International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
SJIF (2024): 6.623

Optimizing Germination Protocols for Juniperus Procera: Effective Strategies for Conservation and Restoration in Saudi Arabia's Mountain Ecosystems

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Abstract: **Juniperus procera** ***(African juniper) populations in Saudi Arabia face severe degradation due to climate change, anthropogenic pressures, and inherently low natural regeneration rates. This study evaluated various seed treatments to optimize germination protocols for large-scale seedling production in the Al-Baha region. Seeds were subjected to chemical treatments (sulfuric acid 40-98%, citric acid 5,000-20,000 ppm, sodium hydroxide 5-20%), physical treatments (cold stratification at 4°C, water soaking, mechanical scarification), and their combinations. Seed viability assessment via tetrazolium staining (68%) proved more accurate than flotation testing (83%). The highest germination rates were achieved through water soaking for 24 hours (70 ± 34.6%) and 5,000 ppm citric acid treatment for 24 hours (66.7 ± 15.3%), followed by 70% sulfuric acid for 5 minutes (63.3 ± 20.8%), and cold stratification at 4°C for 6 weeks (56.7 ± 5.8%). Sodium hydroxide treatment and mechanical scarification yielded inconsistent results. These findings provide practical, cost-effective protocols for breaking the complex dormancy of* **J. procera** *seeds, supporting conservation efforts and Saudi Arabia's Vision 2030 sustainability goals. The study highlights the importance of species-specific approaches to optimize germination in arid-adapted conifers, offering valuable insights for ecological restoration in the region's threatened juniper forests.***

Keywords: Juniperus procera, seed dormancy, germination enhancement, Saudi Arabia, ecological restoration, sustainable forestry

1. Introduction

The juniper plant (*Juniperus* spp.), a genus of evergreen gymnosperms, plays a pivotal ecological role in the mountainous regions of Saudi Arabia, particularly within the Sarawat Mountain range spanning from Taif to Jazan. Characterized by dioecious reproduction (separate male and female individuals), junipers dominate the vegetative cover of these high-altitude ecosystems, providing critical habitat stabilization, soil conservation, and biodiversity support. Among the prominent species, *Juniperus procera* (African juniper) and *Juniperus phoenicea* (Phoenician juniper) are ecologically and culturally significant (. *J. procera*, widely recognized for its bluish-purple berry-like cones, contrasts with *J. phoenicea*, which produces smaller brown cones and thrives at elevations of 2,000–3,000 meters. Both species exude a resinous substance with antimicrobial properties, contributing to their ecological resilience (Abo Hassan et al., 1984; Orwa et al., 2009).

Despite their ecological importance, juniper populations in Saudi Arabia face severe degradation due to climate change, anthropogenic pressures (e.g., overgrazing, urbanization), and inherently low natural regeneration rates (<5%) (Helmersson and Von Arnold, 2009). The Al-Baha region, a juniper biodiversity hotspot, has experienced a 30% reduction in forest cover since 1984, exacerbated by seed dormancy mechanisms—a combination of physical (hard seed coat) and physiological (embryo dormancy) barriers (Aref & El-Juhany, 2004; El-Juhany et al., 2008). Natural regeneration is further hindered by disrupted water pathways from infrastructure development, habitat fragmentation, and invasive species (Aref & El-Juhany, 2000).

Previous studies have explored artificial propagation techniques to address these challenges. For instance, cold stratification (3°C for 60 days) and sulfuric acid scarification (40–98% for 5–20 minutes) have improved germination rates to 70% in controlled settings (Laurent & Chamshama, 1987; Chambers et al., 1999). Similarly, citric acid (5,000–20,000 ppm) and mechanical scarification (1–2 mm seed coat punctures) have shown promise, though results vary regionally (Van Haverbeke & Comer, 1985; Scianna, 2001). Despite these advances, scalable, cost-effective protocols for commercial nurseries remain underdeveloped, particularly for *J. procera*, which exhibits complex dormancy traits (Khalofah, 2022; Tylkowski, 2011).

This study aims to identify an optimized seed treatment protocol for *Juniperus procera* that enhances germination efficiency and enables large-scale seedling production. By evaluating synergistic chemical (e.g., citric acid, sodium hydroxide) and physical (e.g., cold stratification, mechanical scarification) treatments, we seek to develop a commercially viable propagation method tailored to the ecological and climatic conditions of the Al-Baha region. The findings will directly support Saudi Arabia's Vision 2030 sustainability goals, addressing juniper population decline and promoting ecological restoration in its native habitats.

2. Materials and Methods

The study was conducted in the research laboratory of the Department of Biology at Qilwah College of Science and Arts. Mature seeds of *Juniperus procera* were collected from natural forests surrounding Baljurashi Governorate, Al-Baha region, Saudi Arabia. Seeds exhibiting a crimson coloration, indicative of full maturity, were selected for experimentation. The experimental design followed a randomized complete block design (RCBD) with the following workflow:

1) **Seed Viability Assessment**

a) **Tetrazolium Test**: Seed viability was quantified using a tetrazolium TTC) test (ISTA, 2017). Embryos staining red were classified as viable, a method proven more accurate than flotation assays (non-viable seeds float in water).

b) **Seed Metrics**:
- **Dimensional Analysis**: Seed length, width, and height were measured using piacolis (mean ± SD, n = 100).
- **Weight-Based Count**: Seeds per gram were determined using a high-precision analytical balance (0.001 g sensitivity).
- **Moisture Content**: Calculated as the percentage weight loss after oven-drying seeds at 105°C for 24 hours.

2) **Experimental Treatments**

To break seed dormancy and induce germination, the following treatments were applied:

a) **Cold Stratification**: Seeds stored at 4°C in moist sterile sand for 2 months.

b) **Cold Water Immersion**: Seeds soaked in distilled water at 4°C for 24 hours.

c) **Acid Treatments**:
- **Sulfuric Acid (H_2SO_4)**: Concentrations of 40%, 70%, and 98% applied for 5, 10, or 20 minutes.
- **Citric Acid ($C_6H_8O_7$)**: Solutions of 5000, 10,000, and 20,000 ppm with immersion durations of 24, 48, or 96 hours.

d) **Alkaline Treatment**: Sodium hydroxide (NaOH) at 5%, 10%, and 20% concentrations applied for 10, 20, or 30 minutes.

e) **Mechanical Scarification**: Seed coats punctured (1–2 mm depth) using a sterilized scalpel.

f) **Control Group**: Untreated seeds germinated under standard conditions.

3) **Germination Protocol**

a) **Growing Medium**: Seeds sown on sterilized wet filter papers in Petri dishes.

b) **Environmental Conditions**: Maintained at 25°C (day)/18°C (night), 60% relative humidity, and a 12-hour photoperiod.

c) **Monitoring**: Germination (radicle emergence ≥2 mm) recorded daily for 8 weeks.

4) **Statistical Analysis**

Data were analyzed using statistical software (IBM and IBM SPSS Inc., 2012). Results expressed as means ± standard deviation (SD). One-way ANOVA and Tukey's HSD post-hoc tests identified significant differences between treatments ($p < 0.05$). Linear regression models assessed trends in germination rates over time.

3. Results and Discussion

a) **Seed Viability**

The study evaluated seed viability using two methods: the flotation test excluded **17%** of seeds, while the tetrazolium staining method (1% concentration) excluded **32%**, demonstrating the latter's superior sensitivity in identifying non-viable embryos ($P < 0.05$). This aligns with established protocols in seed physiology, where tetrazolium staining is favored for its ability to distinguish viable embryos through red coloration of live tissues.

b) **Number of seeds per unit weight**:

100 grams of air-dried seeds were taken from the laboratory and divided into ten groups, each group containing 10 grams. The number of seeds in each group was calculated, with the average number of seeds in each group being 10 grams. (Table 1)

c) **Physical measurements**

3D measurements of 100 seeds using a piacolis device revealed an average length of **4.22 ± 0.42 mm**, width of **2.66 ± 0.48 mm**, and height of **2.23 ± 0.40 mm** (n = 100; Table 2). These dimensions reflect morphological uniformity, though minor variations may arise from genetic diversity or environmental influences.

d) **Moisture Content of seed and fruits**

Moisture content analysis showed **6.21%** in seeds and **4.81%** in fruits, consistent with prior studies on *Juniperus* populations in arid regions of Saudi Arabia. The lower moisture in fruits suggests an adaptive strategy to minimize water loss, enhancing seed longevity in dry habitats.

Table 1: Number of seeds per unit weight

Groups	Weight	Number
Group 1	10 g	694
Group 2	10 g	681
Group 3	10 g	686
Group 4	10 g	690
Group 5	10 g	686
Group 6	10 g	687
Group 7	10 g	688
Group 8	10 g	687
Group 9	10 g	688
Group 10	10 g	691
Mean ± SE	100 g	6892.8 ± 39

Table 2: Three-dimensional seed measurements (length, width, height)

Dimensions (mm)	Mean ± SE
Length	4.22±0.42
Width	2.66±0.48
Height	2.23±0.40

Values: mean ± SD (n = 100).

Germination Response to Chemical Treatments

Sulfuric Acid (H_2SO_4):

Seeds treated with **70% sulfuric acid** for **20 minutes** exhibited the earliest germination (7 days), while the highest germination rate (**63.3 ± 20.8%**) occurred after 8 weeks with a **5-minute exposure** to the same concentration (n = 3 replicates; Table 3). Significant differences were observed between concentrations ($P < 0.05$), but not exposure times ($P > 0.05$). These results mirror findings by Laurent and Chamshama (1987), who attributed sulfuric acid's efficacy to seed coat degradation, facilitating water uptake.

Table 3: Germination rates under sulfuric acid (40%, 70%, 98%) at 5–20 minutes.

Sulphoric acid	98%			70%			40%			Control
Time	5 min	10 min	20 min	5 min	10 min	20 min	5 min	10 min	20 min	
1st week	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2nd week	0±0	0±0	0±0	0±0	0±0	3.3±5.8	0±0	0±0	0±0	0±0
3rd week	0±0	3.3±5.8	0±0	0±0	0±0	13.3±15.3	3.3±5.8	0±0	0±0	0±0
4th week	0±0	13.3±11.5	16.7±5.8	20±17.3	3.3±5.8	20±20	6.7±5.8	0±0	10±10	4.7±4
5th week	0±0	13.3±11.5	16.7±5.8	36.7±20.8	13.3±11.5	23.3±15.3	10±0	3.3±5.8	10±10	8.7±7.5
6th week	0±0	20±10	16.7±5.8	50±20	13.3±11.5	26.7±20.8	10±0	6.7±11.5	13.3±15.3	22±10.1
7th week	6.7±11.5	20±10	16.7±5.8	56.7±25.2	26.7±15.3	26.7±20.8	10±0	6.7±11.5	20±10	42.3±13.6
8th week	10±10	20±10	26.7±5.8	63.3±20.8	33.3±15.3	30±17.3	13.3±5.8	10±10	23.3±11.5	49±10.1

Data: mean ± SD (n = 3).

Citric Acid:

The **5000 ppm citric acid** treatment with **24-hour exposure** produced the highest germination (**66.7 ± 15.3%**) after 7 weeks (n = 3; Table 4). Unlike sulfuric acid, citric acid's chelating properties may neutralize inhibitors in the seed coat. However, exposure time had no significant impact ($P > 0.05$), emphasizing concentration as the critical factor. This contrasts with Van Haverbeke and Comer (1985), who reported optimal germination for *J. virginiana* at **10,000 ppm**, highlighting species-specific responses.

Table 4: Germination response to citric acid (5000–20000 ppm) at 24–96 hours

Citric acid	5000ppm			10000ppm			20000ppm			Control
Time	24h	48h	96h	24h	48h	96h	24h	48h	96h	
1st week	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	10±10	0±0
2nd week	0±0	3.3±5.8	13.3±11.5	0±0	0±0	0±0	0±0	3.3±5.8	20±10	0±0
3rd week	10±10	13.3±5.8	40±10	0±0	10±0	26.7±25.2	13.3±15.3	13.3±5.8	30±10	4.7±4
4th week	40±17.3	30±10	60±17.3	23.3±20.8	26.7±5.8	26.7±25.2	36.7±15.3	20±10	33.3±1.5	8.7±7.5
5th week	46.7±20.8	33.3±5.8	60±17.3	33.3±11.5	40±20	30±20	50±10	30±17.3	33.3±11.5	22±10.1
6th week	46.7±20.8	43.3±15.3	66.7±15.3	46.7±20.8	46.7±23.1	33.3±15.3	60±17.3	43.3±23.1	33.3±11.5	42.3±13.6
7th week	60±17.3	50±20	66.7±15.3	56.7±15.3	53.3±20.8	33.3±15.3	60±17.3	46.7±20.8	33.3±11.5	49±10.1

Data: mean ± SD (n = 3).

Sodium Hydroxide (NaOH):

The **20% NaOH** treatment with **20-minute exposure** triggered germination within 16 days, peaking at **63.3 ± 15.3%** (10% concentration, 30-minute exposure; n = 3; Table 5). However, no significant differences were observed between concentrations or exposure times ($P > 0.05$), indicating limited reliability compared to acidic treatments.

Table 5: Sodium hydroxide effects on seed germination (5–20%) at 10–30 minutes

Na OH	5%			10%			20%			Control
Time	10 min	20 min	30 min	10 min	20 min	30 min	10 min	20 min	30 min	
1st week	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
2nd week	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
3rd week	3.3±5.8	3.3±5.8	3.3±5.8	10±10	13.3±5.8	0±0	3.3±5.8	16.7±11.5	6.7±5.8	4.7±4
4th week	23.3±20.8	26.7±15.3	16.7±11.5	33.3±11.5	26.7±11.5	36.7±15.3	13.3±5.8	30±10	23.3±20.8	8.7±7.5
5th week	26.7±15.3	33.3±11.5	30±10	36.7±15.3	36.7±20.8	43.3±15.3	23.3±11.5	40±17.3	30±30	22±10.1
6th week	36.7±25.2	33.3±11.5	33.3±11.5	40±10	40±26.5	53.3±15.3	43.3±15.3	50±10	40±26.5	42.3±13.6
7th week	63.3±11.5	40±17.3	53.3±15.3	53.3±5.8	40±26.5	63.3±15.3	46.7±15.3	60±0	46.7±25.2	49±10.1

Data: mean ± SD (n = 3).

Stratification and Physical Treatments

Cold Stratification:

Stratifying seeds at **4°C for 6 weeks** yielded **56.7 ± 5.8%** germination, significantly higher than the control ($P < 0.05$; n = 3; Table 6). This aligns with Scianna (2001), who emphasized cold stratification's role in breaking physiological dormancy. Stratifying cones (fruits) showed minimal improvement, suggesting dormancy mechanisms are seed-specific.

Table 6: Cold stratification outcomes (seeds and cones at 4°C)

Stratification	Cooled seed	Cooled cones	Control
1st week	0±0	0±0	0±0
2nd week	6.7±5.8	0±0	6.7±5.8
3rd week	36.7±15.3	0±0	6.7±5.8
4th week	53.3±5.8	6.7±11.5	10±0
5th week	56.7±5.8	10±10	13.3±5.8
6th week	56.7±5.8	10±10	13.3±5.8

Data: mean ± SD (n = 3).

Soaking and Mechanical Scarification:

Soaking seeds in distilled water for **24 hours** achieved the highest germination (**70 ± 34.6%**) after 8 weeks (n = 3; Table 7), likely due to rehydration of desiccated tissues. Mechanical scarification yielded **26.7 ± 25.2%**, inconsistent with El-Juhany et al. (2008) and Khalofah (2022) reports of 71% and 47% germination, respectively. This possibly due to differences in scarification intensity or seed batch variability.

Table 7: Germination rates for soaked and scarified seeds.

Treatment	Soaked seeds in water	Scratched seeds	control
1st week	0±0	0±0	0±0
2nd week	0±0	0±0	4.7±4
3rd week	6.7±5.8	0±0	8.7±7.5
4th week	16.7±5.8	3.3±5.8	22±10.1
5th week	33.3±11.5	13.3±15.3	42.3±13.6
6th week	50±26.6	13.3±15.3	49±10.1
7th week	63.3±30.6	20±17.3	51.3±7.5
8th week	70±34.6	26.7±25.2	51.3±7.5

Data: mean ± SD (n = 3).

Comparative Analysis of Treatments

Soaking and citric acid treatments outperformed other methods, with sulfuric acid and cold stratification showing moderate efficacy. Sodium hydroxide and mechanical scarification were less reliable, underscoring the need for species-specific protocols.

4. Conclusion

The highest germination rates for *Juniperus* seeds were achieved through soaking in water (70%) and citric acid treatment (5000 ppm). These methods effectively address physical and physiological dormancy, offering practical solutions for conservation and propagation. While sulfuric acid and stratification showed moderate success, sodium hydroxide and mechanical scarification were less reliable. These results underscore the importance of selecting species-specific treatments to optimize germination in arid-adapted conifers.

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