REVISTA CIENTÍFICA - RECIMA21 ISSN 2675-6218

EFFECTS OF DIFFERENT CONCENTRATIONS OF GIBBERELLIC ACID (GA_3) ON THE GERMINATION OF 'CLEOPATRA' MANDARIN SEEDS (Citrus reshni Hort. ex Tanaka)

EFEITOS DE DIFERENTES CONCENTRAÇÕES DE ÁCIDO GIBERÉLICO (GA_3) NA GERMINAÇÃO DE SEMENTES DE TANGERINA 'CLEÓPATRA' (Citrus reshni Hort. ex Tanaka)

EFECTOS DE DIFERENTES CONCENTRACIONES DE ÁCIDO GIBERÉLICO (GA_3) EN LA GERMINACIÓN DE SEMILLAS DE MANDARINA 'CLEOPATRA' (Citrus reshni Hort. ex Tanaka)

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e727252
https://doi.org/10.47820/recima21.v7i2.7252

PUBLISHED: 02/2026

ABSTRACT

The 'Cleopatra' mandarin (Citrus reshni Hort. ex Tanaka) is an economically important rootstock widely used in citrus production for its productivity, vigor, and ability to induce earlier fruit ripening. Its complex taxonomy and preservation by nucellar embryony underscore the need for specific studies on its propagation. Research indicates that growth regulators such as gibberellic acid (GA_3) can enhance germination, although gaps remain regarding the physiological responses. Therefore, this study evaluated different GA_3 concentrations (0, 1000, 2000, 3000 and 4000 mg L^{-1}) on the germination of 'Cleopatra' mandarin seeds under controlled BOD germination-chamber conditions. Germination percentage (%G), index of germination velocity (GSI) and mean germination time (MGT) were measured, and the data were analyzed by normality tests, ANOVA and polynomial regressions. Results showed a significant treatment effect on GSI, with 1000 mg L^{-1} producing the highest germination velocity, while the shortest MGT was estimated near 2600 mg L^{-1} by polynomial fitting; final germination percentage exhibited a complex nonlinear response with no clear differences among all treatments, suggesting the need for greater sample size to confirm these trends. It is concluded that GA_3 improves aspects of the germination process of Citrus reshni Hort. ex Tanaka at low to intermediate doses—particularly 1000 mg L^{-1} for germination velocity—and that the findings support optimization of seed-imbibition protocols and further investigation of physiological mechanisms and population variability.

KEYWORDS: Citrus reshni Hort. ex Tanaka. Gibberellic acid (GA_3). Seed germination.

RESUMO

A tangerina 'Cleópatra' (Citrus reshni Hort. ex Tanaka) é um porta-enxerto economicamente relevante, amplamente utilizado na citricultura pela produtividade, vigor e indução de maturação precoce de frutos. Sua taxonomia complexa e a preservação por embriogênese nucelar reforçam a necessidade de estudos específicos sobre sua propagação. Pesquisas apontam que reguladores como o ácido giberélico podem favorecer a germinação, embora ainda haja lacunas sobre suas respostas fisiológicas. Diante disso, o estudo avaliou diferentes concentrações de GA_3 (GA_3: 0; 1000; 2000; 3000; 4000 mg L^{-1}) na germinação de sementes de tangerina 'Cleópatra' em condições controladas de câmara de germinação tipo BOD. Foram medidas porcentagem de germinação (%G), índice de velocidade de germinação (IVG) e tempo médio de germinação (TMG) e os dados analisados por testes de normalidade, ANOVA e regressões polinomiais. Os resultados mostraram efeito significativo dos tratamentos sobre o IVG, com 1000 mg L^{-1}

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REVISTA CIENTÍFICA - RECIMA21 ISSN 2675-6218

produzindo a maior velocidade de germinação, enquanto o menor TMG foi estimado próximo a 2600 mg L^{-1} por ajuste polinomial; a porcentagem final de germinação apresentou resposta não linear complexa, sem diferença clara entre todos os tratamentos, sugerindo necessidade de maior poder amostral para confirmação. Conclui-se que o GA_3 melhora aspectos do processo germinativo de Citrus reshni Hort. ex Tanaka em doses baixas a intermediárias, especialmente 1000 mg L^{-1} para velocidade de germinação e que os achados subsidiam a otimização de protocolos de embebição e investigações futuras sobre mecanismos fisiológicos e variabilidade entre populações.

PALAVRAS-CHAVE: Citrus reshni Hort. ex Tanaka. Ácido giberélico (GA_3). Germinação de sementes.

RESUMEN
La mandarina 'Cleopatra' (Citrus reshni Hort. ex Tanaka) es un portainjerto de relevancia económica, ampliamente utilizado en la citricultura debido a su productividad, vigor y capacidad de inducir la maduración precoz de los frutos. Su compleja taxonomía y la propagación mediante embriogénesis nucelar refuerzan la necesidad de estudios específicos sobre su germinación. Investigaciones previas indican que reguladores vegetales como el ácido giberélico pueden favorecer este proceso; sin embargo, aún existen vacíos en cuanto a sus respuestas fisiológicas. En este contexto, el presente estudio evaluó el efecto de diferentes concentraciones de GA_3 (0; 1000; 2000; 3000 y 4000 mg L^{-1}) sobre la germinación de semillas de mandarina 'Cleopatra', en condiciones controladas, utilizando una cámara de germinación tipo BOD. Se determinaron el porcentaje de germinación (%G), el índice de velocidad de germinación (IVG) y el tiempo medio de germinación (TMG). Los datos fueron sometidos a pruebas de normalidad, análisis de varianza y regresiones polinomiales. Los resultados evidenciaron un efecto significativo de los tratamientos sobre el IVG, destacándose la dosis de 1000 mg L^{-1} como la que promovió la mayor velocidad de germinación. El menor TMG fue estimado alrededor de 2600 mg L^{-1} mediante ajuste polinomial. El porcentaje final de germinación presentó una respuesta no lineal, sin diferencias consistentes entre todos los tratamientos, lo que sugiere la necesidad de un mayor tamaño muestral. Se concluye que el GA_3 mejora el proceso germinativo de Citrus reshni en dosis bajas a intermedias, contribuyendo a la optimización de protocolos de imbibición y a futuras investigaciones fisiológicas.

PALABRAS CLAVE: Citrus reshni Hort. ex Tanaka. Ácido giberélico (GA_3). Germinación de semillas.

INTRODUCTION

The 'Cleopatra' mandarin is a citrus variety scientifically classified as *Citrus reshni* Hort. ex Tanaka and belongs to the taxonomically complex genus Citrus (Marçal *et al.*, 2014). Citrus taxonomy is particularly intricate, as many species originated through interspecific hybridization and are maintained by nucellar embryony (Weiler *et al.*, 2010). Although its precise geographic origin is not clearly established, this variety has been extensively studied as a rootstock in citrus production systems, especially in Brazil (Brugnara; Sabio; Maro, 2021). 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) exhibits high economic potential as a citrus rootstock, showing favorable agronomic performance. Research indicates that this rootstock is highly productive when combined with different sweet orange cultivars. In a long-term study, Pompeu Júnior; Blumer; Pompeu (2008) reported that the mandarin rootstocks 'Xienkhouanga', 'South Africa', '117.477',

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'Cleopatra', 'Empress', 'Wildt', and 'Szinkon × Tizon' achieved the highest cumulative fruit yields across multiple harvests. Although no significant differences were observed in fruit commercial traits, the 'Cleopatra' rootstock promoted earlier fruit maturation. These attributes reinforce its relevance for commercial citrus production by offering robust rootstock performance and high productive potential.

According to Freitas *et al.,* (2015), citrus rootstocks such as 'Cleopatra' mandarin are essential for both genetic improvement and agricultural production efficiency. Despite this importance, studies specifically addressing seed germination of this variety remain limited. Related research, however, suggests that germination can be enhanced by plant growth regulators, including gibberellic acid, and by biostimulants (Souza *et al.*, 2015).

Research on 'Cleopatra' mandarin is therefore relevant for preserving genetic diversity, developing more robust rootstocks, and improving citrus resilience. Nevertheless, further studies are required to fully understand its germination behavior and to define optimal conditions for seedling establishment. In this context, the present study aimed to evaluate the effects of different concentrations of gibberellic acid on the germination and early development of 'Cleopatra' mandarin seeds under controlled BOD-type germination chamber conditions. Specifically, the study sought to address the lack of information regarding the physiological responses of 'Cleopatra' mandarin seeds to gibberellic acid application by evaluating germination percentage, germination speed index, and mean germination time, thereby contributing to the optimization of seed imbibition protocols and the understanding of germination behavior under controlled conditions.

MATERIALS AND METHODS

The experiment was conducted in 2022 at the Plant Propagation Laboratory of the Federal Institute of Espírito Santo (IFES), Santa Teresa Campus, located in the district of São João de Petrópolis, municipality of Santa Teresa, Espírito Santo State, Brazil (19°56′12″ S; 40°35′28″ W) (INCAPER, 2011).

A completely randomized design was adopted, consisting of five gibberellic acid (GA_3) concentrations (0, 1000, 2000, 3000, and 4000 mg L^{-1}), with four replicates of 25 seeds each, totaling 500 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) seeds.

Seeds were obtained from fully mature fruits collected from trees grown in an orchard at the IFES Santa Teresa campus. Seeds were obtained from a single orchard and harvest, which should be considered when extrapolating the results to other seed lots, growing regions, or production conditions. Seed extraction and preparation followed the methodology described by Santos *et al.,* (2019). Fruits were cut using a bamboo saw to avoid seed damage, manually squeezed over a sieve to separate seeds from juice, washed with 5% lime water to remove the mucilage surrounding the seeds, rinsed under running water to remove excess lime, and air-dried in the shade on newspaper.

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After drying, 500 seeds were manually counted and placed into containers containing GA_3 solutions at the specified concentrations, totaling 100 seeds per treatment (four replicates of 25 seeds each). Subsequently, treated seeds were arranged on germination paper moistened with distilled water at a volume equivalent to 2.5 times the dry weight of the paper. The material was maintained in BOD-type germination chambers at 25 ± 1 °C, under a 12-h intermittent light photoperiod throughout the experimental period.

From the appearance of the first radicle, germinated seeds were counted daily for 30 days. After this period, germination percentage (%G), germination speed index (GSI), and mean germination time (MGT) were calculated.

Data were subjected to the Shapiro–Wilk normality test and Bartlett's test for homogeneity of variances using Rbio software and transformed when necessary. Analysis of variance (ANOVA) and regression analyses were performed using Sisvar® software. For GSI, Welch's ANOVA and nonparametric tests were applied using JASP 0.95.4.0 due to violation of variance homogeneity assumptions.

RESULTS AND DISCUSSION

Gibberellic acid (GA_3) is widely recognized as a key regulator of seed germination, acting through the activation of metabolic pathways associated with the transition from dormancy to active embryo growth (Bewley *et al.*, 2013; TAIZ *et al.*, 2017). One of its most studied effects is the stimulation of hydrolytic enzymes, particularly α-amylase, which promotes the degradation of stored starch reserves into soluble sugars that sustain embryonic development. In addition, gibberellins interact antagonistically with abscisic acid (ABA), a hormone primarily associated with dormancy maintenance; thus, shifts in the GA/ABA balance are fundamental for the onset of germination (Yamaguchi, 2008; Bewley *et al.*, 2013). Beyond reserve mobilization, GA signaling also contributes to embryo expansion and the activation of gene expression involved in early developmental processes. However, hormonal responses are frequently dose-dependent, and excessive concentrations may lead to diminished efficiency or saturation effects, potentially explaining nonlinear response patterns observed in germination variables (Taiz *et al.*, 2017). In this physiological context, the effects observed in the present experiment can be interpreted considering the known mechanisms of GA-mediated regulation of germination.

For the germination speed index (GSI), the violation of variance homogeneity indicated by Levene's test ($F(4,15) = 10.12$; $p < 0.05$) justified the use of robust procedures, and Welch's ANOVA confirmed a significant treatment effect ($F(4,6.821) = 5.207$; $p = 0.030$). Descriptive statistics showed that the 1000 mg L^{-1} dose achieved the highest mean GSI (Mean = 0.989; SD = 0.013) and the lowest relative variability (CV = 0.013), followed by 2000 mg L^{-1} (Mean = 0.962; SD = 0.039), 4000 mg L^{-1} (Mean = 0.935; SD = 0.024), 3000 mg L^{-1} (Mean = 0.912; SD = 0.071), and

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the control (0.0 mg L^{-1}) (Mean = 0.803; SD = 0.125).

Multiple comparisons using Tukey's HSD test revealed significant differences between 0.0 mg L^{-1} and 1000 mg L^{-1} ($p = 0.011$) and between 0.0 mg L^{-1} and 2000 mg L^{-1} ($p = 0.032$). In parallel, the Kruskal–Wallis test confirmed an overall distinction among groups ($H(4) = 10.20$; $p = 0.037$), while Dunn's post hoc test with Bonferroni correction identified a significant difference only between 0.0 mg L^{-1} and 1000 mg L^{-1} ($p = 0.022$).

These results suggest a response pattern compatible with a ceiling effect (i.e., a plateau-type response in which increasing GA_3 concentrations no longer result in proportional biological gains), in which the 1000 mg L^{-1} concentration appears to represent the most efficient range within the tested doses, providing the greatest mean increase with higher consistency. In contrast, further dose increments did not produce statistically meaningful gains in the germination speed index. This plateau response may be associated with physiological saturation mechanisms, receptor sensitivity thresholds, or feedback regulation within GA-mediated signaling pathways, limiting additional stimulatory effects at higher concentrations. Regression analysis for the GSI variable suggested a cubic pattern, with a maximum at 1000 mg L^{-1} and a slight recovery at 4000 mg L^{-1}. However, the fitted curve exhibited very low explanatory power ($R^2 \approx 0.04$), indicating limited predictive capacity. Therefore, the regression model should be interpreted cautiously and primarily as an exploratory description of tendencies rather than as a robust dose–response relationship.

Overall, the 0.0 mg L^{-1} treatment exhibited the lowest GSI response, demonstrating that imbibition without GA_3 supplementation was not optimal for maximizing the germination speed of *Citrus reshni* Hort. ex Tanaka seeds. Moreover, under the conditions of this study, lower doses tended to outperform intermediate ones. From an applied perspective, the 1000 mg L^{-1} dose represents the best balance between efficacy and consistency, with clear implications for protocol optimization and cost–benefit efficiency (Figure 1).

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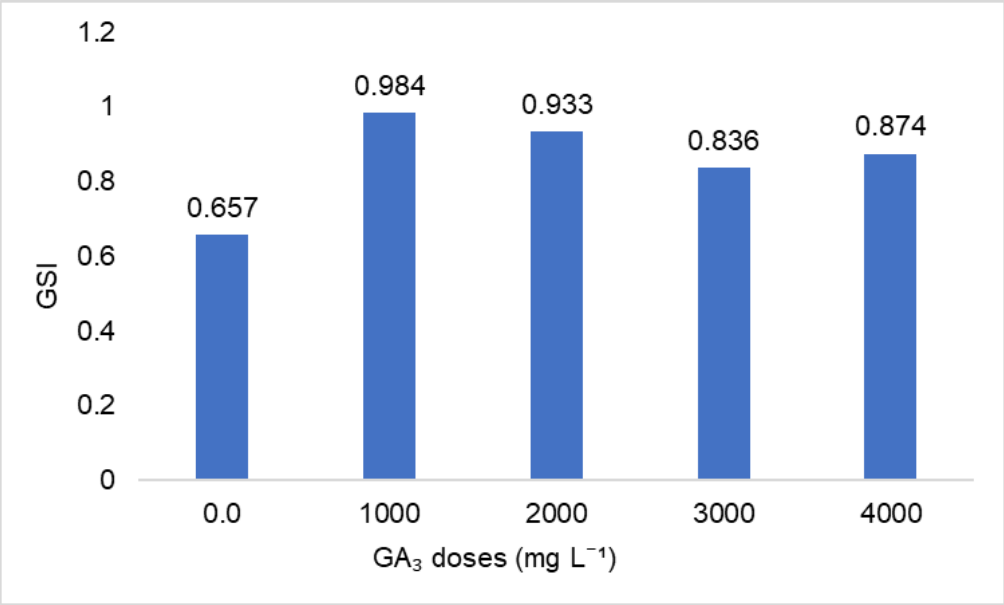
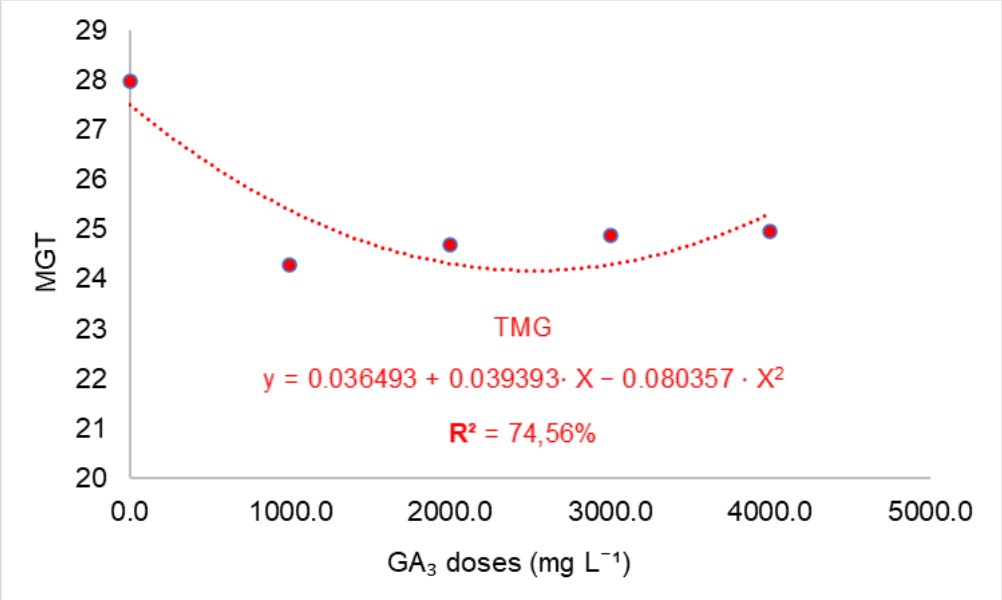
Figure 1. Germination speed index of 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) seeds at different gibberellin concentrations



Source: Elaborated by the authors.

Figure 2. Regression analysis of mean germination time of 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) under different gibberellin doses



Source: Elaborated by the authors.

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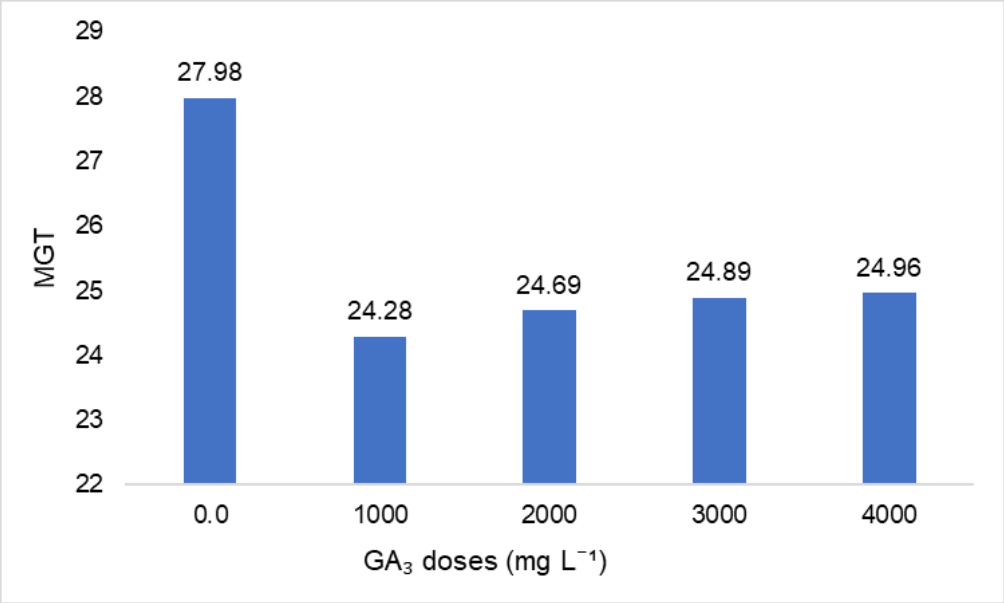
Figure 3. Mean germination time of 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) seeds at different gibberellin concentrations



Source: Elaborated by the authors.

For germination percentage (%G), the global ANOVA did not detect a statistically significant treatment effect ($p = 0.0962$), with a coefficient of variation of 13.58% and an overall mean of 68.51%. Nevertheless, polynomial regression substantially improved model fit (quadratic $R^2 = 60.59\%$; cubic $R^2 = 98.77\%$), although the cubic term did not reach significance at the 5% level ($p = 0.0749$), and sequential (Type I) analysis indicated a significant contribution only from the quadratic term ($p = 0.038$). Although the cubic model presented a high R^2 value, this should not be interpreted as strong evidence of a biological response pattern, since the global ANOVA did not detect a statistically significant treatment effect. High coefficients of determination in polynomial models may reflect overfitting to sample variability rather than a stable physiological response. Therefore, the regression results for %G should be interpreted cautiously and regarded as indicative of possible nonlinear tendencies that require confirmation through experiments with greater statistical power and broader sampling conditions.

This combination of results suggests the presence of an underlying nonlinear trend in germination percentage that was not captured by the global F-test, plausibly due to the higher intrinsic variability of this variable and the limited statistical power of the experiment. Based on the quadratic regression model, the maximum point of the trend curve occurred at approximately 2500 mg L^{-1} of GA_3, with concentrations of 1000 and 2000 mg L^{-1} showing the most favorable overall responses (Figure 4). These findings are consistent with the patterns observed for the other germination-related variables analyzed.

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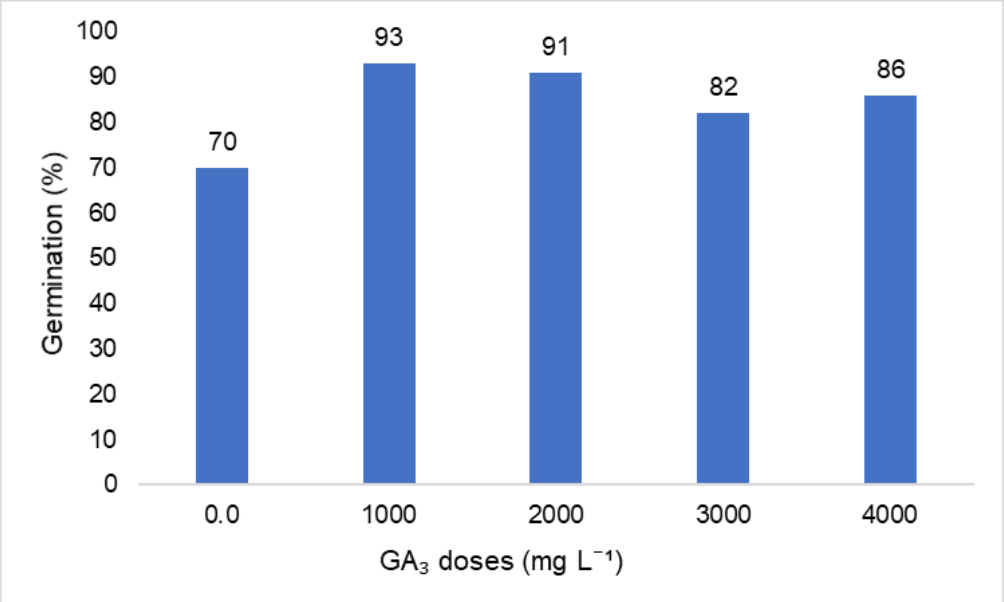
Figure 4. Germination percentage of 'Cleopatra' mandarin (*Citrus reshni* Hort. ex Tanaka) under different gibberellin concentrations



Source: Elaborated by the authors.

Corroborating these findings in Citrus, Mahmoud; Abo-Eid; Khodier (2022) reported that GA_3 treatment of *Citrus reshni* Hort. ex Tanaka seeds led to a progressive increase in germination percentage over a 60-day period: 24.59% (control), 53.65% (200 ppm), 73.12% (250 ppm), and 84.60% (300 ppm). In addition, the authors observed greater seedling height, a higher number of leaves, and increased leaf area in seedlings derived from GA_3-treated seeds, indicating positive effects not only on germination but also on early seedling vigor. This dose-dependent response up to higher concentrations reported by Mahmoud; Abo-Eid; Khodier (2022) is consistent with the non-linear pattern identified in the present study, in which low to intermediate doses showed greater effectiveness relative to the control.

Conversely, Soren *et al.,* (2025) note that excessive GA_3 doses may not result in additional gains, possibly due to saturation of physiological mechanisms or metabolic constraints. This observation aligns with the pattern found here, in which mean germination time (MGT) decreased up to an optimal point (2600 mg L^{-1}) and subsequently increased at higher concentrations. Overall, the data from this study are consistent with existing evidence, indicating that GA_3 primarily enhances germination speed and reduces germination time, with greater efficiency observed at low to intermediate dose ranges.

In summary, the body of evidence consistently points to the 1000 mg L^{-1} dose as the optimal level for maximizing the germination speed index (GSI), with high reproducibility, whereas MGT proved to be sensitive and exhibited a non-linear response to treatment levels. Although polynomial adjustments suggested a nonlinear trend for germination percentage (%G), this

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evidence should be interpreted cautiously, since the global ANOVA did not detect a significant treatment effect and %G typically shows higher intrinsic variability. Therefore, further studies with increased statistical power (e.g., larger sample sizes and additional seed lots/populations) are recommended to confirm the robustness and generalizability of these patterns under different conditions, particularly considering that the present experiment was conducted using a single seed lot under controlled environmental conditions.

CONSIDERATIONS

Mean germination time (MGT) and the germination speed index (GSI) showed a significant response to treatment levels. Overall, lower concentrations between 1000 and 3000 mg L^{-1} resulted in better responses for these variables, with 2600 mg L^{-1} corresponding to the lowest mean germination time. Therefore, gibberellic acid (GA_3) improved the germination process primarily at low to intermediate doses and can be considered a useful pre-germinative strategy for optimizing germination performance under controlled conditions.

From an applied standpoint, these results provide practical guidance for seed-imbibition protocols under controlled conditions. The 1000 mg L^{-1} GA_3 concentration was associated with the highest and most consistent germination speed index (GSI), while the lowest mean germination time (MGT) was estimated near 2600 mg L^{-1} by polynomial fitting. These reference values can support protocol optimization; however, validation across different seed lots and environmental conditions is recommended before broad extrapolation. Additionally, some regression models exhibited limited predictive strength, reinforcing that the interpretation of nonlinear dose–response patterns should be approached cautiously. Further studies with increased sample size and multiple seed sources are necessary to confirm the consistency and generalizability of these findings.

USE OF ARTIFICIAL INTELLIGENCE

The authors declare that generative artificial intelligence tools, specifically ChatGPT (OpenAI) and Elite AI, were used throughout the preparation of this manuscript exclusively to support language editing, translation, and stylistic refinement. All experimental design, data collection, statistical analyses, interpretation of results, and scientific conclusions were performed solely by the authors, who take full responsibility for the accuracy, originality, and integrity of the content presented.

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