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SYNERGISTIC EFFECT OF IMBIBITION TIME AND GA_3 CONCENTRATIONS ON THE GERMINATION OF POMEGRANATE SEEDS (Punica granatum L.)

EFEITO SINÉRGICO DO TEMPO DE EMBEBIÇÃO E DAS CONCENTRAÇÕES DE GA_3 NA GERMINAÇÃO DE SEMENTES DE ROMÃ (Punica granatum L.)

EFECTO SINÉRGICO DEL TIEMPO DE IMBIBICIÓN Y DE LAS CONCENTRACIONES DE GA_3 SOBRE LA GERMINACIÓN DE SEMILLAS DE GRANADA (Punica granatum L.)

Thiago Gratz Spinassé[1], Marcus Vinicius Sandoval Paixão[2], Hector Alexis Miranda[3]

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ABSTRACT

Pomegranate (Punica granatum L.) is a species of agronomic and pharmacological relevance, whose successful propagation depends on strategies capable of overcoming seed dormancy. Although gibberellic acid (GA_3) has been reported to stimulate germination, its effects vary according to dose and exposure time. The objective of this study was to evaluate the synergistic effect of different soaking times (0, 6, 12, 18, and 24 hours) and GA_3 concentrations (0, 1000, 2000, 3000, and 4000 mg L^{-1}) on seed germination and vigor. The experiment followed a completely randomized design, totaling 2,500 seeds distributed across treatments. Evaluations included germination percentage, germination speed index, and mean germination time under controlled laboratory conditions. The results demonstrated that the application of GA_3, especially with prolonged soaking periods, provided no benefits; on the contrary, it reduced germination and vigor compared to the untreated control, which achieved a higher germination percentage, germination speed index, and germination uniformity. It is concluded that, for the seed lot and experimental conditions tested, the use of GA_3 within the evaluated concentration range is not recommended for dormancy overcoming in Punica granatum L. seeds. The traditional method without growth regulator and without prolonged imbibition proved more efficient for seedling establishment.

KEYWORDS: Punica granatum L. Gibberellic acid (GA_3). Dormancy.

RESUMO

A romã (Punica granatum L.) é uma espécie de destaque agronômico e farmacológico cuja propagação eficiente depende de estratégias que promovam a superação da dormência de suas sementes. Estudos sugerem que o ácido giberélico (GA_3) pode estimular a germinação, mas seus efeitos variam conforme dose e tempo de exposição. Assim, o objetivo deste trabalho foi avaliar o efeito sinérgico de diferentes tempos de embebição (0, 6, 12, 18 e 24 horas) e concentrações de GA_3 (0; 1000; 2000; 3000; e 4000 mg L^{-1}) na germinação e vigor das sementes de romã. O experimento foi realizado em delineamento inteiramente casualizado, totalizando 2.500 sementes distribuídas nas combinações testadas. As avaliações englobaram a porcentagem de germinação, índice de velocidade de germinação e tempo médio de germinação em condições controladas de

[1] Engenheiro Agrônomo pelo Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo (IFES), Santa Teresa-ES, Brasil.
[2] Engenheiro Agrônomo, Doutor em Produção Vegetal pela Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes-RJ, Brasil.
[3] Engenheiro Agrícola, Doutor em Agronomia (Irrigação e Drenagem), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Jaboticabal-SP, Brasil.

laboratório. Os resultados demonstraram que a aplicação de GA_3, especialmente em embebições prolongadas, não trouxe benefícios; pelo contrário, reduziu a germinação e o vigor em relação à testemunha sem tratamento, que obteve maior percentual de emergência, índice de velocidade de germinação e uniformidade de germinação. Conclui-se que, para o lote e condições avaliadas, o uso de ácido giberélico nas faixas testadas não é recomendado para a superação da dormência em sementes de *Punica granatum L*., sendo preferível adotar o método tradicional, sem regulador ou embebição prolongada, visando maior eficiência e qualidade na germinação de plântulas.

PALAVRAS-CHAVE: Punica granatum L. Ácido giberélico (GA_3). Dormência.

RESUMEN
El granado (Punica granatum L.) es una especie de relevancia agronómica y farmacológica, cuya propagación exitosa depende de la adopción de estrategias capaces de superar la dormancia de las semillas. Aunque el ácido giberélico (GA_3) ha sido reportado como estimulante de la germinación, sus efectos varían en función de la dosis y del tiempo de exposición. El objetivo de este estudio fue evaluar el efecto sinérgico de diferentes tiempos de imbibición (0, 6, 12, 18 y 24 horas) y concentraciones de GA_3 (0, 1000, 2000, 3000 y 4000 mg L^{-1}) sobre la germinación y el vigor de las semillas. El experimento se realizó bajo un diseño completamente aleatorizado, con un total de 2.500 semillas distribuidas entre los tratamientos. Las evaluaciones incluyeron el porcentaje de germinación, el índice de velocidad de germinación y el tiempo medio de germinación, bajo condiciones controladas de laboratorio. Los resultados demostraron que la aplicación de GA_3, especialmente en embebiciones prolongadas, no reportó beneficios; por el contrario, redujo la germinación y el vigor en relación al testigo sin tratamiento, el cual obtuvo un mayor porcentaje de germinación, índice de velocidad de germinación y uniformidad de germinación. Se concluye que, para el lote de semillas y las condiciones experimentales evaluadas, el uso de GA_3 dentro del rango de concentraciones estudiado no se recomienda para la superación de la dormancia en semillas de Punica granatum L. El método tradicional, sin regulador de crecimiento y sin imbibición prolongada, resultó más eficiente para el establecimiento de las plántulas.

PALABRAS CLAVE: Punica granatum L. Ácido giberélico (GA_3). Dormancia.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit species native to the Mediterranean coast as well as Western and Eastern Asia, botanically classified as a woody shrub belonging to the family Punicaceae (Santos; Azevedo, 2010). The plant may reach up to seven meters in height and produces spherical fruits containing numerous angular seeds embedded in a pink to reddish aril with a slightly acidic taste (Santos; Azevedo, 2010). Historically, the species has been widely cultivated in arid regions of the Middle East and North Africa, where it holds strong economic and cultural significance (Oliveira *et al*., 2012).

Its chemical profile is complex, comprising various bioactive compounds such as tannins, flavonoids, alkaloids, and organic acids (Bacelar *et al*., 2023). Scientific evidence indicates that these constituents contribute to several pharmacological activities, including anti-inflammatory, antimicrobial, antioxidant, anticancer, neuroprotective, and cardioprotective effects. The available evidence is moderately robust, supported by *in vitro* studies and preliminary clinical trials (Pereira Rodrigues; Carvalho de Alencar, 2023). Among the most widely reported applications are those related to oral health, cardiovascular disease prevention, and modulation of inflammatory processes.

In Brazil, pomegranate is cultivated successfully across several states—including São Paulo, Bahia, Pernambuco, and Ceará (Oliveira *et al*., 2012)—demonstrating its adaptability to a range of environmental conditions. Numerous studies have characterized the fruit's physical, chemical, and nutritional properties (Santos; Azevedo, 2010), highlighting its potential for both fresh consumption and production of high-value derivatives.

Gibberellic acid (GA_3) has been widely investigated for its potential to stimulate pomegranate seed germination. Paixão *et al*., (2022) reported that GA_3 at 3000 mg L^{-1} resulted in the highest germination rate (85%). Conversely, Takata *et al*., (2014) showed that simple water soaking was the most effective method, with no advantage from GA_3 application, indicating variability in the physiological response to the regulator.

Overall, studies suggest that GA_3 may assist in overcoming physiological dormancy by modifying endogenous hormonal balance during early germination. However, ideal concentrations and application protocols appear to depend on intrinsic seed characteristics and experimental conditions. Thus, further research is required to establish standardized and broadly applicable germination protocols (Paixão *et al*., 2022).

Given this context, expanding knowledge on the use of gibberellins in pomegranate seeds is essential to clarify their effects on germination and early seedling vigor, considering the lack of standardized and consistent pre-germinative protocols reported in the literature. Therefore, this study aimed to assess the influence of different GA_3 soaking times on the germination of pomegranate seeds (*Punica granatum* L.), contributing to the optimization of germination practices for the species.

MATERIALS AND METHODS

The study was conducted 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, Brazil (19°56′12″S; 40°35′28″W), between October and December 2023.

A completely randomized design (CRD) was adopted, with five soaking times in GA_3 solution (0, 6, 12, 18, and 24 hours) and five GA_3 concentrations (0, 1000, 2000, 3000, and 4000 mg L^{-1}), with four replicates of 25 seeds each, totaling 2,500 *Punica granatum* L. seeds.

Seeds from mature fruits harvested in the IFES orchard were washed under running water with the aid of a sieve to remove impurities and arils. They were then dried in the shade on paper towels and manually counted. Groups of 500 seeds were placed into flasks containing the respective GA_3 concentrations.

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At every 6-hour interval, corresponding to the predefined soaking times, seeds were transferred to germination paper moistened with distilled water at 2.5 times the paper's dry weight. The material was placed in BOD germination chambers at 25 ± 1 °C under a 12-hour photoperiod.

From radicle germination onward, germination was recorded for 30 days. The following variables were measured: germination percentage (%G), germination speed index (GSI), and mean germination time (MGT).

Data were tested for normality (Shapiro–Wilk) and homogeneity of variances (Bartlett), using Rbio, and transformed when necessary. ANOVA and regression analyses were subsequently performed using Sisvar®.

RESULTS AND DISCUSSION

The results consistently indicate that the combination of soaking time and the tested gibberellic acid (GA_3) concentrations (0, 1000, 2000, 3000, and 4000 mg L^{-1}) did not improve the germination of *Punica granatum* L. seeds under the experimental conditions adopted. On the contrary, GA_3 application—particularly when associated with prolonged imbibition periods—was frequently correlated with reduced germination percentage and loss of vigor (GSI), whereas the non-soaked control (0 h, 0 mg L^{-1}) exhibited the best overall performance and the greatest uniformity.

For germination percentage (%G), the time-based ANOVA showed dose effects only at 6 h ($p = 0.0298$), 18 h ($p = 0.0006$), and 24 h ($p = 0.0020$), with no significant effects at 0 h ($p = 0.1136$) or 12 h ($p = 0.1773$). In the soaking periods with significant effects, responses were nonlinear: a quadratic fit at 6 h ($R^2 = 42.4\%$) and 24 h ($R^2 = 88.6\%$), and a cubic fit at 18 h ($R^2 = 89.8\%$), indicating complex dose–response patterns and clear signs of inhibition within certain concentration ranges. At 0 h, the linear model ($R^2 = 61.4\%$) showed a negative trend in %G as GA_3 concentration increased; that is, GA_3 application reduced germination compared with the control (Figure 1). In absolute terms, the mean germination observed in the control (0 h, 0 mg L^{-1}) was 84.1%, higher than the means obtained in most regulator treatments and exhibiting lower variability (CV ≈ 17.8%).

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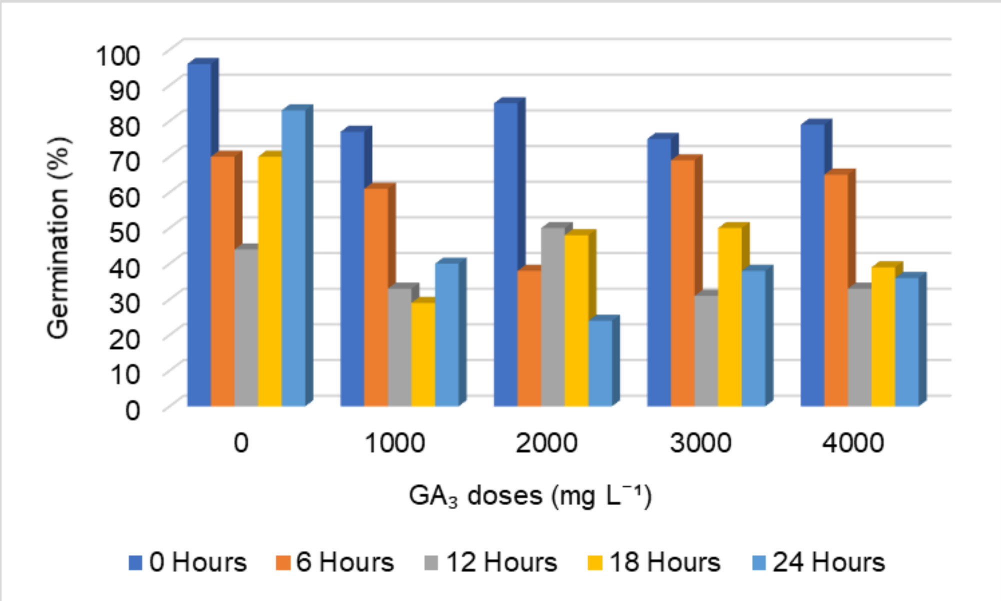
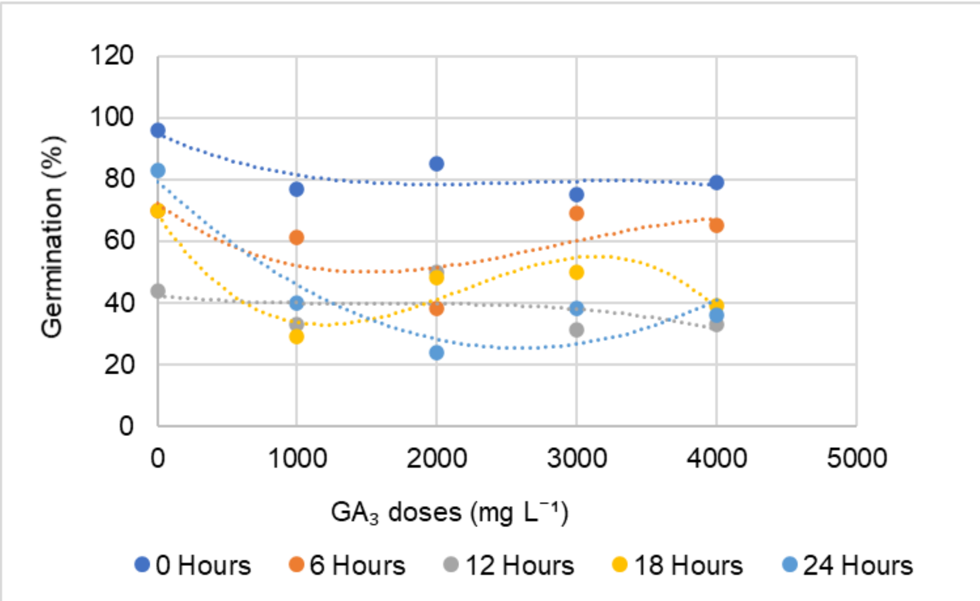
Figure 1. Germination percentage as a function of GA_3 concentration at 0, 6, 12, 18, and 24 hours of imbibition



Source: Elaborated by the authors.

Figure 2. Germination percentage by imbibition time and GA_3 concentration



Source: Elaborated by the authors.

The germination speed index (GSI) and mean germination time (MGT) reinforce this unfavorable pattern regarding GA_3 use. GSI was significantly affected by GA_3 doses at 6 h, 12 h, 18 h, and 24 h ($p \leq 0.01$ in multiple analyses), whereas no significant effect was observed at 0 h (p

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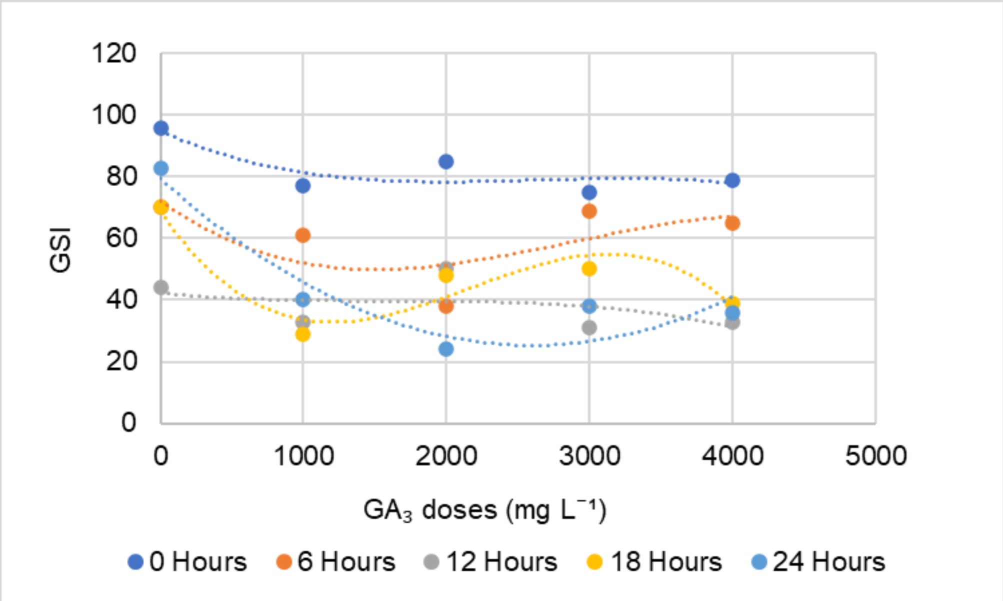
= 0.1312), which maintained the highest GSI among all treatments. For MGT, no consistent reduction associated with the regulator was detected; although the model fitted for the 24 h treatment exhibited high explanatory power ($R^2 \approx 98.8\%$), this variation resulted in an increase in mean germination time and a decrease in total germination—outcomes contrary to the practical goal of accelerating emergence.

Figure 3. Germination speed index as a function of GA_3 concentration at 0, 6, 12, 18, and 24 hours of imbibition



Source: Elaborated by the authors.

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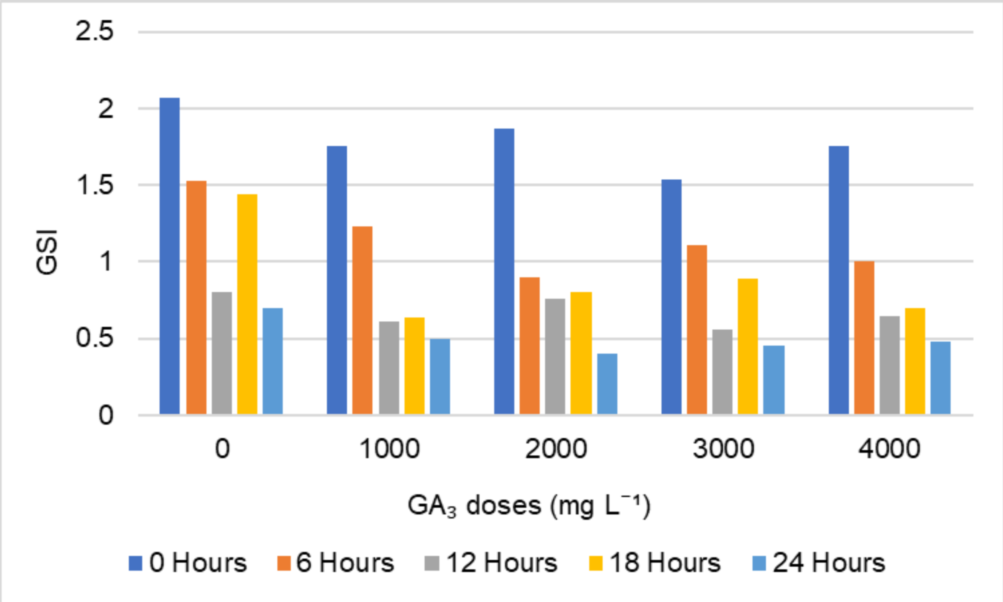
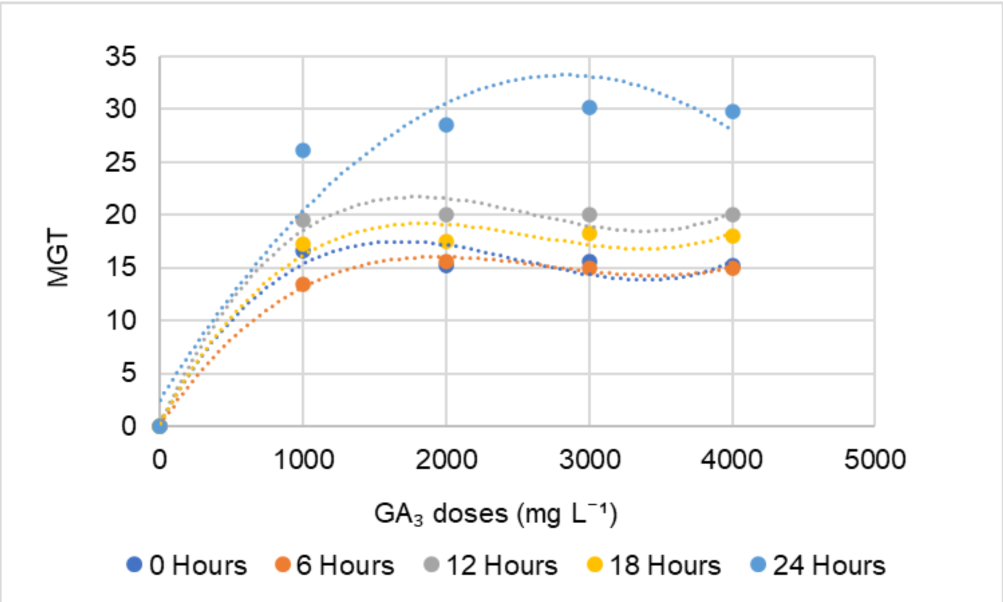
Figure 4. Germination speed index by imbibition time and GA_3 concentration



Source: Elaborated by the authors.

Figure 5. Mean germination time as a function of GA_3 concentration at 0, 6, 12, 18, and 24 hours of imbibition



Source: Elaborated by the authors.

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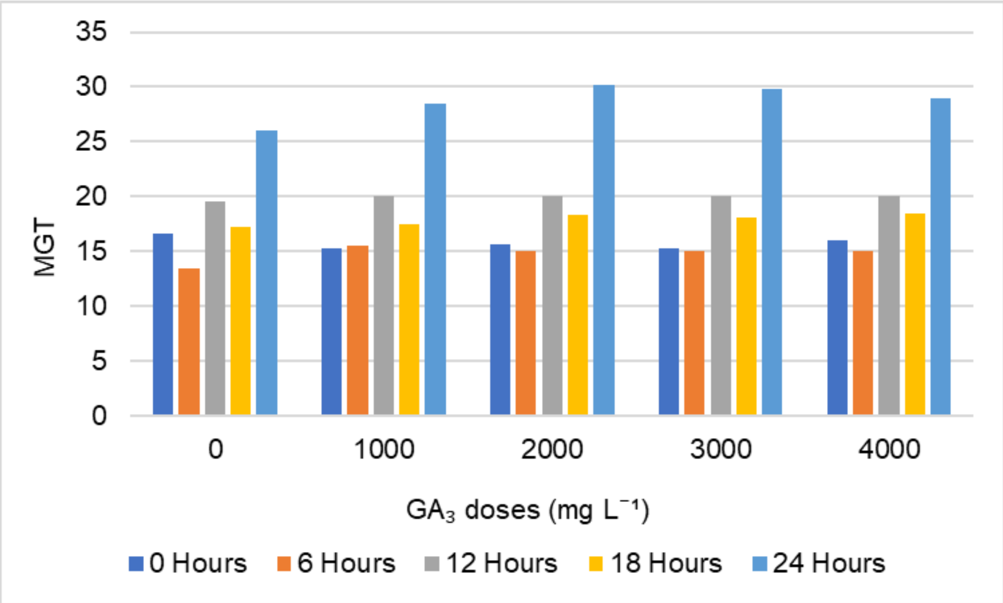
Figure 6. Mean germination time by imbibition time and GA_3 concentration



Source: Elaborated by the authors.

Similar results were reported by Takata *et al*., (2014), who investigated the germination of pomegranate seeds (*Punica granatum* L.) and concluded that soaking in pure water is an effective method, with no need for gibberellin application, and that periods of up to 4 hours are ideal for the germination process. This pattern suggests that, at higher doses, GA_3 requires shorter absorption times to exert its physiological effects, whereas prolonged soaking tends to be deleterious.

The results obtained in the present study are consistent with the known physiological role of gibberellins. At appropriate doses and exposure times, GA_3 promotes germination by stimulating reserve mobilization and embryo activation, a process regulated by the balance between gibberellins and abscisic acid (Shu *et al*., 2018). However, prolonged exposure can induce osmotic stress and leaching of reserve compounds, thereby reducing seed viability (Pompelli; Jarma-Orozco; Rodríguez-Páez, 2023).

Supporting this interpretation, Custódio *et al*. (2002) observed that extended flooding periods in bean seeds significantly reduced germination and vigor, particularly beyond 8 hours. Similarly, Sales *et al*. (2015) found in pumpkin seeds that shorter imbibition times (0 and 24 h) favored germinative performance, corroborating the results obtained here.

From a physiological standpoint, the findings suggest that the tested doses and soaking durations induced hormonal imbalances or physiological stresses capable of impairing the resumption of embryonic development. Two plausible hypotheses include: (i) disruption of the ABA/GA balance within the seeds, whereby exogenous GA_3 applied at inadequate levels interferes with signaling pathways involved in breaking dormancy; and (ii) phytotoxic or osmotic effects associated with prolonged soaking, potentially causing leaching of protective aril compounds or excessive uptake of the regulator, thus compromising seed viability. Furthermore, the high variability observed in some treatments (e.g., CV ≈ 25.9% for %G at 24 h) suggests that seed-lot heterogeneity may amplify adverse responses, reinforcing the need for strict quality control in germination experiments.

Based on the results, it is recommended that pomegranate seeds from the evaluated lot, under the laboratory conditions described here (BOD at 25 ± 1 °C, 12-hour photoperiod), should not be subjected to GA_3 soaking within the tested concentration range (1000–4000 mg L^{-1}). The most efficient and reproducible protocol was the absence of soaking and growth regulator (0 h, 0 mg L^{-1}), which yielded the highest germination percentage, greatest uniformity, and best germination speed. Avoiding prolonged imbibition (> 6 h) is also advised, as these treatments reduced both %G and GSI.

CONSIDERATIONS

The application of different soaking times and GA_3 concentrations did not improve germination or vigor of pomegranate seeds under the conditions tested. The treatment without growth regulator and without soaking produced the best results in terms of germination percentage, germination speed, and uniformity. Prolonged imbibition and GA_3 concentrations within the evaluated range significantly reduced germination, increased mean germination time, and decreased vigor, likely due to hormonal imbalance or physiological stress. For seeds of the same lot and similar laboratory conditions, the use of GA_3 or soaking longer than six hours is not recommended; the traditional, untreated method remains the most efficient approach.

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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