

## Acclimatization of *Argania spinosa* in Thal Desert of Pakistan: Unlocking Its Potential for Climate Resilience

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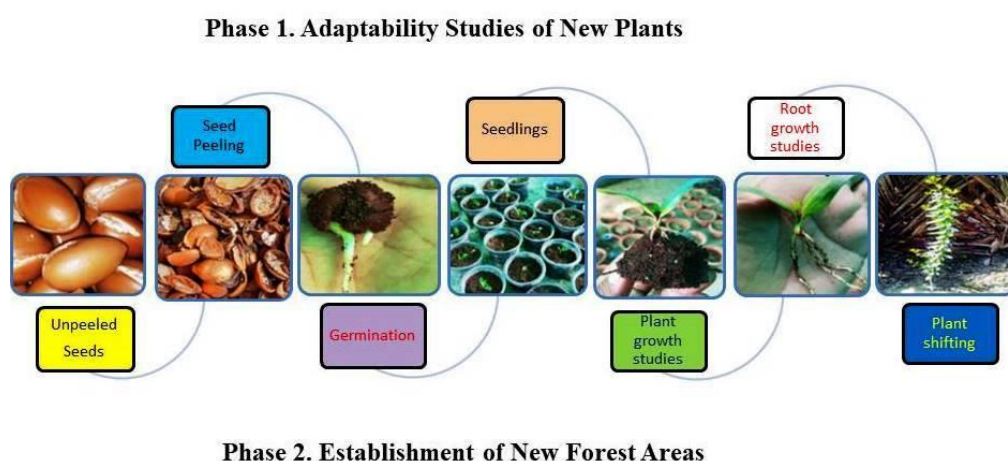
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### ABSTRACT

Argan (*Argania spinosa*) oil is used for culinary, cosmetics, and medicinal purposes worldwide. The purpose of study is to enhance oil production and local farmer's earnings. Initial attempts were made to introduce it into the arid regions of Pakistan. Effects of different seed sowing techniques and soil types on plant growth parameters were evaluated throughout the year. Seed sowing techniques were compared based on their impact on germination (%), germination period (days), plant growth (cm), plant height at 45 and 90 days after germination (DAG), number of leaves at 45 and 90 DAG, root length at 45 and 90 DAG and survival rate with different combinations of soil types (sandy, loamy, organic matter, farm yard manure, coconut peat, sand: peat and sand: organic matter (1:1)). Results revealed that seed cracking and soaking for 96 hours and improved germination rates (59.2 and 38.6%), reduced germination periods (14 and 26 days), better overall plant growth compared and enhanced survival rate of 75.16 and 60.18%, respectively as compared to un-soaked and un-cracked seeds. Coconut peat showed the most favorable conditions for seedling establishment and early growth with highest germination (71.65%), survival rate (85.62%), lowest germination period (10 days) and the lowest seed deterioration (28.64%) among all treatments. Coconut peat had the highest plant growth rate per day of 0.36 cm/day, followed by sandy soil (0.23 cm/day) and the lowest growth rate exhibited by loamy soil of 0.10 cm/day during the suitable environmental conditions. Seed germination and plant growth are strong negative correlation with the higher temperatures (>30°C) and lower humidity. Overall, argan plant have shown good potential for its growth and climate resilience in Thal area.

**Keywords:** Argan nuts, arid climate, edible oil, medicinal plant.

### Graphical Abstract



## INTRODUCTION

Argan tree (*Argania spinosa* L.) is a member of family *Sapotaceae*, which is an endemic specie of Morocco forest ecosystem, capable of reaching heights of 8-10 meters under arid and hot conditions. They are native to the semi-desert regions of Southwestern Morocco covering over 8,000 km<sup>2</sup> and Algeria (Zohra *et al.*, 2014) across various altitudes from sea level up to 1,500 m (Charrouf & Guillaume, 2009; Mateille *et al.*, 2016). It serves multiple purposes, primarily as a source of fodder and oil production (Chakhchar *et al.*, 2020). The argan tree produces small, oval-shaped fruits, roughly the size of an olive, which takes about a year to mature. Each fruit contains oval shaped somewhat similar to almond-shaped nuts surrounded by unpleasant-tasting pulp. These nuts can be yields into oil, which are a rich source of vitamin E, oleic acid, linoleic acid, palmitic acid and stearic acid (Sadiqqi *et al.*, 2021). Oil extracted from these nuts is known for its nutritional, medicinal as well as cosmetic qualities (Charrouf *et al.*, 2008). In Berber culture, argan trees is widely utilized as feed for livestock and produce oil (Nouaim *et al.*, 2002). Like other plants as olive, walnut or almond tree, the fruit of the argan tree is a stone-fruit, distinguished by pulp that encompass a hard endocarp. Within this argan nut, one to three kernels can be found, from which edible oil is extracted (Nouaim *et al.*, 2002). Except this their socio-economic significance, The argan plays a crucial role in environmental protection owing to their deep root systems enabling them to absorb nutrients and water from poor as well as arid soils (Justamante *et al.*, 2017). Despite its significance, low seed germination and early seedling development are impetuous in multiplication and establishment of viable argan plants. These practices are strongly affected by several environmental factors i.e., light duration, temperature as well as moisture contents (Benaouf *et al.*, 2016). Propagation of argan plants through seeds is challenging and not commercially inefficient due to number of factors like as variations

in seed size, viability and dormancy (Miloudi & Belkhdja, 2009).

Temperatures between 25 and 28 °C is ideal for argan nuts germination (Elmandouri *et al.*, 2020). According to Alouani & Bani-Aameur, (2003), this process should take place over a photoperiod of 16 hours of light and 8 hours of darkness. Fresh harvested seeds are more viable. Whereas, after a storage of one or more year, there viability significantly reduces, which leads to a decline in germination percentage ranging from 76% to 70%. With each additional year of storage, germination percentage usually reduces by approximately 6% (Hamani *et al.*, 2018). However, it's crucial to remember that the germination rate might changes depending on number of variable including origin of the mother plants, the date of fruit harvest (Hamani *et al.*, 2018), on the other hand duration of storage also (Azizi *et al.*, 2022). As the exocarp of argan nuts is hard shells which hinder their germination under natural conditions. Most of the studies had shown that these trees can be propagated by vegetative means using various nursery and in vitro techniques (Bellefontaine *et al.*, 2010). For instance, imbibition and gibberellic acid treatments have been found to be successful (Ikinici, 2014), along with mechanical scarification. However, chemical pretreatments such as those involving acetic acid and hydrogen peroxide appear to have an inhibitory effect on germination. The efficacy of these pretreatments is influenced by the origin of the nuts (Zunzunegui *et al.*, 2013). Developing simple and practical methods that combine mechanical, chemical and physical pretreatments is essential for breaking the dormancy of argan seeds and improving their establishment during the nursery stage. The climatic conditions of Thal in Bhakkar are suitable for the introduction and propagation of argan tree. Therefore, a series of efforts were made to grow argan tree through seeds under various growing techniques and media for its adoptability throughout the year to find out suitable time and conditions.

## MATERIALS AND METHODS

Study was conducted during 2022-23 at Arid Zone Research Institute (31.6344° N, 71.1202° E), Bhakkar, Pakistan with an aim to introduce argan plant in Thal desert.

### Medium Preparation

*Argania spinosa* seeds were sourced from online stores like ebay and itsy. Healthy and disease free seeds were selected for germination. A variety of growing media mixes, including sandy loam, organic matter, coconut peat, farmyard manure, sand + peat (1:1), and sand + organic matter (1:1), were examined during this project. There were various stages to the

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study strategy. In phase one, the seeds were classified as cracked, peeled, and unpeeled, while in phase two, the seeds were classified as soaked and un-soaked.

**Sowing Techniques**

Unpeeled seeds with no soaking, unpeeled seeds with soaking for 48 hours, unpeeled seeds with soaking for 96 hours, cracked seeds without soaking, and peeled seeds without soaking each with three replications. For the seed sowing techniques there were three replications for following treatments: cracked seeds without soaking, peeled seeds without soaking, unpeeled seeds without soaking, unpeeled seeds with soaking for 48 hours, and unpeeled seeds with soaking for 96 hours.

**Seedling Preparation**

The peeled, un-peeled and cracked seed were sown in above given media mixtures filled in plastic pots (4 × 4 inches depth: width). Seeds were sown 1-2 cm below the media surface covered with sand and irrigated with an interval of two days. Different parameters i.e., germination (%).

The cracked, peeled, and unpeeled seeds were planted in the above Peeled, unpeeled, and cracked seeds were seeded in prepared media combinations. All media mixtures were placed in plastic pots that were 4 × 4 inches deep and 4 inches wide. The seeds were planted 1-2 cm below the sand-covered media surface in plastic pots (4X4 inches). The seeds were then watered every 2-3 days to maintain optimum moisture conditions for seed germination. Various factors, such as the effects of these various treatments, were evaluated based on the percentage of germination. The various parameters such as seed germination (days), seed deterioration (%), seedling survival rate (%), plant growth (cm) per days and monthly, plant height and number of leaves at 45 and 90 days after

germination (DAG) and root length at 45 and 90 DAG Figure 1. Plant propagation from seeds were recorded. Effects of temperature (°C) and humidity (%) on seed germination and plant growth were tested throughout the year to find out best condition for the seed germination, plant growth and development.

**Statistical Analysis**

ANOVA followed by Tukey's HSD test was conducted to compare the means of different seed sowing techniques for all parameters measured. A significance level of  $p < 0.05$  was used by using statistics software 8.1 (Steel & Torrie, 1960). Pearson correlation coefficients were calculated to determine the relationships between temperature, humidity, seed germination, and growth rate (White *et al.*, 2016). Linear regression models were fitted to assess the impact of temperature and humidity on seed germination and growth rate over time by using Minitab 18.1 (Barbara & Slovin, 2024).

*Plant Growth Rate (cm)*

$$= \frac{1st\ Measurement - 2nd\ Measurement}{Days\ Between\ two\ Measurements}$$

*Seed Deterioration (%)*

$$= \frac{Total\ Seed\ Sown - Total\ Germinated\ Seeds}{Total\ Seed\ Sown} \times 100$$

*Plants Survival (%)*

$$= \frac{Total\ Plants - Plants\ Dried}{Total\ Plants} \times 100$$



Figure 1. Plant propagation from seeds

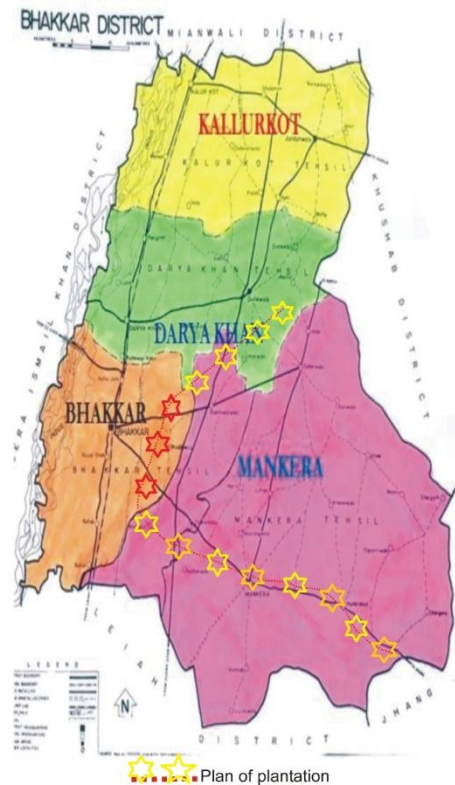


Figure 2. Plan of tree plantation in Thal area of Bhakkar

## RESULTS AND DISCUSSION

Effects of various seed sowing techniques on plant growth parameters are listed under table 1. Each technique is evaluated based on its impact on percent germination, germination days, plant height at 45 and 90 days after germination (DAG), number of leaves at 45 and 90 DAG, root length at 45 and 90 DAG and survival rate. Unpeeled seeds with no soaking technique yielded germination of 18.69% with 35 days germination period. The resulting plant height at 45 and 90 DAG was 8.96 cm and 11.69 cm, respectively, with 15 leaves at 45 DAG increasing to 27 at 90 DAG. Root length also increased from 6.32 cm at 45 DAG to 18.63 cm at 90 DAG with a survival rate of 65.38%. When the unpeeled seeds were soaked for 48 hours, the percent germination increased to 32.64% with a shorter germination period of 31 days. This led to a plant height of 9.64 cm at 45 DAG and 12.72 cm at 90 DAG, with 17 leaves at 45 DAG increasing to 30 at 90 DAG with a survival rate of 59.61%. the root length also rose from 5.69 cm at 45 DAG to 21.69 cm at 90 DAG. With a germination rate of 38.57% and a germination period of 26 days, the results were further enhanced by extending the soaking time to 96 hours. This resulted as with 16 leaves at 45 DAG and 38 at 90 DAG, the plants height was 10.27 cm at 45 DAG and 14.38 cm at 90 DAG. With a survival rate of 60.18%, the root length also rises from 8.19 cm at 45 DAG to 25.37 cm at 90 DAG on the other hand, the

cracked seeds that were not soaked exhibited quickest germination time of 14 days and the highest germination percentage of 59.22 % with 18 leaves at 45 DAG and 40 at 90 DAG, the plants height was 8.72 cm at 45 DAG and 13.44 cm at 90. At 45 DAG, root length was 10.55 cm; at 90 DAG it was 28.34 cm, and the survival rate was 75.16%. The lowest germination percentage (15.13%) and germination period (18 days) were observed in the peeled seeds that were not soaked. With 14 leaves at 45 DAG and 21 at 90 DAG, the plants height was 7.65 cm at 45 DAG and 9.85 cm at 90 DAG. With the lowest survival rate of 35.82% the root length also rose from 4.92 cm at 45 DAG to 7.74cm at 90 DAG. Seed sowing techniques on plant growth parameters, with soaking and cracking seeds leading to improved germination rates, shorter germination periods and better overall plant growth compared to un-soaked and un-cracked seeds. These findings are valuable for optimizing seed sowing practices to enhance plant growth.

Table 1. Effect of different seed sowing techniques on plant growth parameters

Seed Sowing Techniques	GP ± S.E	GT± S.E	PH (cm) 45 DAG ± S.E	PH (cm) 90 DAG ± S.E	NOL 45 DAG ± S.E	NOL 90 DAG ± S.E	RL (cm) 45 DAG ± S.E	RL (cm) 90 DAG ± S.E	Survival Rate (%) ± S.E
Unpeeled Seeds-No Soaking	18.69 ±1.2c	35± 0.7a	8.96±0.5a	11.69±0.3a	15±0.2	27±1.5b	6.32±0.2b	18.6±1.6b	65.38±3.6b
Unpeeled Seed-Soaking 48 HRS	32.64 ±0.9b	31± 1.2ab	9.64±0.9a	12.72±0.6a	17±1.1	30±2.6b	5.69±0.5b	21.7±2.5b	59.61±2.8b
Unpeeled Seed-Soaking 96 HRS	38.57 ±3.7b	26± 0.6c	10.27±0.3a	14.38±0.1a	16±0.6	38±3.9a	8.19±0.9a	25.4±0.7a	60.18±5.7b
Cracked Seeds-No Soaking	59.22 ±4.2a	14± 1.3de	8.72±0.7a	13.44±0.8a	18±0.5	40±2.8a	10.5±0.7a	28.3±0.9a	75.16±6.2a
Peeled Seeds-No Soaking	15.13 ±0.6c	18± 0.8d	7.65±0.2b	9.85±0.4b	14±0.3	21±1.5c	4.92±0.3c	7.7±0.3c	35.82±3.1c

Where GP: Percent germination, GT: Growth time (day), PH: Plant height, NOL: No of leaves, RL: Root length, DAG: Days after germination.

The impact and performance of various soil types on seed germination, seedling establishment and early plant growth has described in table 2. The findings indicate that sandy soil demonstrates a moderate percent germination of 40.89% and a relatively short germination period of 17 days. However, the seed deterioration percentage was measured at 25.6%. On the other hand loamy soil exhibits a lower percent germination (33.64%) and a longer germination period of 24 days, indicating less favorable conditions for seed germination compared to other soil types, with a higher seed deterioration percentage of 41.9%. Organic Matter treatment showed higher percent germination (51.34%) and a shorter germination period (14 days), indicating favorable conditions for seed germination and early growth. However, the seed deterioration percentage was high (60.45%). Farm yard manure demonstrated 59.63% seed germination and germination period of 15 days. However, it has a

lower survival rate of 36.54% as compared to other treatments, indicating challenges in seedling establishment and early growth. In contrast, Coconut Peat showed highest seed germination (71.65%), survival rate (85.62%), lowest germination period (10 days) and the lowest seed deterioration (28.64%) among all treatments, indicating the most favorable conditions for seedling establishment and early growth. Additionally, combinations like sand+peat (1:1) and sand+organic matter (1:1) exhibited germination of 62.64% and 45.81%, relatively short germination periods (13 and 16 days) and survival rates (73.68% and 44.38%). In terms of plant growth rate per day, coconut peat had the highest rate at 0.36 cm, followed by sandy soil (0.23 cm), sand+peat (0.21 cm), farm yard manure (0.19 cm), organic matter (0.15 cm), sand+organic matter (0.14 cm) and loamy soil (0.10 cm).

Table 2. Impact of various soil types on plant growth parameters

Soil Types	Germination (%) ± S.E	Germination Time (Days) ± S.E	Seed Deterioration (%) ± S.E	Survival Rate (%) ± S.E	Plant Growth Rate (cm) ± S.E
Sandy Soil	40.89±2.3c	17±0.4ab	25.60±0.9d	75.63±3.7ab	0.23±0.01b
Loamy Soil	33.64±1.6d	24±1.3a	41.90±3.5c	55.31±5.6bc	0.1±0.00d
Organic Matter	51.34±3.6b	14±0.3bc	60.45±5.7ab	62.64±2.1b	0.15±0.02c
Farm Yard Manure	59.63±1.7b	15±1.3b	76.92±6.3a	36.54±3.6d	0.19±0.03bc
Coconut Peat	71.65±4.5a	10±0.2cd	28.64±3.4d	85.62±4.9a	0.36±0.10a
Sand + Peat	62.64±5.1ab	13±0.6bc	36.51±2.8cd	73.68±3.2ab	0.21±0.07b
Sand + Organic Matter	45.81±3.6c	16±0.7b	51.12±7.6b	44.38±1.7c	0.14±0.03c

The performance of seed germination and plant growth throughout the year under different environmental conditions is described in Figure 2. January marked a low point in seed germination at 5.25%, with a relatively slow growth rate of 0.59 cm and a cool temperature of 15.64°C. As the year progressed, February showed improvement in germination at 17.45%, with a higher growth rate of 1.5 cm and a slightly warmer temperature of 17.69°C. March demonstrated significant progress in germination of 36.54% and growth rate 2.3 cm. April maintained relatively high germination at 22.69% and a growth rate of 1.6 cm, despite a rise in temperature to 26.48°C. A decline in both germination and growth rate of 8.63% and 0.4 cm was observed in May at 29.64°C. June and July experienced minimal germination and growth 0.42: 0.31% and 0.1 cm and no growth, respectively. October exhibited a notable increase in germination at 21.63% with growth rate of 0.9 cm due to continued temperature drop to 29.42°C.

November marked a significant peak in germination of 42.94% and a growth rate of 2.6 cm. December maintained relatively high germination at 17.34% and a growth rate of 1.4 cm, with a further drop in temperature to 18.41°C. The correlation coefficient between temperature, seed germination and growth rate indicating a significant negative correlation as shown in table 3. As temperature increases, seed germination and growth rate tends to decrease. While humidity indicating a significant positive correlation. Increases in humidity increases seed germination growth rate. Temperature had 18.95 and 25.0% impact on per unit change in germination and growth rate of argan plants. While humidity had 75.96 and 75.0% impact on per unit change in germination and growth rate. Overall, the results suggest that higher temperatures of more than 30°C and lower humidity negatively impact seed germination and plant growth, with the best performance observed in cooler months with higher humidity.

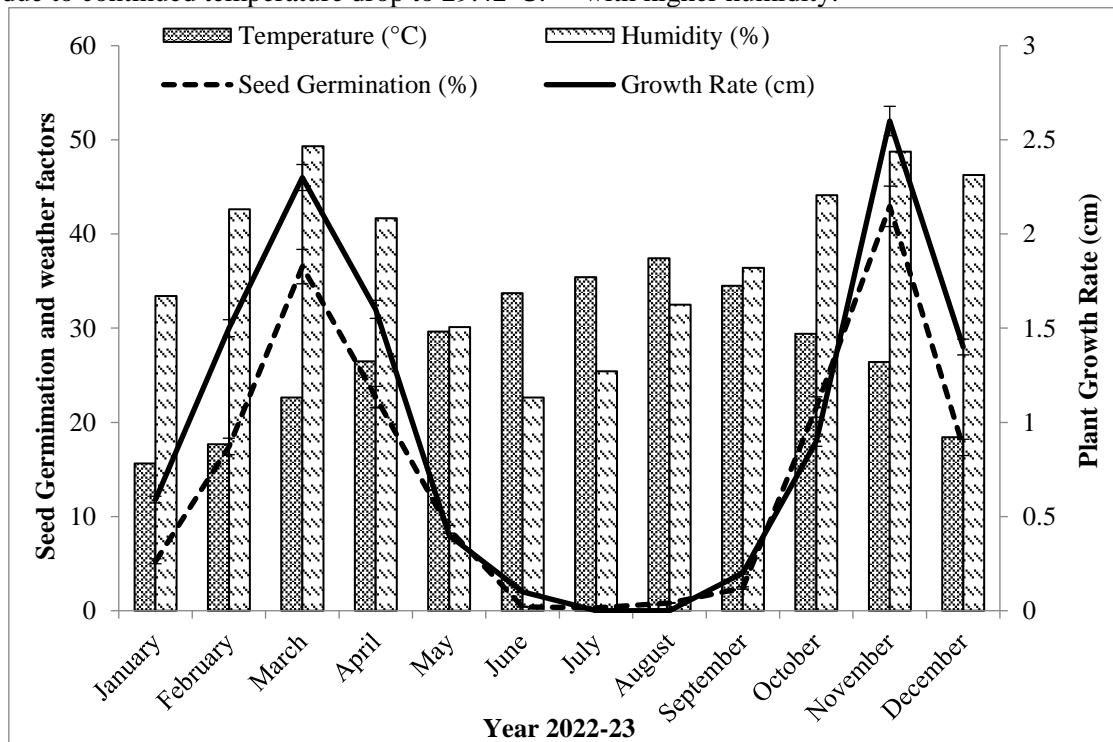


Figure 2. Performance of seed germination and plant growth

Table 3. Correlation and regression analysis of plant growth parameters with weather factors

Growth Parameters	Temperature (°C)	Humidity (%)
Seed Germination (%)	-0.435 (0.00) $Y_1 = 37.8 - 0.848(X = 18.95\%)$	0.872 (0.00) $Y_1 = -37.87 + 1.392(X = 75.96\%)$
Growth Rate Monthly (cm)	-0.584 (0.05) $Y_2 = 2.913 - 0.0714(X = 25.0\%)$	0.879 (0.00) $Y_2 = -2.364 - 0.882(X = 75.0\%)$

Where Y<sub>1</sub>: Seed germination, Y<sub>2</sub>: growth rate, X: Impact (%)

The most challenging phase in argan production is breaking the seed dormancy. They noted that the physical characteristics of the seeds pose complications for germination, suggesting that achieving a 30% germination rate would be considered a good result for argan seeds (Al-Menaie *et al.*, 2008). While removing the seed coat helped quicker moisture absorption by the seed, but it is more vulnerable to pathogenic attacks, resulting in deterioration during germination. Consequently, the seed coat was deemed essential for protecting against fungal attacks (Hamani *et al.*, 2018). Soaking seeds in water for four days before germination facilitates water absorption and accelerates the germination process. These effects are linked to enzyme activation as well as the swelling and softening of the seed coat Qadir *et al.* (2012). Cracked seeds without soaking exhibited the highest percent germination at 59.22% and the shortest germination period of 14 days. But sowing seeds in this way, there is always chance of fungal attacks. When the unpeeled seeds were soaked for 48 hours, the percent germination increased to 32.64% with a shorter germination period of 31 days, with a survival rate of 59.61%. Extending the soaking period to 96 hours further improved the results, with a percent germination of 38.57% and a germination period of 26 days, with a survival rate of 60.18%. Several scientists confirmed our findings to soak seeds for 96 hours before sowing for best germination. Soaking seeds for a minimum of four days resulted in a successful germination rate of 95% for seeds soaked in water for 96 and 120 hours at temperatures ranging from 30°C to 25°C. Seeds soaked for 120 hours at a temperature of 22°C exhibited germination rates between 50% and 70% (Benaouf *et al.*, 2016). Al-Menaie *et al.* (2007) achieved 16-30 % seed germination after soaking in freshwater and 44 % in no soaking. Zunzunegui *et al.* (2013) found 30-55 % seed germination after presoaking for 24 hours in water. Alouani and Bani-Aameur (2003) reported *Argania spinosa* seed germination under nursery conditions with mean germination period of 13.4 days and seed deterioration of 30.1 % during cold storage. In a study by Ikinici (2014), argan seeds were subjected to pre-sowing soaking durations of 24, 48, 72, and 96 hours. The highest germination rate (56.67%) was observed in argan seeds that had been soaked in water for 96 hours prior to sowing. Miloudi and Belkhodja (2009) found 55% germination rate with argan seeds that had been soaked for 120 hours and germinated at 25°C. However, they noted that an even higher germination rate (70%) was observed in seeds soaked for 96 hours and germinated at 30°C. In our findings the root length and plant height in different treatments were ranged from 7.7-28.3 and 9.85-14.38 cm, respectively. These findings are at par with Zohra *et*

*al.* (2014), who soaked the seeds for 96 hours resulted in more than 95% seed germination. After two weeks, the seedlings reached a height of 0.8 cm, which increased to 6.5, 18, and 40 cm after one, two and nine months, respectively. Moreover, the root length of the seedlings was 3 cm after two weeks, 8, 29 and 35 cm after one, five and nine months, respectively. These results differed from Nouaim *et al.* (2002) who reported total root length of 15 to 60 times that of the shoot length. After 38 days, the shoot lengths were 12.5-8 cm, corresponding to root lengths of 48-53 cm, respectively. This difference was due to different ecological conditions for the adoptability. Different planting media are very important for the germination and plant growth. Coconut Peat showed highest seed germination (71.65%) and survival rate (85.62%), indicating the most favorable conditions for seedling establishment and early growth. Additionally, combinations like sand+peat (1:1) and sand+organic matter (1:1) also exhibited satisfactory germination of 62.64% and 45.81% and survival rates (73.68% and 44.38%), respectively. These findings confirmed by Al-Menaie *et al.* (2008) who evaluated various techniques for seed germination in Kuwait. Best treatments sand: peat moss: humus (2:1:1) gave seed germination (16 and 56 %), root length (16.3 and 19.9 cm) and shoot length (3.3 and 3.2 cm) under no soaking and water soaking technique, respectively. Elmandouri *et al.* (2020) conducted tests to assess the germination of argan seeds in various substrates (sand, peat, and a 1:1 mixture of sand and peat) with the aim of developing efficient propagation protocols for this plant. The results indicated that using the 1:1 mixture of sand and peat as a substrate significantly improved the germination rate of argan seeds from 60-70%. In the current findings average plant growth was ranged from 0.10-0.36 cm/day in various treatments. This was in confirmity with Azizi *et al.* (2022) who propagated *Argania spinosa*. He concluded 75 % seed germination at 25 °C, 0.36 cm plant growth and 5.96 leaves/week. In our region the months February-April and October-December were favorable for the seed germination and plant growth. These conditions are variable in different ecological zones. According to Hamani *et al.* (2018), the germination percentage ranged from 53.3-86.7%, varying with the month and year of harvest. The germination time varied from 2 to 12 days. The study noted that seeds harvested in June exhibited the highest frequency of early germination of 20.83-35.83% with 10-day periods of germination.

## CONCLUSION

The study results indicate that soaking and cracking seeds significantly enhance germination rates, reduce germination periods and enhance overall plant growth. Additionally, coconut peat proved to be the most

effective soil medium for seedling establishment and early growth, exhibiting the highest germination percentage, survival rate, and lowest seed deterioration compared to other soil types. Optimum temperature (20-30°C) temperature and humidity levels (50-65 %) were found to positively influence seed germination and plant growth. These findings provide practical insights for introducing the Argan plant in Thal desert by implementing appropriate seed growing techniques and selecting the most suitable soil types for improved adoptability. The successful establishment and cultivation of Argan in Arid region will not only enhance oil production and local farmer's but it will also contribute to ecological restoration and climate change resilience in arid regions.

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**Data Availability** Not applicable.

#### Declarations

**CONFLICT OF INTEREST** The authors report there are no competing interests to declare.

**Author contribution** M.A. and S.Y.A. conducted research and wrote the manuscript. M.T.J. revised and edited the manuscript. M.A. and N.H. made a research plan, A.G., M.N., and S.A. reviewed the literature and manuscript.

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