contrary to expectations, treatments with higher doses of MWCNT had increased fungal proliferation during germination. There are studies demonstrating the opposite, such as Gorczyca et al. (2022), who observed in the germination test in peas that the use of MWCNT at a concentration of 1 mg mL^{-1} limited the effect of disease caused by pathogens present in the natural microbiota of the seeds.

Seeking to understand the structural transformations in *C. ternatea* seeds during germination, resulting from treatment with MWCNT, scanning electron microscopy was used (Fig. 4) at the moment the seed is in the soaking phase.

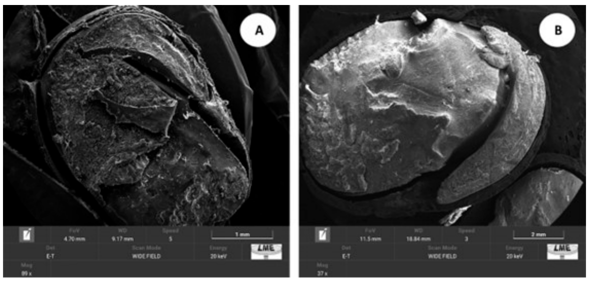

Fig. 4. Scanning electron micrograph. A) Interior of the seed, with 24 hours of soaking in distilled water; B) Interior of the *Clitoria ternatea* seed, with 24 hours of soaking in MWCNT (200 mg L^{-1}).

Observing Fig. 4 obtained in the SEM, in the treatment where the seed was soaked in water, excessive dehydration inside the seed is observed, indicating that it may have been affected by the dehydration procedure, but it can also be explained that the seed did not sufficiently imbibe in the region near the embryo, demonstrating slow water penetration, in addition to limited cellular development in the area. This is confirmed, as slow water penetration means the seed takes longer to transition from phase 1 to phase 2, leading to a delay in development and resulting in a low germination percentage as observed in other experiments.

When the seed was soaked in MWCNT 200 mg L^{-1} (Fig. 4B), higher activity is observed compared to when it is treated only with water, due to the size of the embryo relative to the other treatments. This may indicate the presence of MWCNT inside the seed, as well as an interaction with the seed coat that allowed water entry into the seed.

Even though MWCNT did not have a significant effect on overcoming dormancy, at the concentration of 200 mg L^{-1}, all evaluated parameters in seedlings were superior to other treatments, where, for example, the average total size (cm) was greater than all others (Table 5, Fig. 5).

Table 5. Morphological parameters of normal seedlings from intact seeds. Treatments with means followed by the same letter do not differ from each other by the Scott-Knott test, at 5% significance for germination of *Clitoria ternatea* L.

Treatments	Hypocotyl (cm)	Primary root (cm)	Hypocotyl/primary root	Full size (cm)
No soaking	7.80 ± 0.45 B	7.44 ± 0.55 B	0.97 ± 0.08 A	15.24 ± 0.72 B
Soaking at water	8.77 ± 0.45 A	5.17 ± 0.55 C	0.63 ± 0.08 B	13.94 ± 0.72 B
100 mg L^{-1} MWCNT	9.00 ± 0.45 A	6.91 ± 0.55 B	0.77 ± 0.08 B	15.91 ± 0.72 B
200 mg L^{-1} MWCNT	9.28 ± 0.45 A	9.88 ± 0.55 A	1.07 ± 0.08 A	19.17 ± 0.72 A
400 mg L^{-1} MWCNT	6.67 ± 0.45 B	4.55 ± 0.55 C	0.74 ± 0.08 B	11.23 ± 0.72 C
800 mg L^{-1} MWCNT	7.00 ± 0.45 B	7.44 ± 0.55 B	1.15 ± 0.08 A	14.43 ± 0.72 B

Seedlings from seeds treated with 200 mg L^{-1} MWCNT showed better results compared to the control and other MWCNT concentrations in the parameters of primary root length and total size (Table 5). When treated with 200 mg L^{-1} MWCNT, they achieved a primary root length of 9.88 cm, demonstrating excellent root growth, in addition to a hypocotyl/root of about 115 and a total average size of 19.17 cm in length at the final count (Fig. 5).

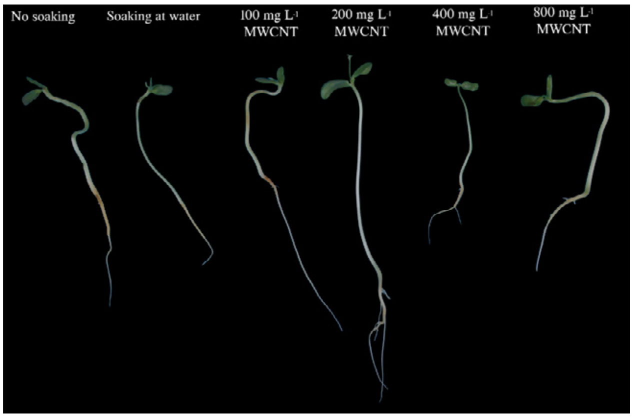

Fig. 5. Normal Seedlings from the germination experiment using Multi-walled carbon nanotubes (MWCNT) at different concentrations.

Seedlings from the treatment with 200 mg L^{-1} MWCNT had better results in terms of germination, but especially in seedling development, demonstrating it to be the most suitable concentration in this experiment.

The way MWCNT interacts with the seed is not yet clear, however, there is evidence that this nanoparticle creates new pores for water penetration through the seed coat, creating a cellular interaction that allows greater germination and seedling development (Mathew et al., 2020).

In a study with corn seeds (*Zea mays*), it was proven that when seeds are treated with MWCNT, there is a drastic improvement in seedling growth due to a higher rate of water absorption compared to the control medium. Seedlings exposed (Tiwari et al., 2023).

Traditionally, overcoming seed dormancy in *C. ternatea* L. involved chemical and mechanical scarifications, but these approaches have limitations. The use of MWCNT, however, proves to be a promising alternative to overcome dormancy and improve germination. Image analysis and scanning electron microscopy were essential to understanding the physicochemical characteristics of the seeds and their changes when exposed to nanotechnologies.

Conclusions

Clitoria ternatea L. seeds have an oblong shape and possess distinct coloring, characterizing heterochromia in the species. The centesimal composition demonstrated that the seed is high in proteins, carbohydrates, crude fiber and lipids. Additionally, the mineral composition highlights some important nutrients, such as potassium, calcium, magnesium, iron, zinc, and copper.

Regarding the use of MWCNT, when seeds are soaked in this product at a concentration of 200 mg L^{-1}, their germination characteristics and seedling development are improved.

Therefore, according to the composition data referred to in this study, *C. ternatea* seeds can be considered a source of nutrients for the food industry, as well as in the supplementation of agricultural products. Moreover, the use of nanotechnology in overcoming seed dormancy in this species has proven promising and encourages its utilization, suggesting potential advances in agriculture and the development of new research.

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Author Contribution

LTB: Writing – Original Draft, Investigation, Methodology, Project and Administration. **ARCN:** Data Curation, Formal Analysis, Writing – Review & Editing. **PDOP:** Conceptualization, Visualization. **RP:** Conceptualization, Visualization. **FCN:** Investigation, Methodology, Validation and Visualization. **MVR:** Administration Resources, Writing – Review & Editing, Software

Conflict of Interest

The authors declare no conflict of interests.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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