



The Effects of Prechilling, Chemical and Physical Scarification and Preheating Shock on Seed Germination in *Capparis spinosa*

Solmaz NAJAFI ^{1*}, Sebahattin ÖZCAN ², Mehdi TAJBAKHS ³

¹ Van Yuzuncu Yil University, Faculty of Agriculture, Department of Field Crops, Van

² Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara

³ Urmia University, Faculty of Agriculture, Department of Plant Production and Genetics, Urmia

*Corresponding author: solmaznajafi@yyu.edu.tr

Abstract

This study evaluated various seed treatments to identify optimal methods for enhancing the germination of *Capparis spinosa* (*C. spinosa*) seeds. Four treatments were tested: (1) sulfuric acid (98%) for 1 hour followed by 250 ppm gibberellic acid (GA₃) for 1 hour; (2) soaking in water for 4 hours followed by chilling at 4°C for 24 hours; (3) soaking in water for 4 hours, chilling at -10°C for 7 days, and GA₃ treatment for 1 hour; (4) soaking in hydrochloric acid (37%) for 6 hours followed by GA₃ treatment for 1 hour. A completely randomized design with three replicates was used. Analysis of variance (ANOVA) revealed significant differences in germination percentage, mean germination time (MGT) at the 0.05 level, and germination rate index (GRI) at the 0.01 level. Treatments 1, 3, and 4 resulted in the highest germination percentages of 73%, 71%, and 73%, respectively, while Treatment 2 had the lowest at 51%. For MGT, Treatments 1, 2, and 3 ranked highest with values of 7.53, 7.38, and 7.21 days, respectively, whereas Treatment 4 ranked lowest at 8.4 days. The highest GRI values were observed in Treatments 1 and 3, with values of 4.89 and 5.14, respectively, and the lowest in Treatment 2 at 3.64. Results suggest that combining sulfuric acid scarification or chilling at -10 °C with GA₃ significantly enhances germination. These findings provide practical insights for improving the germination of *Capparis* seeds, beneficial for large-scale cultivation and conservation efforts.

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1. Introduction

Caper (*Capparis spinosa* L.), a member of the Capparidaceae family, is widely recognized for its diverse applications in nutrition, pharmaceuticals, erosion control, and ornamental purposes (Coşge et al., 2005). The flower buds of caper are highly valued commercially, containing significant amounts of essential nutrients, including 67 mg of calcium, 65 mg of iron, 9 mg of zinc, and 24.01 g of protein per 100 g of dry weight. Typically pickled, caper buds are incorporated into salads, vegetarian dishes like izas, and various meat-based meals. Caper buds are known for their antioxidant properties, with specific

chemical compounds that actively suppress cancer cells and mitigate damage from carcinogenic agents. Following studies by the International Agency for Research on Cancer, caper extracts have been utilized in antitumor treatments (Akgül, 1996, Kara et al., 1996). Additionally, certain compounds in caper exhibit diuretic and antihypertensive effects, while substances in its seeds contribute to the enhancement of liver, spleen, and kidney functions, as well as the management of conditions such as asthma and hemorrhoids. The fruits of caper possess analgesic properties, and its bark contains anti-inflammatory agents (Tansi et al., 1997). (Figure 1)



Figure 1. *C. spinosa* flower (a); *C. spinosa* seeds (b)

Caper is considered endemic to the Mediterranean region but is also cultivated in regions along the Atlantic coast, including the Canary Islands, Morocco, Spain (particularly Almeria, Granada, and the Balearic Islands), France (Provence), Italy, Greece, Cyprus, Turkey, and Iran under both cultivated and natural rainfall conditions (Zohary, 1959; Soyler and Khawar, 2007). Historically, caper has been valued for its medicinal and aromatic qualities, with uses that reduce bloating, exhibit anti-rheumatic effects, support liver protection, and serve as treatments for arteriosclerosis, anemia, arthritis, and gout (Güleyüz et al., 2009). Capers are also known for their diuretic and antiseptic properties, making them useful as tonics for kidney health.

Caper plants can propagate from seeds or stem cuttings; however, both methods present challenges that limit the commercial expansion of this valuable crop (Bhojar et al., 2010). Seed dormancy is a common occurrence in the plant life cycle, and several structural components contribute to dormancy in caper seeds (Rao et al., 1976). There are two primary dormancy mechanisms: embryonic dormancy and coat-imposed dormancy. In caper, dormancy is generally not attributed to embryonic dormancy, as partial removal of seed coat structures, scoring, or perforating the seed coat has proven effective in stimulating germination (Sozzi and Chiesa, 1995). Although caper seeds can germinate, they typically do so at low rates (1-2%), and dormancy intensifies with seed drying,

presenting a significant obstacle to natural germination (Olmez et al., 2004). The hard seed coat forms an external barrier that restricts oxygen diffusion to the embryo, thereby maintaining dormancy (Soyler and Khawar, 2007).

Pre-chilling, scarification, and treatments with gibberellic acid (GA₃) or nitric acid (KNO₃) are commonly employed to improve germination rates in dormant caper seeds. Various approaches have shown success in enhancing germination percentages, including combinations of gibberellic acid and KNO₃ (Perez et al., 2002; Puppala and Fowler, 2003), sulfuric acid (H₂SO₄) pretreatment (Kara et al., 1996), H₂SO₄ combined with GA₃ (Sozzi and Chiesa, 1995), and warm water followed by chilling (Kontaxis, 1997).

Due to the high demand for caper seedlings, research has focused on refining propagation techniques. Seed propagation remains the most straightforward method for cultivating capers; however, it is constrained by issues such as low germination rates, variability in fruit quality, and high genetic diversity, which can affect commercial productivity. During germination, seed mucilage can obstruct oxygen diffusion to the embryo, forming a barrier to successful sprouting. Additionally, dried seeds enter a dormant state that hinders germination, requiring specific treatments to break this dormancy (Al-Oudat, 2008; Coskun and Yaman, 2024).

Recent studies have investigated various combinations of scarification, seed soaking, GA₃ pretreatments, and temperature regimes to address these challenges. For instance, Chiboub et al. (2024) tested treatments under two temperature conditions: T₁ (9/35.7 °C in the laboratory) and T₂ (1/43 °C in the greenhouse). Their findings revealed the highest germination rate (68.33%) for control seeds in the laboratory setting, with both pretreatments and temperature regimes applied.

Despite extensive research on the effects of acids on seed germination rates in agricultural and ornamental plants, studies on medicinal species like *C. spinosa* L. remain limited.

Additionally, current literature lacks detailed data on key germination metrics, such as germination percentage, mean germination time, and germination rate index, particularly for medicinal plants. This study hypothesizes that targeted chemical and mechanical scarification treatments can effectively break dormancy in *C. spinosa* seeds. By enhancing germination rates, this approach aims to address existing knowledge gaps and promote sustainable propagation techniques for this valuable medicinal species.

2. Material and Methods

The seeds used in this study were collected from the Kirikhan area of the Hatay Province, Türkiye. The experiments were conducted in the Department of Field Crops, Faculty of Agriculture, Ankara University. The experiments followed a completely randomized design with 14 treatments and 3 replications, resulting in a total of 4200 *Capparis* seeds. The seeds were first disinfected using 20% sodium hypochlorite for 30 minutes, followed by thorough washing with distilled water (3 times). The 14 treatments applied were as follows:

- a. Soaking in water for 24 hours
- b. Soaking for 12 hours, drying for 24 hours, then soaking for 12 hours
- c. Soaking for 4 hours, followed by pre-chilling for 24 hours at 4 °C
- d. Mechanical scarification
- e. Heat shock treatment
- f. Soaking in water at 40 °C for 4 hours
- g. Soaking in 0.02% KNO₃
- h. Soaking in 37% hydrochloric acid (HCl) for 6 hours, followed by treatment with 250 ppm gibberellic acid (GA₃) for 1 hour
- i. Soaking in sulfuric acid (H₂SO₄) for 1 hour, followed by treatment with 250 ppm gibberellic acid (GA₃) for 1 hour
- j. Soaking in water for 4 hours, then chilling at -10 °C for 7 days, followed by treatment with 250 ppm gibberellic acid (GA₃)
- k. Treatment with 400 ppm indole-3-acetic acid (IAA) for 1 hour
- l. Exposure to 40 °C for 7 days
- m. Soaking in 1% NaCl solution for 12 hours
- n. Soaking in water for 48 hours

For each of these treatments, 300 seeds were used (100 seeds per replication), resulting in a total of 1200 seeds. In each replication, 100 treated seeds were placed in 9 cm Petri dishes.

2.1. Germination Conditions

Once the seeds have been pre-treated, they are placed under controlled environmental conditions in a growth chamber:

2.1.1. Temperature

The optimal temperature range for germination is typically between 25-30 °C. Studies suggest that *C. spinosa* seeds show improved germination at 25 °C under controlled conditions, although lower temperatures (e.g., 4 °C for chilling) might be used for stratification.

2.1.2. Light Intensity

Moderate light intensity is ideal, with a photoperiod of 12-14 hours of light per day. This mimics natural conditions, where the seeds receive exposure to sunlight during the day.

2.1.3. Humidity

A relative humidity of 60-80% is generally maintained to prevent the seeds from drying out. Higher humidity helps maintain moisture content in the seeds, promoting the activation of enzymes responsible for seedling growth.

2.1.3. Watering

Seeds should be kept moist but not submerged, as over-saturation can lead to fungal growth or rotting, so, ten ml of distilled water was added to each petri dish. Seeds were observed daily and germinated seeds were counted until no further germination was observed for two consecutive days. Seeds were considered germinated when the seedling length reached 2 mm (Nichols and Heydecker, 1968). At the end of the experiment, the following indices were measured:

a. 2.2. Germination Percentage (GP)

The percentage of germinated seeds was calculated using the following formula (Hartmann and Kester, 1983; Camberato and Mccarty, 1999)

$$\%GP = \frac{\sum G}{N} \times 100$$

Where:

G: the number of germinated seeds.

N: the total number of seeds.

2.3. Mean Germination Time (MGT)

This index was calculated using the formula by Nichols and Heydecker (1968)

$$MGT = \frac{\sum (nt)}{\sum n}$$

Where:

N = number of germinated seeds each day

T = number of days corresponding to each count

2.4. Germination Rate Index (GRI)

The GRI was calculated using the formula by Throneberry and Smith (1955):

$$RI = \frac{\sum Ni}{\sum Ti}$$

Where:

Ni = number of germinated seeds on day i

Ti = total time (number of days) from the start of the experiment

The results were statistically analyzed using SPSS software, applying a completely randomized design. The means were compared using Duncan's Multiple Range Test at a 5% significance level (SPSS, 2016).

3. Results and Discussion

The preliminary experiment revealed that

10 out of the 14 treatments applied had negative effects on seed germination, resulting in complete seed burning (Figure 2; Table 1).

Table 1. Preliminary experiment results of different treatments on *C. spinosa* seeds

Treatment	Result
a Soaking in hot water for 24 hours	Burned
b Soaking for 12 hours, drying for 24 hours, then soaking for 12 hours	Burned
c Soaking for 4 hours, followed by pre-chilling for 24 hours at 4 °C	Germinated
d Mechanical scarification	Burned
e Heat shock treatment	Burned
f Soaking in water at 40 °C for 4 hours	Burned
g Soaking in 0.02 % KNO ₃	Burned
h Soaking in 37% hydrochloric acid (HCl) for 6 hours, followed by treatment with 250 ppm gibberellic acid (GA ₃) for 1 hour	Germinated
i Soaking in sulfuric acid (H ₂ SO ₄) for 1 hour, followed by treatment with 250 ppm gibberellic acid (GA ₃) for 1 hour	Germinated
j Soaking in water for 4 hours, then chilling at -10 °C for 7 days, followed by treatment with 250 ppm gibberellic acid (GA ₃)	Germinated
k Treatment with 400 ppm indole-3-acetic acid (IAA) for 1 hour	Burned
l Exposure to 40 °C for 7 days	Burned
m Soaking in 1% NaCl solution for 12 hours	Burned
n Soaking in water for 48 hours	Burned

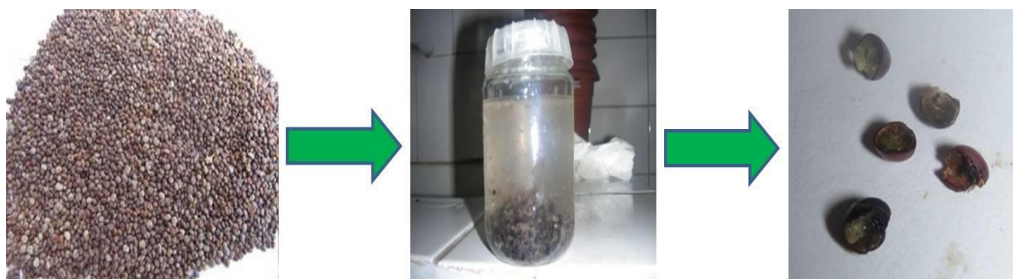


Figure 2. Seed burning due to different treatments in the preliminary experiment.

Based on these results, only four treatments were selected for the main experiment and further statistical analysis:

Treatment (T₁)

Soaking in sulfuric acid (H₂SO₄) for 1 hour, followed by treatment with 250 ppm gibberellic acid (GA₃) for 1 hour

Treatment (T₂)

Soaking in water for 4 hours, followed by chilling at 4 °C for 24 hours

Treatment (T₃)

Soaking in water for 4 hours, followed by chilling at -10°C for 7 days, and treatment with 250 ppm gibberellic acid (GA₃)

Treatment (T₄)

Soaking in 37% hydrochloric acid (HCl) for 6 hours, followed by treatment with 250 ppm gibberellic acid (GA₃). Several treatments were excluded due to their detrimental effects on *C. spinosa* seeds. For instance, soaking in water for 24 hours (Treatment 1), followed by a drying and re-soaking process (Treatment 2),

caused the seeds to burn, likely due to the prolonged soaking and lack of additional pre-treatment measures. Similarly, mechanical scarification (Treatment 4) and heat shock treatment (Treatment 5) both resulted in burned seeds, indicating that these methods caused severe damage. Soaking in water at 40 °C for 4 hours (Treatment 6) and in a 1% NaCl solution (Treatment 13) also led to seed burning, possibly from excessive heat and salinity stress, which impaired the seeds' viability. The 400-ppm indole-3-acetic acid (IAA) treatment (Treatment 11) produced similar results, causing burning, which suggests that this auxin concentration was too high for the seeds. Finally, exposing seeds to 40 °C for 7 days (Treatment 12) caused irreversible damage, while soaking in water for 48 hours (Treatment 14) led to burning, likely due to prolonged soaking without the benefit of other treatments to protect the seeds. Analysis of variance (ANOVA) revealed significant differences in germination percentage, mean germination time at the 0.05, and germination rate index at the 0.01 levels. (Table 2).

Table 2. Analysis of variance on percentage of germination, mean germination time and germination rate index of Capparis seed

Source of variation	Df	Means of squares		
		Percentage of germination	Mean germination time	Germination rate index
Treatments	3	323*	0.842*	1.571**
Error	8	51.25	0.072	0.025

*: significant at P < 0.05 **: significant at P < 0.01

Comparison of means for measured properties in Capparis seeds was done based on Duncan's Multiple Range Test (Table 3, Figure 3).

Table 3. The means comparison for different treatments based on duncan's multiple range test

Treatment	T ₁	T ₂	T ₃	T ₄
Index ¹				
Percentage of germination	71 <i>a</i>	51 <i>b</i>	73 <i>a</i>	59 <i>ab</i>
Mean germination time	7.53 <i>a</i>	7.38 <i>a</i>	7.21 <i>a</i>	8.4 <i>b</i>
Germination rate index	4.89 <i>a</i>	3.64 <i>c</i>	5.14 <i>a</i>	3.95 <i>b</i>

Treatment with sulfuric acid for 1h + 250 ppm gibberellic acid for 1h; T₂: soaking in water for 4h+ chilling at 4 °C for 24h; T₃: soaking in water for 4h then chilling (Chilling

at -10 °C for 7days) + treatment with 250 ppm gibberellic acid for 1h; T₄: soaking in chloridric acid 37% + treatment with 250 ppm gibberellic acid.

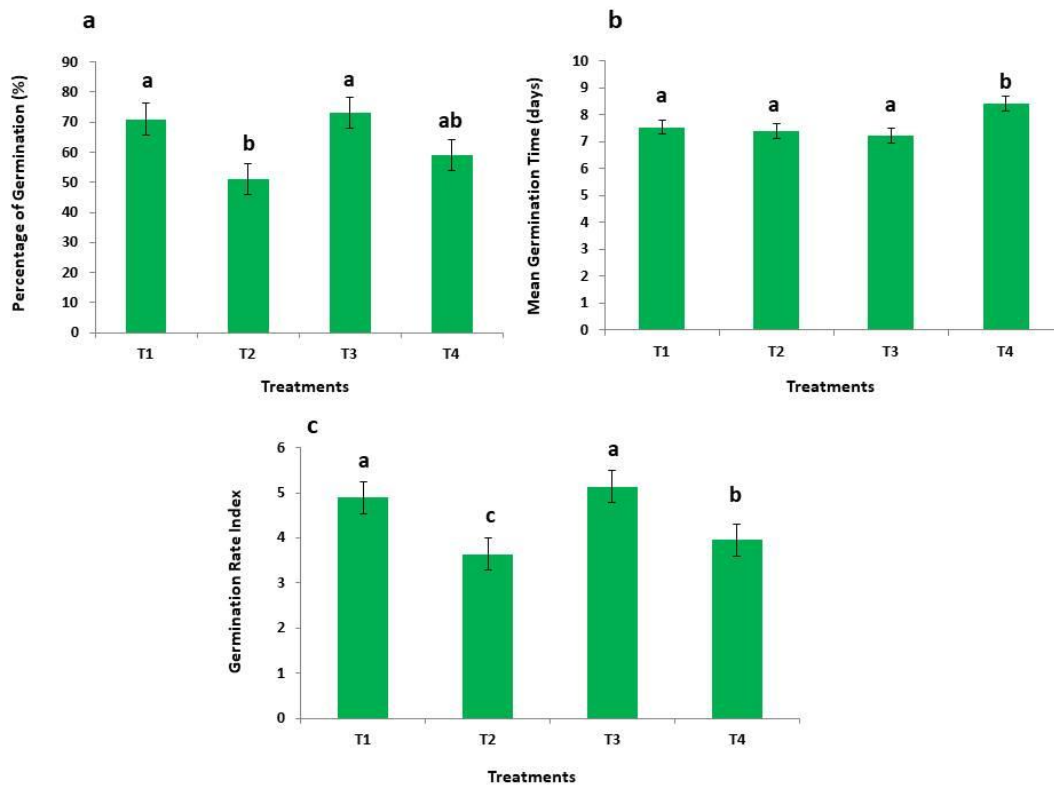


Figure 3. The effects of different treatments (T₁, T₂, T₃, and T₄) on Percentage of Germination (a), Mean Germination Time (b) and Germination Rate Index (c) of Capparid Seeds.

The effects of different treatments (T₁, T₂, T₃, and T₄) on seed germination of *C. spinosa* across three parameters: (a) Percentage of Germination, (b) Mean Germination Time (MGT), and (c) Germination Rate Index (GRI). Data indicate that Treatments T₁, T₂ and T₄ resulted in the highest germination percentages, while Treatment T₂ showed significantly lower germination. Treatments T₁, T₃ exhibited the shortest MGT, while Treatment T₄ had the longest. Regarding GRI, Treatments T₁ and T₃ led to the highest values, highlighting their effectiveness in promoting seed vigor. Statistical significance was determined using ANOVA at the 0.05 and 0.01 levels for germination percentage, MGT, and

GRI. The highest germination percentage was observed with the treatments of sulfuric acid (98%) for 1 hour followed by 250 ppm gibberellic acid (GA₃) for 1 hour, soaking in water for 4 hours followed by chilling at -10 °C for 7 days, and the GA₃ treatment for 1 hour. The lowest germination percentage was observed in the treatment of soaking in water for 4 hours followed by chilling at 4 °C for 24 hours (Figure 3a). Regarding mean germination time, the treatments with sulfuric acid (98%) for 1 hour followed by 250 ppm gibberellic acid (GA₃) for 1 hour, soaking in water for 4 hours followed by chilling at 4 °C for 24 hours, and soaking in water for 4 hours, chilling at -10 °C for 7 days, and GA₃ treatment

for 1 hour ranked the highest, while the lowest was in the treatment with hydrochloric acid (37%) for 6 hours followed by GA₃ treatment for 1 hour (Figure 3b). As for the germination rate index, the highest values were obtained from the treatments with sulfuric acid (98%) for 1 hour followed by 250 ppm gibberellic acid (GA₃) for 1 hour and soaking in water for 4 hours, chilling at -10°C for 7 days, and GA₃ treatment for 1 hour, while the lowest value was observed in the treatment of soaking in water for 4 hours followed by chilling at 4 °C for 24 hours (Figure 3c). Seed germination of *C. spinosa* L. is highly important for ecology, medicine, and economics. For this reason, various researchers have conducted numerous studies on the effects of different pre-treatments and temperature regimes, as well as pre-treatments such as physical and chemical scarification, to facilitate the germination of Capparis seeds, which typically have germination challenges. These studies primarily aim to break seed dormancy, enhance water absorption capacity, and soften the seed coat using various priming techniques. In the current study as shown in Table 3, all sulfuric acid (98%) for 1 hour followed by 250 ppm gibberellic acid (GA₃) for 1 hour, soaking in water for 4 hours, chilling at -10 °C for 7 days, and GA₃ treatment for 1 hour and soaking in hydrochloric acid (37%) for 6 hours followed by GA₃ treatment for 1 hour treatments statistically resulted in the highest germination percentage, which in germination percentage in this level also soaking in water for 4 hours, chilling at -10 °C for 7 days, and GA₃ was the higher than the other ones (%73). The lowest germination percentage related to the soaking in water for 4 hours followed by chilling at 4 °C for 24 hours. In the mean germination time, all three treatments including sulfuric acid (98%) for 1 hour followed by 250 ppm GA₃ for 1 hour, soaking in water for 4 hours followed by chilling at 4 °C for 24 hours, and soaking in water for 4 hours, chilling at -10 °C for 7 days, and GA₃ treatment for 1 hour treatments statistically rank one, which in mean germination time in this level also sulfuric acid (98%) for 1 hour followed by 250 ppm GA₃ for 1 hour was the

higher than the other ones (7.53 days). The lowest value is observed in soaking in hydrochloric acid (37%) for 6 hours followed by GA₃ treatment for 1 hour. And for germination rate index, the highest value obtained from both treatments sulfuric acid (98%) for 1 hour followed by 250 ppm GA₃ for 1 hour (4.89) and soaking in water for 4 hours, chilling at -10 °C for 7 days, and GA₃ treatment for 1 hour (5.14) and the lowest value was in soaking in water for 4 hours followed by chilling at 4 °C for 24 hours treatment. These results are consistent with the findings of Soyler and Khawar (2006), who reported that treatment of *C. spinosa* seeds with 100 ppm GA₃ for 24 hours enhances germination. Similarly, our results align with Fivaz (1971) and Pfister (1974), who demonstrated that soaking *Ribes rotundifolium* seeds in 1N sulfuric acid for 35 minutes improves germination. However, their studies did not examine the combined effect of sulfuric acid treatment and GA₃, which is a key factor in the present research. Chiboub et al. (2024) studied the effect of gibberellic acid (GA₃) and temperature on *C. spinosa* seed germination and reported different results. In their study, various pretreatments, including scarification and seed imbibition in water and gibberellic acid, were tested. Their findings showed that the highest germination rate (68.33%) occurred in control seeds, which contradicts the present study. Additionally, they observed lower germination rates for seeds soaked in water or GA₃ solutions (e.g., 58.33% for seeds soaked in water for 48 hours and 56.67% for seeds soaked in 200 ppm GA₃). In contrast, the lowest germination rate (12.86%) was recorded for seeds soaked in 600 ppm GA₃ under greenhouse conditions. Their results also indicated that seed germination latency was reduced after soaking the seeds in water for 24 hours, which is similar to the findings of the current study, where the germination time was shorter for seeds pretreated with water. In contrast, our study observed that the best results were achieved with specific treatments such as sulfuric acid followed by gibberellic acid, which provided higher germination rates compared to water soaking or other GA₃

treatments. This highlights the complexity of *C. spinosa* seed germination, where different pre-treatments, including acid scarification and GA₃, interact with environmental factors such as temperature and incubation duration. Thus, seed germination of *C. spinosa* is influenced by multiple factors that may vary across different studies, such as treatment types and temperature regimes. Our findings are also in agreement with Seifi et al. (2014), who reported that pre-treatment of Capparis seeds resulted in higher germination rates compared to untreated seeds. Similarly, Orphanos (1983) found that gibberellic acid significantly enhanced germination rates, and soaking seeds in water for 24 hours slightly reduced the lag period. In line with our results, Suleiman et al. (2008) reported that a combination of treatments—scratching seeds with 1% H₂SO₄ for 20 minutes, followed by 0.04% GA₃ and chilling at 4 °C for one week had a positive effect on *C. spinosa* seed germination.

The observed optimal germination at laboratory temperatures (10–30 °C), supported by Lebafi (2018), further strengthens our findings. The germination process in *C. spinosa* seeds appears to be influenced by a series of factors, including membrane permeability, associated protein activity, and cytosolic enzyme activity, as described by Bewley and Black (2013). Moreover, other factors such as plant genotype, culture medium composition, plant growth regulators (PGRs), seed coat characteristics, pre-treatment methods, culture conditions (Mezri et al., 2022), and seed storage duration (Fuschi et al., 2022) have also been shown to impact seed germination in various species. Finally, it is worth mentioning that different treatments may interact in ways that alter germination outcomes, as evidenced by the study of gamma irradiation by Ngoenngam et al. (2019), which enhanced seed germination and seedling growth. These findings further support the idea that seed germination of *C. spinosa* is a complex process, influenced by both external treatments and intrinsic seed characteristics.

4. Conclusion

The present study emphasizes the significance of pre-treatment and temperature conditions in enhancing the germination rate and overcoming the dormancy of *C. spinosa* seeds. The findings demonstrate that germination can be improved by pre-treating seeds and exposing them to temperatures ranging from 4 °C to -10 °C, which are optimal for breaking dormancy, boosting germination capacity, and ensuring uniformity in germination. To further enhance the germination rate, exploring additional hormonal treatments may be beneficial. In conclusion, this study offers valuable insights for farmers, providing practical methods to improve the germination rate of *C. spinosa* seeds, with potential economic and environmental benefits.

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