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Overcoming dormancy in *Coffea arábica* seeds with different immersion times in sodium hypochlorite

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ABSTRACT

Germination of coffee seeds (*Coffea arabica* L.) can take between 90 and 120 days, making the process slow and impacting production. This study evaluated the effect of sodium hypochlorite (NaClO) immersion time on dormancy breaking in seeds, aiming to optimize coffee crop management to improve productivity and quality. The experimental design was completely randomized in a 2 × 6 factorial scheme with four replications. The first factor was immersion in NaClO and H_2O, while the second factor corresponded to the immersion time in each solution (03h, 06h, 09h, 12h, 15h, and 18h). The analyzed variables included germination percentage (G%), germination speed index (GSI), mean germination time (MGT), mean germination rate (MGR), as well as seedling length and dry (DM) and fresh mass. NaClO yielded better G%, GSI, and DM results, while immersion in H_2O stood out for MGT, MGR, and root length. The results highlight the need to consider the effectiveness of dormancy breaking and its effects on early seedling development.

Keywords: Coffee; Germination; Bleach water.

Superação de dormência em sementes de *Coffea arábica* com diferentes tempos de imersão no hipoclorito

RESUMO

A germinação de sementes de café (*Coffea arábica* L.) pode levar de 90 a 120 dias, tornando o processo lento e afetando a produção. Esse estudo avaliou o efeito do tempo de imersão em hipoclorito de sódio (NaClO) na superação da dormência em sementes, buscando otimizar o manejo do café para melhorar a produtividade e a qualidade. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 2 x 6, com quatro repetições. O primeiro fator foi a imersão em NaClO e H_2O e o segundo fator correspondeu ao tempo de imersão em cada solução (03h, 06h, 09h, 12h, 15h, 18h). As variáveis analisadas incluíram a porcentagem de germinação (G%), índice de velocidade de germinação (IVG), tempo médio de germinação (TMG), velocidade média de germinação (VMG), além do comprimento e massa seca (MS) e verde das plântulas. O NaClO mostrou melhores resultados para G%, IVG% e MS, enquanto a imersão em H_2O destacou-se para TMG, VMG% e comprimento das raízes. Os resultados enfatizam a necessidade de considerar, além da eficácia na superação da dormência, os efeitos no desenvolvimento inicial das plântulas.

Palavras-chave: Café; Germinação; Água sanitária.

1. Introduction

Coffee beans obtained from the *Coffea* L. plant, belonging to the *Rubiaceae* family, hold significant importance in the global economic landscape, particularly the species *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* (Robusta or Conilon coffee), which are widely cultivated (Stoffelen et al., 2021).

In Brazil, coffee consumption continues to grow steadily. Data from the Brazilian Coffee Industry Association (ABIC, 2024) indicate a 4.10% increase in 2024 despite rising coffee bean prices. The International Coffee Organization (ICO, 2022) also reports that Brazilians consume an average of three to four cups daily, highlighting a behavioral shift: many consumers have started preparing coffee at home, whereas previously, 90% frequented coffee shops. Coffee holds immense economic value for Brazil, particularly in states such as Minas Gerais, São Paulo, and Espírito Santo, which lead national production. The country remains the world's largest coffee bean exporter (CONAB, 2024).

According to the National Supply Company (CONAB, 2024), the cultivated area of *Coffea arabica* is expected to reach 1.82 million hectares in 2024, with an estimated production of 58.08 million 60-kg bags, representing a 5.5% increase compared to the previous year. Although Brazil stands out in global coffee production and consumption, Finland leads the world in per capita coffee consumption, with 12 kg per inhabitant, while Brazil ranks second.

Despite its economic importance, coffee production faces challenges, particularly concerning seed germination, which can take 90 to 120 days before root development begins. Abiotic factors, such as temperature and biotic factors, including phenolic processes in seeds, can delay germination and influence the absorption of essential gases (Silva et al., 2023).

The slow germination of coffee seeds is not yet fully understood, but evidence suggests that the endocarp (or parchment) interferes with this process by limiting water and oxygen absorption. According to Souza & Carrasco (2021), seeds can exhibit two types of dormancy that influence germination. Primary dormancy, which is physiological, results from characteristics such as seed coat impermeability. Conversely, secondary dormancy is related to environmental factors and can be triggered by unsuitable temperature and humidity conditions.

To overcome these obstacles, dormancy-breaking techniques have been researched and applied. Scarification methods mechanical, chemical (using acids and other chemical substances), and temperature variations are commonly used to accelerate germination (Aguiar et al., 2021).

Among these techniques, chemical scarification with sodium hypochlorite (NaClO) has shown efficiency in various crops, although immersion time and solution concentration vary between species. Studies on *Juerana branca* seeds (*Albizia pedicellaris* (DC) L. Rico) in the Eastern Amazon have demonstrated that NaClO immersion is effective in breaking seed coat dormancy and improving germination rates (Barata et al., 2024). However, the relationship between NaClO immersion time and its effectiveness still requires further investigation.

Given this, this study aims to evaluate the effect of immersion in sodium hypochlorite and distilled water on dormancy breaking in *Coffea arabica* L. seeds.

2. Material and Methods

The experiment was conducted in the seed laboratory of the Engineering Center at Uniguaçu College, located in São Miguel do Iguaçu, PR (Figure 1). The coffee seeds used in the study belonged to the species *Coffea arabica* L., cultivar Catuaí Vermelho IAC 144, sourced from the farm of Mr. Deoclecio de Souza, a farmer from the Foz do Iguaçu region, PR. The seeds were manually harvested at the maturation stage, known as the cherry stage (Figure 2A), when they exhibit a red or yellow coloration depending on the variety and contain the optimal balance of sugars and essential oils.

After harvesting, *Coffea arabica* L. seeds underwent a manual depulping process without adding water. Following depulping, the seeds were washed under running water to remove as much mucilage as possible. Next, the seeds were placed in a plastic tray and kept in the shade to allow excess water to evaporate. Finally, the seeds were taken to the laboratory to initiate the experiment (Figure 2B).

The experiment was conducted using a completely randomized design (CRD) in a 2 × 6 factorial scheme with four replications. The first factor corresponded to the solution used for seed immersion, consisting of 200 mL of sodium hypochlorite (household bleach) containing 2.5% active chlorine or 200 mL of distilled water (H_2O). A total of 2,400 coffee seeds were used. The second factor was the immersion time, with six different durations (3, 6, 9, 12, 15, and 18 hours). The seeds were distributed at a rate of 50 per Gerbox container, corresponding to each treatment, and remained immersed in the solutions for the designated periods (Figure 3).

The seed moisture content was determined using oven-drying at 105 ± 3º C for 24 hours before conducting vigor and germination tests (Gonçalves et al., 2023). Approximately 5 g of seeds were randomly selected for each treatment to ensure representativity.

Figure 1. The satellite image of the Uniguaçu College campus highlights the Engineering Center and the laboratory used in the research.

Figure 2. *Coffea arabica* seeds with the hull at the cherry phenological stage (A), *Coffea arabica* seeds after manual depulping (B).

After immersion in NaClO or H_2O for the predetermined durations, the seeds were immediately arranged between sheets of germination paper (Germitest®), previously moistened with distilled water at 2.5 times their dry weight using a dispensing bottle. Each treatment consisted of eight rolls containing 25 seeds, properly labeled and placed in plastic bags to maintain moisture.

The photoperiod was set to 8 hours of light and 16 hours of darkness, and the plastic bags were placed in a BOD chamber at 30 °C for 30 days, following the Rules for Seed Analysis (Brasil, 2009). Substrate moisture was periodically monitored, with distilled water added whenever necessary. During the experiment, fungal presence in some treatments required replacing the germination paper in affected cases.

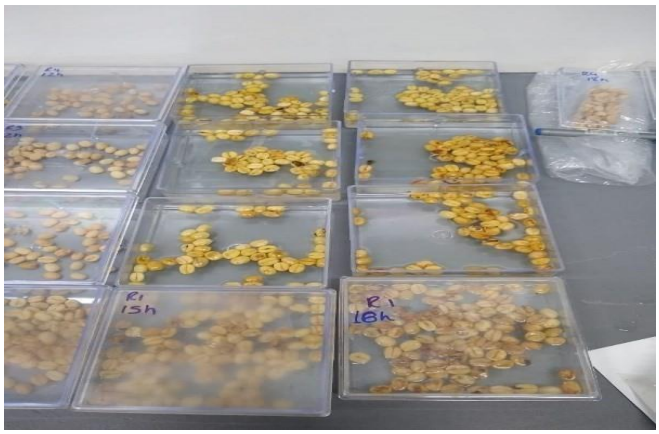

Figure 3. *Coffea arabica* seeds were treated with different immersion times in sodium hypochlorite (NaClO).

Evaluations for germination percentage (G%) and first germination count (FGC%) were conducted daily, starting on the 18th day after the experiment was set up (July 14, 2024), when the first signs of germination, such as radicle protrusion, were observed. Germination percentage (G%) was calculated based on the number of seeds that germinated by the end of the test, following the equation proposed by Tunes et al. (2020). The germination speed index (GSI) was also calculated using the equation by Borges et al. (2024), and the mean germination time (MGT) was determined using the equation proposed by Roweder et al. (2020).

Radicle length and fresh and dry mass were assessed after 30 days, with five root samples randomly collected per replication and measured using a millimeter ruler. Fresh mass was weighed on an analytical balance, while dry mass was determined after drying in an oven at 65 °C for 48 hours (Schmidt et al., 2017).

Data normality was verified using the Shapiro-Wilk test ($p \leq 0.05$). Analysis of variance was performed using the Tukey test ($p \leq 0.05$) with the SISVAR statistical software (Ferreira, 2019).

3. Results and Discussion

The results demonstrated a significant interaction between the evaluated factors for germination percentage (G%), first germination count (FGC%), germination speed index (GSI%), mean germination time (MGT), and mean germination rate (MGR%). This interaction indicates that the performance of *Coffea arabica* seeds was jointly influenced by immersion time and the type of solution used (sodium hypochlorite or distilled water), leading to variations in the analyzed physiological parameters, as shown in Table 2.

The germination percentage (G%) showed the best results in treatments with 15 and 18 hours of immersion in NaClO (Table 2), while for immersion in H_2O, the 6-hour duration resulted in the highest germination rate (Table 2). The other treatments presented lower values, indicating that immersion time and solution type directly influence seed germination performance.

Prolonged immersion in NaClO may have facilitated the removal of germination-inhibiting compounds on the seed surface, promoting better imbibition and metabolic activation. Thus, selecting the appropriate pre-germination treatment is essential to maximize germination and ensure the initial establishment of seedlings, directly impacting the success of *Coffea arabica* seedling production.

These results highlight the effectiveness of sodium hypochlorite (NaClO) in accelerating coffee seed germination. Studies such as that of Menegaes et al. (2022) have shown that the use of NaClO for surface disinfection of safflower (*Carthamus tinctorius* L.) seeds reduced the presence of phytopathogens and increased the germination rate compared to untreated seeds. Thus, sodium hypochlorite is an efficient sanitizing agent and a potential enhancer of seed germination capacity.

NaClO creates a more favorable environment for rapid and uniform germination, possibly due to its role in breaking dormancy and reducing microorganisms on the seed surface. In contrast, Ramos et al. (2023), in a study on dormancy breaking in *Mouriri cearensis* seeds, reported that treatment with H_2O at 50 °C resulted in only 1% germination, highlighting the inefficiency of this method compared to NaClO. This underscores the importance of appropriate treatments to optimize germination, especially in species with physiological dormancy.

For the first germination count (FGC%), the treatment with 18 hours of immersion in NaClO stood out significantly compared to the other treatments (Table 2), indicating that this duration effectively accelerated the germination process. In the treatments with immersion in H_2O, the 3, 6, 9, and 12-hour durations showed germination in the first count, suggesting that pure water, although not as effective as sodium hypochlorite, can also induce germination when applied for specific periods.

This result differs from the study by Carvalho et al. (2020), which demonstrated that salt stress induced by NaClO can reduce first germination count values in other species, such as *Paineira* and *Pau de Balsa*. The differences in observed effects may be related to each species sensitivity to hypochlorite, the composition of the seed cuticle, or even the interaction of NaClO with specific germination-inhibiting compounds. Thus, while some species may benefit from NaClO treatment, others may experience osmotic stress that negatively affects initial germination.

For the germination speed index (GSI%), the treatments with 15 and 18 hours of immersion in NaClO were the most effective (Table 2), indicating that this exposure time to sodium hypochlorite promoted faster and more uniform germination. In contrast, the same immersion times in distilled water resulted in lower GSI values, demonstrating an opposite effect.

These results align with Araújo Neto et al. (2020) findings, who observed an increase in germination and initial growth of *Vigna unguiculata* (L.) Walp. (cowpea) under salt stress, suggesting that certain species may exhibit moderate salinity tolerance, which can positively influence germination.

A decreasing trend was observed for the mean germination time (MGT) as the immersion duration increased, indicating that longer immersion times can accelerate seed germination.

Table 1. Germination (G%), first germination count (FGC%), germination speed index (GSI%), mean germination time (MGT), and mean germination rate (MGR%) for *Coffea arabica* L. seeds at different immersion times in sodium hypochlorite and distilled water.

Time	G (%)		FCG (%)		GSI (%)		MGT		MGR (%)	
	NaClO	H_2O	NaClO	H_2O	NaClO	H_2O	NaClO	H_2O	NaClO	H_2O
3 (h)	42.00 aB	44.00 aAB	24.00 aC	24.00 aAB	4.36 aB	4.59 aAB	5.35 aB	5.23 aABC	0.19 aA	0.19 aABC
6 (h)	46.00 aB	50.00 aA	18.50 bC	34.00 aAB	4.37 bB	5.43 aA	5.87 aB	5.04 bAB	0.17 aA	0.20 bBC
9 (h)	45.50 aB	42.00 aAB	16.00 aC	24.00 aAB	4.35 aB	4.31 aAB	5.72 aB	5.48 aABC	0.18 aA	0.18 aABC
12 (h)	50.00 Ab	42.00 aAB	23.50 aC	26.00 aAB	4.91 aB	4.92 aAB	5.69 aB	5.71 aBC	0.18 aA	0.18 aAB
15 (h)	70.00 aA	38.00 bB	44.00 aB	22.00 bB	7.23 aA	3.71 bB	5.38 aB	5.89 Ac	0.19 bA	0.17 aA
18 (h)	66.50 aA	44.00 bAB	59.50 aA	36.00 bA	7.93 aA	4.98 bA	4.38 aA	4.82 aA	0.23 bB	0.21 aC

Means followed by the same lowercase letter in the columns and the same uppercase letter in the lines do not differ statistically according to the Tukey test ($p < 0.05$).

This pattern was also reported by Ramos et al. (2023) when evaluating the effects of water and salt stress on *Peltophorum dubium* (Spreng.) Taub, reinforcing the hypothesis that controlled exposure to certain conditions can positively influence germination speed. Regarding the mean germination rate (MGR%), the treatments with 15 and 18 hours of immersion in NaClO took longer to initiate germination but were the most effective in uniformity and final germination rate (Table 2).

In contrast, in the treatments with immersion in distilled water, the 12, 3, and 9-hour durations resulted in faster germination, indicating that germination occurred earlier but did not necessarily lead to greater efficiency at the end of the process. These findings contrast with the results of Cruz et al. (2020), who reported that NaCl significantly reduced the germination speed of *Ochroma pyramidale*.

The divergence between studies may be related to species-specific salinity sensitivity and sodium hypochlorite distinct effects, which can act differently depending on seed chemical composition and the presence of inhibitory compounds. This reinforces the need for species-specific studies to determine the best pre-treatment strategy for each crop to optimize germination and early seedling development.

The best results for root fresh mass (RFM) were observed in the treatment with 3 hours of immersion in NaClO, while the lowest RFM was recorded for the same immersion time in H_2O (Table 3). These findings align with the study by Conde et al. (2021), which demonstrated a positive relationship between seed imbibition in distilled water and higher RFM volumes in lettuce, although the response may vary depending on species and treatment conditions.

Table 2. Root fresh mass (RFM), root dry mass (RDM), and radicle length of *Coffea arabica* L. at different immersion times.

Time	RFM (g)		RDM (g)		Length (cm)	
	NaClO	H_2O	NaClO	H_2O	NaClO	H_2O
3 (h)	0.28 aA	0.21 bB	0.27 aBC	0.22 aB	1.69 aA	1.82 aC
6 (h)	0.23 bAB	0.35 aA	0.35 aABC	0.38 aA	1.69 aA	1.97 aBC
9 (h)	0.21 bAB	0.35 aA	0.31 aABC	0.21 bB	1.32 bA	2.32 aAB
12 (h)	0.24 bAB	0.34 aA	0.41 aA	0.23 bB	1.55 bA	2.76 aA
15 (h)	0.16 bB	0.30 aA	0.37 aAB	0.17 bB	1.44 bA	2.40 aA
18 (h)	0.21 bAB	0.29 aA	0.23 aC	0.26 aAB	1.61 aA	1.80 aC

Means followed by the same lowercase letter in the columns and the same uppercase letter in the lines do not differ statistically according to the Tukey test ($p < 0.05$).

For root dry mass (RDM), the treatments with NaClO immersion resulted in the highest values, with 12 hours being the most effective exposure time. In contrast, for immersion in H_2O, the 3, 6, and 18-hour treatments also showed higher RDM values (Table 3). This result differs from the study by Tomazi et al. (2019), who reported an increase in root dry mass in bean seeds treated with NaClO compared to other aseptic methods, suggesting that the response to sodium hypochlorite may vary depending on species and experimental conditions.

Finally, radicle length showed the best results with immersion in H_2O for 12 and 15 hours, while immersion in NaClO resulted in the shortest radicle length, statistically similar to the 3, 6, and 18-hour immersion times in H_2O (Table 3). This result correlates with the study by Aderaldo et al. (2022) on the effects of water and salt stress on *Erythrina velutina* Wild seedlings, in which the control treatment (irrigation with distilled water) was the most recommended for *mulungu* cultivation, minimizing harmful effects on seedling development.

The analysis of the results reveals that the different treatments applied to coffee seeds significantly impacted germination and early seedling development. The variability in the results highlights the importance of carefully selecting treatment methods, considering their effectiveness in breaking seed dormancy and their potential to promote healthy seedling development.

4. Conclusions

Based on the results, immersing *Coffea arabica* L. seeds in NaClO for 15 or 18 hours is the most effective

alternative for overcoming dormancy, considering the higher germination rates and germination speed observed. However, the optimal immersion time may vary depending on the variable of interest, as the 12-hour immersion resulted in the highest root dry mass accumulation, while the 3-hour immersion favored greater root fresh mass.

Additionally, the treatments with NaClO led to shorter radicle lengths compared to immersion in H_2O, which, in turn, produced better results at 12 and 15 hours. This suggests that while sodium hypochlorite effectively breaks dormancy, its effects may vary regarding early seedling growth.

Thus, the evaluation of immersion time in sodium hypochlorite (NaClO) allowed for the identification of 15 and 18 hours as the most efficient durations for germination and initial seedling establishment. However, considering root development, different immersion times may be more advantageous. Therefore, the treatment choice should consider the germination rate and the desired conditions for early seedling growth, assisting in managing and producing high-quality seedlings.

Authors' Contribution

All authors contributed equally to this manuscript. Pablo Coutinho, Danielle Cadorin de Fraga, and Fernando Cologni performed data analysis, writing, and revision. Gabriel Matsuda and Liane Piacentini conducted text editing. Eduarda de Souza conducted the experiment and data collection.

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