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Research Article

Analysis of Physical Dormancy-Breaking in *Andrographis paniculata* (Burm.f.) Nees

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ABSTRACT

Seed dormancy is a major constraint to uniform germination and crop establishment in *Andrographis paniculata* (Burm.f.) Nees (*A. paniculata*), resulting in low germination. *A. paniculata* is a member of the Acanthaceae family and is propagated via seeds. However, it exhibits poor germination and dormancy. The purpose of this study is to evaluate the effectiveness of two commonly used methods, hot water treatment and sandpaper scarification, on *A. paniculata* seeds. This study aims to optimize seed dormancy-breaking methods and identify a simple yet efficient germination approach useful for both farmers and researchers. Both treatments were selected based on previous reports indicating their potential to enhance germination. To ensure consistency, the procedure is tested under controlled conditions in a plant growth chamber, following established protocols. Germination parameters, including final germination percentage (FGP) and mean germination time (MGT), were recorded. Results indicated that sandpaper scarification significantly enhanced germination, achieving an average FGP 93%, compared with hot water treatment (18.4%) and the control (7.2%) across all accessions. These preliminary findings showed that sandpaper scarification with a moistened tissue towel is an effective method for overcoming physical dormancy in *A. paniculata* seeds.

1. Introduction

Andrographis paniculata (Burm.f.) Nees, a medicinal plant of the Acanthaceae family, is known as the "King of Bitters" due to its bitter taste. In Malaysia, it is known as Hempedu Bumi (Vikram *et al.* 2015; Detpiratmongkol *et al.* 2017). It is widely distributed across tropical Asian countries, including India, Sri Lanka, Thailand, Indonesia, and Malaysia. Traditionally, this well-known Malay ethnomedical plant is used to treat a wide range of ailments, including fever, skin eruptions, ulcers, boils, and the common cold (Yang *et al.* 2020; Jacob *et al.* 2021). Its therapeutic properties are largely attributed to andrographolide and related diterpenoid compounds (Tan *et al.* 2016; Patuhai *et al.* 2023), which exhibit pharmacological properties, including anti-inflammatory, antioxidant, antimalarial, anti-diabetic, anti-hypertensive, anti-leukemia, respiratory conditions, and anticancer activity (Singh and Sehgal 2018; Pandey *et al.* 2019; Mussard *et al.* 2020; Murthy *et al.* 2021; Joshi *et al.* 2022). Recent studies have also demonstrated that *A. paniculata* contains compounds with inhibitory activity against COVID-19, further reinforcing its medicinal importance. Therefore, the pharmaceutical industry is in high demand for this plant.

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Propagation of *A. paniculata* is commonly carried out using seeds due to their cost-effectiveness and suitability for large-scale cultivation. However, seed germination is generally low, even for freshly harvested seeds, and many young seedlings fail to survive under natural conditions (Talei *et al.* 2012a; Vikram *et al.* 2015; Gundala *et al.* 2023). Seed longevity is also limited, especially in regions with high humidity and temperature, which may accelerate aging and reduce viability (Hozayn *et al.* 2015; Nolasco-Guzman *et al.* 2016). Furthermore, substantial variation in germination rates has been observed among different seed lots and accessions, indicating inherent biological differences within the species (Tong *et al.* 2020; Promwee *et al.* 2023). Despite these limitations, seed propagation remains preferred because seedlings produced from seed are more resilient, long-lived, and resistant to drought and disease (Nolasco-Guzman *et al.* 2016).

Dormancy is an intrinsic germination-preventing property of plant seeds that requires external stimulants to overcome. Physical dormancy-breaking methods, such as scarification, have been shown to improve germination in *A. paniculata*. Talei *et al.* (2012b) reported that sandpaper scarification resulted in faster and higher germination, while Kumar *et al.* (2011b) reported that soaking seeds in 50°C water for five minutes increased the germination. Optimal germination has also been reported at 25°C under high humidity conditions (Kumar *et al.* 2011a; Shruti *et al.* 2023). Hence, the experiment will employ these two methods to compare them, as they yield the highest percentage of germination.

Although extensive studies on *A. paniculata* seed dormancy exist, this preliminary study focuses specifically on comparing the most effective physical dormancy-breaking methods under standardized conditions. This study was conducted as a preliminary evaluation to improve seed germination performance in five *A. paniculata* accessions by applying physical dormancy-breaking treatments under optimal conditions. Our primary goal was to identify a simple, direct, and non-chemical method to improve seed germination and ensure reliable seedling establishment.

2. Materials and Methods

2.1. Seed Source

The seeds of five *Andrographis paniculata* (Burm.f.) Nees accessions were collected from nurseries in five different states in Malaysia: Kuala Lumpur (KL), Perak (PE), Selangor (SL), Negeri Sembilan (NS), and Malacca (ML). For this experiment, seeds were visually selected to be uniformly light brown to brown, excluding dark brown or physically damaged seeds. Fifty seeds per accession were used as replicates, with two replicates per treatment due to limited seed availability. The seeds were stored in a sealed plastic bag at 4°C until the experiment was carried out at the Agro Gene Bank, University Putra Malaysia, Serdang, Selangor, Malaysia. Representative images of the seeds from five accessions are shown in Figure 1.

2.2. Physical Dormancy-Breaking

Two dormancy-breaking treatments were applied, selected based on previously reported optimal conditions:
1. Hot water treatment: seeds were soaked at 50°C water for 5 minutes, as identified as optimal by Kumar *et al.* (2011b).
2. Sandpaper scarification: seeds were evenly spread on a P120 sandpaper surface placed on a flat base and covered with a second sheet of sandpaper, forming a sandwich-like arrangement. Gentle pressure was applied using the palm while the upper sandpaper was moved slowly in a circular motion for five rotations, following Talei *et al.* (2012b)

Untreated seeds were used as the control. These methods were chosen because they represent simple, non-chemical, and practical approaches suitable for a preliminary screening. This study compared their effectiveness across five accessions under identical optimal conditions.

2.3. Seed Germination

Fifty seeds per accession per replicate were evenly spread on a moistened tissue towel, which was then folded into two to cover the seeds and maintain consistent moisture. The moistened folded tissue towels were placed in a sealed bag and incubated in a growth chamber at 25°C and 60% relative humidity, under a 16-hour photoperiod of light and an 8-hour photoperiod of darkness.

Every three days, the moistened tissue towels inside sealed bags were opened on a clean table to observe and count germinated seeds. Seeds were considered germinated upon radicle emergence. The moistened tissue towels were replaced as needed to maintain moisture and prevent contamination. Seedlings were gently transferred using forceps to avoid damaging the radicles. Radicle length was not measured because physical treatments did not damage seedlings, and all germinated seeds were allowed to continue growing.

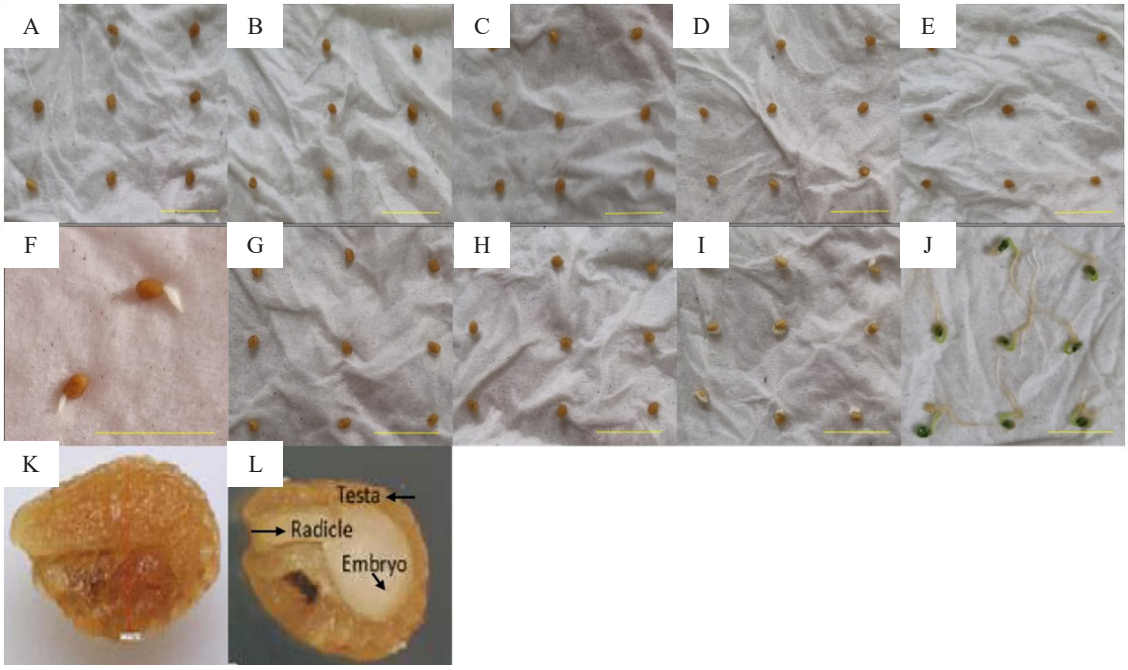

Figure 1. Germination of *Andrographis paniculata* (Burm.f.) Nees seeds from five accessions on moistened tissue. Scale bar = 0.5 cm. (A) Kuala Lumpur (KL), (B) Malacca (ML), (C) Negeri Sembilan (NS), (D) Perak (PE), and (E) Selangor (SL). (F) Radicle emergence indicating germination. Effects of dormancy-breaking treatments on day 6 of NS accession: (G) control, (H) hot water treatment, and (I) sandpaper scarification. (J) Seedlings at the two-cotyledon stage. Morphology of seed adapted from Gundala *et al.* (2023) (K) an entire seed, (L) a cross section. These figures illustrate seed appearance and germination behaviour in this preliminary study; detailed seed morphological measurements were not conducted

2.4. Experimental Design

The two-factor experiment employed a completely randomized design (CRD) with three dormancy-breaking treatments: hot water, sandpaper scarification, and an untreated control applied to five *A. paniculata* accessions. Each treatment-accession combination consisted of 50 seeds per replicate, with two replicates.

All replicates were maintained under identical conditions in a plant growth chamber at the AgrogeneBank, Universiti Putra Malaysia (UPM). The study was designed as a preliminary screening to identify a simple and effective method for improving seed germination across different accessions.

Germination was observed and recorded at 3-day intervals for 15 days. Final germination percentage (FGP) and mean germination time (MGT) were recorded as the primary response variables. Daily germination counts were derived from cumulative germination data by subtracting the number of seeds germinated during the previous observation. This interval-based counting approach was adopted to minimize seed disturbance and reduce the risk of contamination.

Final germination (FGP) was calculated as the proportion of seeds that germinated by day 15 relative to the total number of seeds sown per replicate. Mean germination time (MGT) was calculated according to the standard formula:

$$MGT = \frac{\Sigma (n \times d)}{N}$$

where n is the number of seeds germinated on day d, d is the number of days from the start of the germination test, and N is the total number of seeds germinated during the experiment. Standard deviation (SD) was calculated for each accession treatment group.

2.5. Data Analysis

Mean germination time (MGT) and final germination percentage (FGP) were analyzed using a two-way ANOVA (Analysis of variance) conducted with the aov function in RStudio (version 4.5.1). The effect sizes of variables (accession, treatment, and interaction effects) were calculated as partial eta squared (η^2). Tukey's HSD test ($p < 0.05$) was performed using the multcompView package to compare means within each treatment, where different letters indicate significant differences.

3. Results

3.1. Effects of Physical Scarification on Seed Germination

The dormancy-breaking treatments significantly influenced seed germination. In Table 1, the mean germination time (MGT) is significantly different among all treatments and accessions. Two-way ANOVA indicated a strong effect of treatment (partial $\eta^2 = 0.756$) and accession (partial $\eta^2 = 0.596$), as well as a significant treatment × accession interaction (partial $\eta^2 = 0.673$), indicating accession-dependent responses to the applied treatments. The accession-dependent responses observed in this study may be associated with differences in seed coat properties among accessions. However, seed coat thickness and geographical adaptation were not directly investigated in the present study.

FGP was also significantly influenced by both treatments and accession (Table 2). Two-way ANOVA showed that treatment accounted for nearly all observed variance in FGP (partial $\eta^2 = 0.999$), with similarly high effect sizes for accession (partial $\eta^2 = 0.990$) and the treatment x accession interaction (partial $\eta^2 = 0.989$).

For all accessions, sandpaper scarification resulted in high final germination percentages, ranging from 84% to 100% (Table 3). Among the treatments, sandpaper scarification produced the shortest mean germination time (MGT), followed by control and hot water treatment (Figure 2). Across accessions, the average MGT was approximately 7 days for sandpaper scarification, 8

Table 1. Two-way ANOVA for MGT

	dF	Mean square	E-value	p-value	Partial η^2
Accession	4	3.501	5.522	6.15E-03 **	0.596
Treatment	2	14.755	23.275	2.52E-05 ***	0.756
Accession: Treatment	8	2.44	3.851	0.01183 *	0.673
Residuals	15	0.634			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2. Two-way ANOVA for FGP

	dF	Mean square	E-value	p-value	Partial η^2
Accession	4	304.9	381.08	6.94E-15 ***	0.990
Treatment	2	21753.7	27192.17	< 2.2e-16 ***	0.999
Accession: Treatment	8	135.6	169.49	2.70E-13 ***	0.989
Residuals	15	0.8			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3. Summary of mean germination time (MGT) and final germination percentage (FGP) of five *A. paniculata* accessions expressed as mean ± standard deviation under different treatments

Accession	Treatment	MGT (days) ± SD	FGP (%) ± SD
KL	Control	5.63±0.530	8.00±0.000
	HotWater	9.32±0.152	27.00±0.000
	SandPaper	6.45±0.451	94.00±0.000
ML	Control	8.50±1.414	4.00±0.000
	HotWater	10.00±1.414	6.00±0.000
	SandPaper	7.75±0.146	84.00±1.410
NS	Control	8.13±0.177	7.00±1.410
	HotWater	8.85±1.909	9.00±1.410
	SandPaper	7.99±0.495	100.00±0.000
PE	Control	10.50±0.707	6.00±0.000
	HotWater	10.13±0.530	8.00±0.000
	SandPaper	6.85±0.091	91.00±1.410
SL	Control	6.70±0.424	11.00±1.410
	HotWater	10.03±0.297	42.00±1.410
	SandPaper	7.67±0.029	96.00±0.000

days for the control, and 9 days for hot water treatment, indicating that germination times among treatments were closely comparable, with sandpaper scarification showing a slight advantage in promoting earlier germination.

3.2. Variation in Treatment Response among *A. paniculata* Accessions

Seed germination varied among accessions within each treatment. Across all accessions, sandpaper scarification consistently resulted in higher germination percentages compared with hot water treatment and the control. The NS accession achieved the highest final germination percentage under sandpaper scarification (100.00 ± 0.000%), while other accessions ranged from 84.00 ± 1.410% to 96.00 ± 0.000% (mean ± standard deviation) (Figure 3; Table 3).

In contrast, hot water treatment resulted in low FGP, ranging from 6.00 ± 0.000% to 42.00 ± 1.410%,

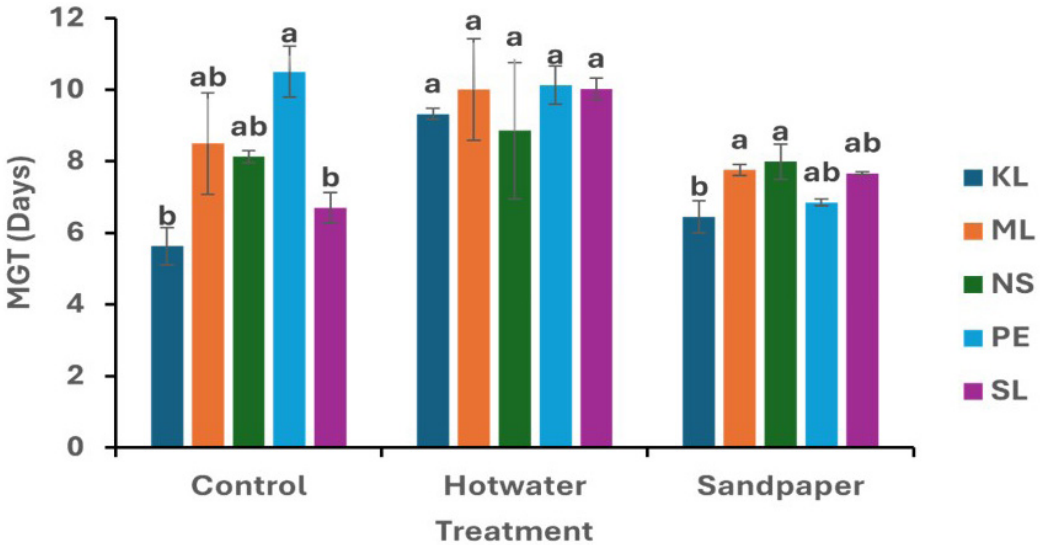

Figure 2. Mean germination time (MGT, days) of five *A. paniculata* accessions under different treatments. Error bars represent mean ± standard deviation. Different letters above bars indicate significant differences among accessions within the same treatment based on Tukey's HSD test ($p < 0.05$)

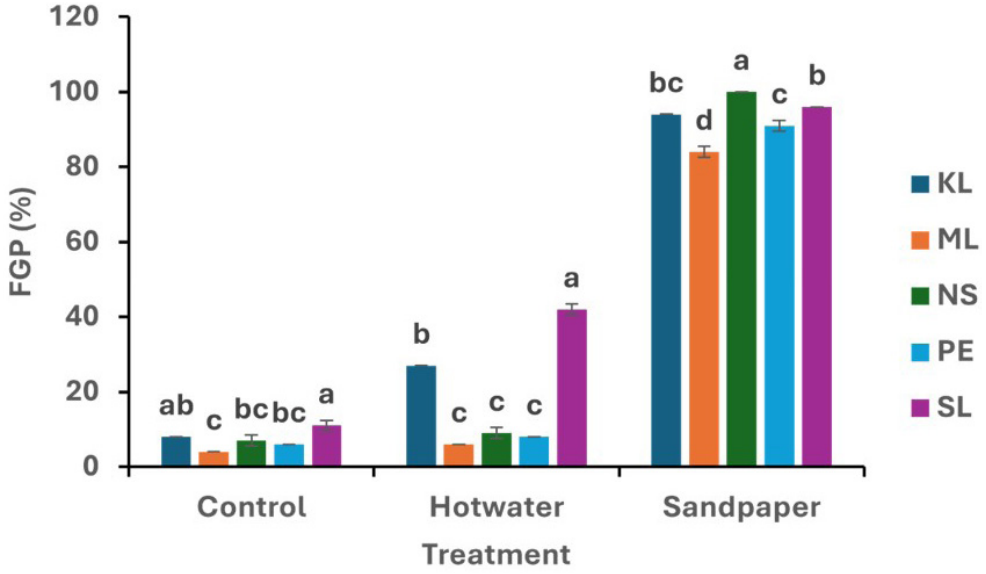

Figure 3. Final germination percentage (FGP, %) of five *A. paniculata* accessions under different treatments. Error bars represent mean ± standard deviation. Different letters above bars indicate significant differences among accessions within the same treatment based on Tukey's HSD test ($p < 0.05$)

while the control exhibited the lowest germination percentages, ranging from 4.00 ± 0.000% to 11.00 ± 1.410%. Significant differences among accessions were detected for both FGP and MGT regardless of treatment (Tables 1 and 2). A significant treatment x accession interaction further indicated that the effectiveness of dormancy-breaking treatments varied by accession.

With respect to MGT, the average of sandpaper scarification resulted in the fastest germination (7.34 days), followed by the control (7.89 days), whereas hot water treatment showed the slowest germination (9.66 days). This indicates that sandpaper scarification enhanced the rate of germination, while hot water treatment delayed germination, possibly due to heat stress. The significant treatment-by-accession interaction of MGT indicates variation in seed dormancy among accessions. This suggests that different accessions may respond differently to dormancy-breaking treatments, not only in germination percentage but also in germination speed.

4. Discussion

4.1. Effects of Physical Scarification on Seed Dormancy

The ability to reduce the time required to establish *Andrographis paniculata* (Burm.f.) Nees seedlings and increasing the germination percentage are important from an industrial perspective. In this study, physical dormancy-breaking treatments significantly influenced germination performance, confirming that scarification methods can effectively overcome the seed dormancy in *A. paniculata*.

The dormancy of *A. paniculata* is influenced by both physical and physiological mechanisms, and hot water soaking is commonly used to overcome physical dormancy by softening the seed coat, thereby facilitating water uptake by the embryo. The seed coat of *A. paniculata* protects the embryo and its environment from water and any external threats. Therefore, hot-water treatment could soften the seed coat and allow the water to be absorbed by the embryo. Previous work by Kumar *et al.* (2011b) reported a maximum germination rate of 93% with hot-water treatment, indicating the effectiveness of thermal scarification. In contrast, the present study recorded a much lower average germination percentage after hot water treatment (18.4%). This discrepancy may relate to differences in seed maturity and selection criteria. While Kumar *et al.* (2011b) used seeds collected from mature pods of six-month-old plants, seeds in the present study were harvested from mature siliques, selected for uniform seed color, and stored at 4°C for 1 month before experimentation. Although short-term cold storage is commonly used to preserve seed viability, such post-harvest handling may influence seed physiological status, consequently, its response to hot water treatment. In addition, the 50°C hot water treatment may have induced thermal stress or partial injury in the embryos, as indicated by slower germination (9.66 days) compared to the control (7.89 days), thereby reducing germination performance. However, embryo viability was not directly assessed in the present study.

In contrast, sandpaper scarification effectively accelerated the physiological processes underlying germination. This treatment may enhance the water permeability and gas exchange, thereby promoting early metabolic activation. This effect is supported by the seed anatomy shown in Figure 1, in which the testa serves as the primary barrier to imbibition and gas exchange. Dormancy release in seeds is commonly associated with hormonal changes, including reduced abscisic acid (ABA) levels and increased gibberellin (GA) activity, which promote embryo growth and radicle protrusion. (Shu *et al*. 2016; Nelson *et al*. 2023) Although the hormonal mechanisms were not measured in this preliminary study, the accelerated germination observed with sandpaper scarification may have resulted from activation of physiological processes. Sandpaper scratched the seed coat and simply breaks the dormancy of the seed by exposing the embryo to water. The results align with Talei *et al.* (2012b), who report an overall germination percentage of more than 94% after 15 days of treatment. Similarly, the FGP recorded in the present showed 93% on day 15, further confirming the effectiveness of sandpaper scarification in overcoming dormancy.

The consistently high final germination percentages observed in sandpaper scarification further indicate that dormancy in *A. paniculata* seeds is not absolute and can be effectively alleviated under suitable conditions. Similar improvements in germination and establishment with dormancy-breaking or priming treatments have been reported across several crop species, underscoring the broader relevance of these approaches in seed-based propagation systems. For instance, Hemalatha and Chaudhari (2020) demonstrated that manual scarification with sandpaper in sandalwood (*Santalum album* L.) resulted in the earliest seed emergence and a significantly superior response across all observations.

Seed maturity and storage duration are also known to influence germination performance in *A. paniculata*. Previous reports indicate that the highest germination percentage of *A. paniculata* occurs in mature seeds stored for 12 months, whereas seeds stored for 6 months germinate more rapidly. According to Jayawardhane *et al.* (2021), *A. paniculata* seeds exhibit over 85% germination after one month of dry storage at room temperature. These findings support the importance of seed maturity and storage conditions for achieving high germination.

Overall, sandpaper scarification produced superior results compared with all other treatments, even though the storage duration of the seeds was unknown. By physically abrading the seed coat, sandpaper scarification increases water penetration and effectively breaks physical dormancy. This mechanism explains the high germination percentage (average 93%) and the accelerated germination rate observed. These results demonstrate that sandpaper scarification is a simple, reliable, and non-chemical approach to improving germination in *A. paniculata*, making it suitable for preliminary screening that requires uniform and consistent seedling establishment.

4.2. Effect of Different Treatments Among *A. paniculata* Accessions

Germination percentage varied among accessions. Sandpaper scarification was the most effective treatment across all accessions, with the final germination percentages ranging from 84% to 100%. In contrast, hot water treatment and control resulted in lower germination percentages. Wang *et al.* (2011), studying five wild *Vigna* species, reported that seeds pretreated with sandpaper scarification generally showed higher germination rates than those pretreated with hot or boiling water. These results demonstrate that mechanical scarification provides a more reliable and direct means of dormancy-breaking compared with thermal treatment. This is further supported by Lombraña *et al.* (2024), who reported that mechanical scarification increased the percentage of germinating seeds to nearly 100% in both *Astragalus maritimus* and *A. Verucosus*, regardless of incubation temperature. Han *et al.* (2022) also reported that hard-seededness in "Taitung No.1" winged bean could be effectively removed by sandpaper scarification, which ensured rapid and high germination.

Significant differences in both MGT and FGP were observed among accessions, regardless of treatment. This indicates that inherent genetic or physiological differences may cause some accessions to germinate faster or more successfully than others. The interaction between accession and treatment suggests that the effectiveness of a dormancy-breaking method depends not only on the treatment itself but also on the genetic background of the seed source. Among all accessions, NS performed best with sandpaper scarification, achieving $100.00 \pm 0.000\%$ germination, highlighting its potential for propagation. This response may be attributed to accession-specific traits, such as differences in seed coat properties or permeability, which can influence sensitivity to mechanical scarification. Chaves *et al.* (2017) reported that seed coat permeability is affected by scarification treatments and low-pH solutions. Although seed coat traits were not quantified in the present study, these findings suggest that genetic variation among accessions may underlie the observed difference in germination response.

4.3. Accession-Dependent Responses and Interaction Effects

Despite the overall effectiveness of the treatments, significant accession effects and treatment-by-accession interactions were detected for MGT. This indicates that genetic background plays an important role in determining germination speed and responsiveness to dormancy-breaking treatments.

Villa-Rivera *et al.* (2024) reported that traits such as seed mass and seed volume did not predict germination speed or germination percentage, suggesting that other factors are likely more important in explaining variation among accessions. Physical dormancy is commonly caused by the impermeability of the seed coat, which prevents water uptake and delays germination (Wen *et al.* 2024). Therefore, differences among accessions may reflect variation in seed coat properties, dormancy depth, or endogenous hormone levels, resulting in differential sensitivity to dormancy-breaking treatments.

Effect size analysis revealed high partial η^2 values for final germination percentage, indicating that most of the observed variance was explained by treatment and accession effects. This finding reflects the consistently high germination percentage influence of sandpaper scarification across seed sources.

4.4. Implications For Cultivation and Propagation

The reduced MGT and high FGP across five accessions under sandpaper scarification have practical implications for the cultivation and large-scale propagation of *A.*

paniculata. Faster, more uniform germination can improve seedling establishment, enhance crop uniformity, and shorten production cycles.

Standard germination protocols for *A. paniculata* commonly use a petri dish covered with Whatman paper (Abdullahi *et al.* 2025; Talei *et al.* 2012b). For instance, Kundu *et al.* (2024) sowed *A. paniculata* seeds on moistened filter paper with double-distilled water in sterilized petri dishes to study seed ecology and seedling phenology. In the present study, a minor modification to the germination protocol was applied: a moistened tissue towel in a "sandwich" setup was sealed inside a seal bag. The results align closely with those reported by Talei *et al.* (2012b), who achieved over 94% germination after 15 days. Similarly, the present study recorded an FPG of 93% on day 15, confirming that the modified approach effectively overcomes seed dormancy. This method maintained consistent moisture levels, reduced the risk of contamination, and enabled effective monitoring of germination while minimizing technical requirements. It primarily functions as a moisture-maintaining system that supports germination rather than a dormancy-breaking treatment. Compared with petri dish-based methods, the moistened tissue "sandwich" technique is more practical, cost-effective, and accessible, particularly for large-scale seed testing or for those with limited laboratory facilities.

Although the FGP ranged from 84% to 100% under optimal treatment, this trait was not sufficiently sensitive to distinguish performance differences among accessions. Future studies could incorporate additional physiological or biochemical measurements to elucidate the mechanisms of dormancy further.

In Conclusion, The hard seed coat of *Andrographis paniculata* (Burm.f.) Nees is primarily responsible for the seed dormancy. Using sandpaper for scarification promotes seed germination. In the present experiment, *A. paniculata* effectively germinated when the sandpaper scarification strategy was employed, eventhough the duration of seed storage can alter the rate of growth. Furthermore, this method would be simple and affordable for farmers and nursery workers to implement on a large scale. Based on the results, Accession NS may be most suitable for propagation due to its high germination success (100%), while Accession KL may be preferred where rapid germination (6.45 days) is required. However, further field evaluation is needed before commercial recommendation.

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