

Comparative analysis of seed germination and early growth in *Amaranthus thunbergii* and *Cleome gynandra* as affected by pre-treatment methods

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Background: African Indigenous Leafy Vegetables (AILVs), including *Amaranthus thunbergii* and *Cleome gynandra*, contribute significantly to food and nutrition security in rural African communities. However, their utilisation, conservation and domestication are constrained by poor and inconsistent germination associated with seed dormancy, increasing the risk of genetic erosion and limiting their integration into formal food systems.

Aim: This study evaluated the effects of seed pre-treatment methods and durations on germination and early seedling development of *A. thunbergii* and *C. gynandra*.

Setting: Experiments were conducted under controlled laboratory conditions at the Botswana National Seed Laboratory.

Methods: Factorial experiments arranged in a Completely Randomised Design (CRD) with three replications were conducted in 2022. Seed pre-treatment methods included control, pre-heating and pre-chilling, while durations comprised pre-heating at 0 s, 30 s, 60 s and 90 s and pre-chilling for 24 h, 84 h and 168 h.

Results: Seed pre-treatment methods and durations significantly influenced germination parameters. *Amaranthus thunbergii* responded more positively to pre-treatments than *C. gynandra*. Pre-heating for 90 s increased germination of *A. thunbergii* to 90%, representing a 28% improvement compared with *C. gynandra*. Pre-heated *A. thunbergii* seeds reached full imbibition within 3 days, showing a 40% improvement over *C. gynandra* across all durations.

Conclusion: Extended pre-heating enhanced water uptake, accelerated germination and improved early seedling growth in both species by softening the seed coat. Pre-heating is therefore recommended as a practical, low-cost approach to improve germination of AILVs.

Contribution: This study contributes empirical evidence to support conservation, domestication and sustainable utilisation of AILVs in Botswana and across Africa.

Keywords: *Amaranthus thunbergii*; *Cleome gynandra*; seed pre-treatment methods; pre-heat; pre-chill.

Introduction

The germination of seeds from many indigenous plant species presents significant challenges because of complex dormancy mechanisms and specific ecological requirements (Mkhwanazi, Maseko & Dube 2024; Nonogaki 2014). A comprehensive understanding of a species' life history, native habitat and physiological behaviour is essential to inform effective seed management, propagation techniques and the enhancement of seed vigour and genetic integrity during cultivation (Nonogaki 2014). Each plant species possesses distinct germination cues such as temperature, photoperiod, moisture levels and even microbial interactions that have evolved as ecological adaptations to ensure survival under optimal conditions (Mkhwanazi et al. 2024; Nonogaki 2014; Talluri, Narayani & Babu 2024). Recent findings also emphasise the role of eco-physiological approaches, including water treatments and microbial inoculation, which mimic natural germination triggers and enhance seedling establishment in stress-prone environments (Mkhwanazi et al. 2024).

African indigenous leafy vegetables (AILVs), including *Amaranthus thunbergii* and *Cleome gynandra*, have long served local communities as accessible sources of nutrition and traditional

medicine (Dlamini et al. 2010; Mkhwanazi et al. 2024; Moatshe-Mashiqha et al. 2024). Despite their cultural and nutritional significance, these species remain largely underutilised and are often harvested from the wild, leading to concerns over sustainability (Moatshe-Mashiqha et al. 2024). According to Raselebe (2017), there are over 53 000 underexploited indigenous plant species globally, many of which face threats from habitat degradation, climate change, overharvesting and illegal trade. Furthermore, the cultivation of such species is limited by low germination rates, largely attributable to physiological seed dormancy and the lack of standardised propagation protocols (Moatshe-Mashiqha et al. 2024).

Studies in Botswana and Nigeria confirm that pre-sowing treatments such as hot water soaking, acid scarification and mechanical abrasion significantly improve germination in indigenous species like *Acacia* and *Tamarindus indica*, underscoring the potential for similar interventions in leafy vegetables (Abdulrahman et al. 2023; Mojeremane, Rasebeka & Mathowa 2014).

Seed dormancy, while evolutionarily advantageous in natural ecosystems, poses a major barrier to domestication and commercial cultivation (Debbarma & Priyadarshinee 2017). Overcoming these dormancy traits through appropriate pre-treatment methods is therefore critical to improving germination success and enabling broader adoption of indigenous crops. Empirical research has shown that pre-sowing treatments such as scarification, hydropriming, temperature manipulation and chemical stimulants can significantly influence seed behaviour, emergence rates and early seedling vigour (Baatuuwie et al. 2019; Makuvara, Marumure & Chidoko 2022; Mkhwanazi et al. 2024; Moatshe-Mashiqha et al. 2024; Olatunji, Maku & Odumefun 2013).

In parallel, increased scientific attention on the health-promoting properties of indigenous vegetables has revealed their potential as functional foods. Species like *Amaranthus* are rich in provitamin A carotenoids (β -carotene), antioxidants (lutein) and other nutraceuticals that combat micronutrient deficiencies and age-related diseases (Dlamini et al. 2010). Similarly, *C. gynandra*, which is widely consumed in Southern Africa, is a vital source of vitamins A and C, calcium and iron. These leafy vegetables are traditionally prepared with complementary species such as *Vigna* spp. and *Solanum nigrum* to enhance flavour and nutritional content (Van den Heever & Venter 2007). Beyond nutrition, recent studies highlight their phytochemical diversity, including flavonoids and glucosinolates, which contribute to anti-inflammatory and antimicrobial properties, positioning them as candidates for functional food development (Moatshe-Mashiqha et al. 2024).

Given the urgent need for resilient, nutrient-dense crops in the face of food insecurity, promoting the domestication of indigenous vegetables such as *A. thunbergii* and *C. gynandra* through improved seed germination practices represents a viable pathway towards sustainable agriculture in Botswana

and beyond. This study investigates the comparative effectiveness of different pre-treatment methods on seed germination and early growth performance in these two species, with the aim of identifying optimal strategies for their successful cultivation and wider integration into agroecological systems. By integrating traditional knowledge with modern seed biology, this research contributes to the broader agenda of conserving biodiversity while enhancing food and nutritional security in sub-Saharan Africa.

Materials and methods

Experimental design

Seeds of *A. thunbergii* and *C. gynandra* were collected from natural veld populations in south-eastern Botswana. The experiment was conducted under controlled laboratory conditions at the Botswana National Seed Laboratory using a germination chamber maintained at temperatures of 20 °C – 30 °C, with regulated light availability and a photoperiod of 8 s/16 h (light/dark). Seed germination was assessed using the top-of-paper method, with absorbent paper as the substrate, following standard germination testing protocols (Rao et al. 2006).

Experiments were arranged as a factorial Completely Randomised Design (CRD) with three replications. Two experimental factors were evaluated. Factor A consisted of seed pre-treatment method, including soaking (control), pre-heating and pre-chilling. Factor B comprised pre-treatment duration, with three levels specific to each method. Control seeds were soaked in tap water for 12 h. Pre-heating treatments involved exposing seeds to heat for 60 s, 90 s and 120 s, while pre-chilling treatments were conducted at 6 °C for 24 h, 72 h and 168 h in petri dishes.

For each treatment combination and species, 30 seeds were randomly selected and evenly distributed across replications. Germination counts were recorded at 2-day intervals, with the first count conducted 4–5 days after sowing (DAS) and the final count at 14 days, in accordance with the International Rules for Seed Testing (International Seed Testing Association [ISTA] 2013).

Germination data analysis

After 4 days of pre-treatment, germination performance was evaluated using several standard germination and vigour parameters. Germination percentage (GP%) was determined by recording daily seed germination counts for 21 DAS. Final GP% was calculated as the ratio of the total number of germinated seeds to the total number of seeds sown (20), multiplied by 100, following the methods of Maguire (1962) and Hossain et al. (2005).

Seed imbibition time was assessed to quantify the duration required for seeds to absorb water and reach full hydration, a critical physiological process initiating germination. Imbibition time (T) was calculated using the equation:

$$T = (W_f - W_i)/R,$$

where W_f represents the final seed weight after water uptake, W_i is the initial dry seed weight and R is the rate of water absorption expressed as g h^{-1} or $\% \text{ h}^{-1}$, in accordance with Woodstock (1988).

The germination rate index (GRI) was calculated to describe the speed and uniformity of germination over time. This index reflects the cumulative proportion of seeds germinating on successive days and was computed using the formula:

$$\text{GRI} = G_1/1 + G_2/2 + \dots + G_x/x,$$

where G_x is the percentage of seeds germinated on day x (Esechie 1994; Maguire 1962). Higher GRI values indicate faster and more uniform germination.

Germination speed (GS) was calculated as the sum of germinated seeds recorded per day across the entire germination period, providing an overall measure of germination rapidity. Germination value (GV), an integrated measure of germination capacity and speed, was calculated as the product of peak value (PV) and mean daily germination (MDG), as described by Czabator (1962). Peak value represents the highest number of seeds germinated on a single day, while mean daily germination reflects the average daily germination over the testing period.

Statistical analysis

Normality assumptions were checked using PROC UNIVARIATE on residuals. Data were analysed using analysis of variance (ANOVA) through the general linear model (PROC GLM) in SAS software (2009). Regression analyses explored responses of seedlings to pre-treatment conditions. Mean comparisons were made using protected least significant difference (LSD) at a 0.05 significance level.

Ethical considerations

This article followed all ethical standards for research without direct contact with human or animal subjects.

Results and discussion

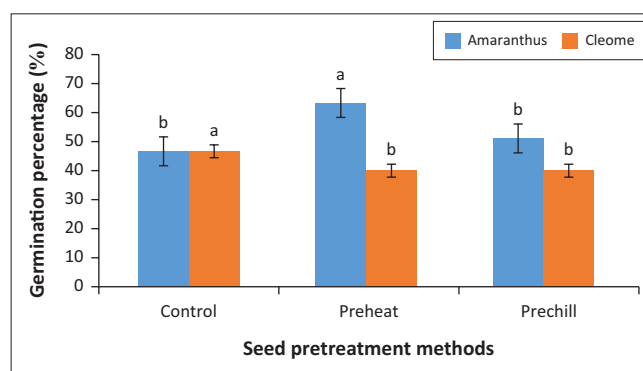
The degree of dormancy and the effectiveness of treatment techniques vary across species, influenced by factors such as pre-treatment method, duration and concentration. These differences are critical in determining germination matrix responses. A significant comparative effect ($p < 0.05$) was observed between *A. thurnbergii* and *C. gynandra* in terms of

germination performance (Table 1). Notably, *A. thurnbergii* outperformed *C. gynandra* in GP%, imbibition period, germination period, GS and plant height, indicating more rapid and efficient seed activation. However, no significant difference ($p > 0.05$) was found in seed vigour metrics such as number of leaves and germination value (Table 1). Despite this, *A. thurnbergii* produced significantly ($p < 0.05$) taller plants, suggesting enhanced post-germination growth (Table 1). Similar findings were reported in other indigenous leafy vegetables (Rhaman, 2025; Hossain et al. 2005; Moatshe-Mashiqah et al. 2024), where species-specific dormancy mechanisms dictate pre-treatment success (Mangena 2022).

The comparative germination responses of *A. thurnbergii* and *C. gynandra* under different seed pre-treatment methods indicated that GP% of both species was significantly ($p < 0.05$) affected by pre-treatment methods (Figure 1). Overall, *Amaranthus* consistently outperformed *Cleome* across all pre-treatment methods, with exception of the control group, where no significant difference was observed ($p < 0.05$) (Figure 1). This highlights the species-specific nature of dormancy-breaking mechanisms.

Among the treatments, pre-heating proved most effective for *Amaranthus*, enhancing GP% by 16.66% compared to the control (Figure 2). In contrast, *Cleome* did not benefit significantly from either pre-heating or pre-chilling, both of which resulted in a 6.67% decline in GP%. This suggests that these pre-treatment methods may not be suitable for enhancing *Cleome*'s germination. This is supported by Mangena (2022), who emphasised that *C. gynandra* requires chemical priming (e.g. gibberellic acid or potassium nitrate) rather than thermal or chilling treatments to overcome dormancy.

Seed pre-treatment methods significantly ($p < 0.05$) influenced germination value for both *A. thurnbergii* and *C. gynandra*



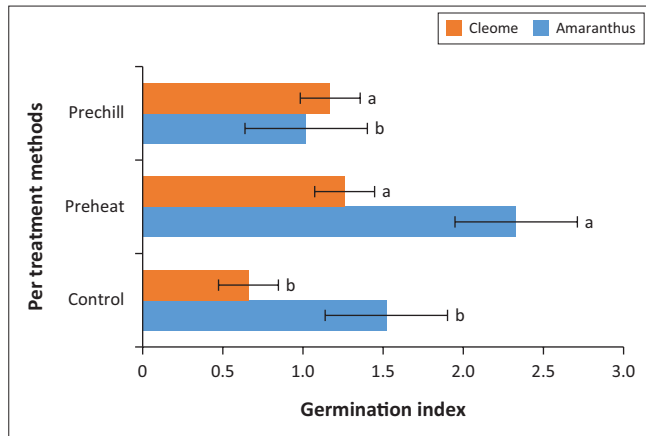
Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 1: Effect of seed pre-treatment methods on germination percentage of *Amaranthus thurnbergii* vs *Cleome gynandra*.

TABLE 1: Comparison of germination responses of *Amaranthus thurnbergii* and *Cleome gynandra*.

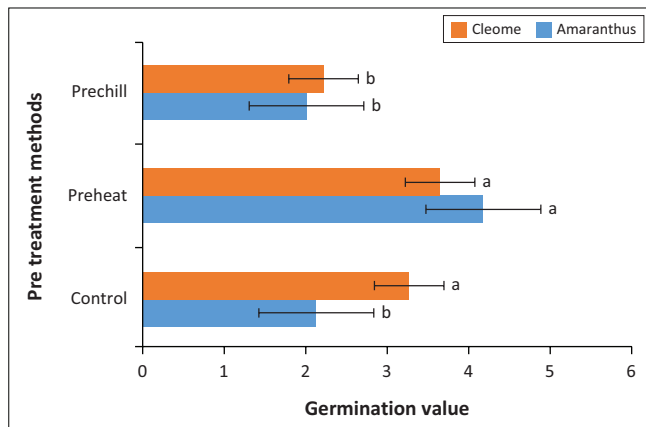
Treatments	Germ %	Imbibition period	Germ period	Germ index	Germ value	Germ speed	Number of leaves	Plant height (cm)
<i>Amaranthus</i>	61.33a	4.38b	0.71b	1.59a	2.96a	1.39a	1.905a	4.85b
<i>Cleome</i>	50.09b	6.05a	2.43a	1.00b	2.98a	0.88b	1.905a	4.00a
LSD	4.68	0.77	0.87	0.43	0.47	0.50	0	0.52
Significance	**	***	***	*	NS	**	NS	***

*, 0.05; **, 0.01; ***, 0.0001. Means of the same letter are not significantly different from each other.



Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 2: Effect of seed pre-treatment methods on germination index of *Amaranthus thurnbergii* and *Cleome gynandra*.

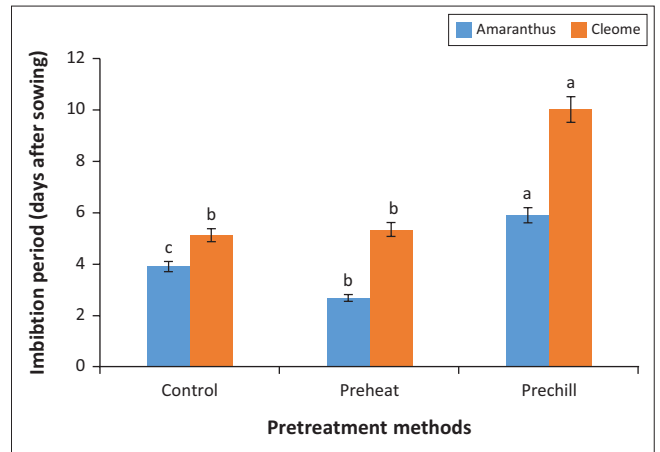


Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 3: Effect of seed pre-treatment methods on germination value of *Amaranthus thurnbergii* and *Cleome gynandra*.

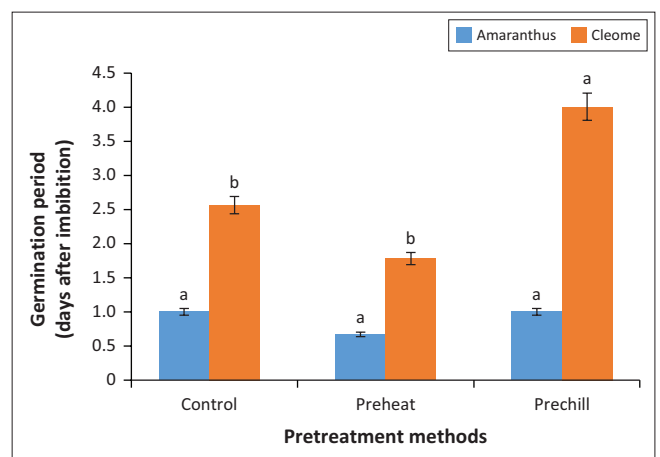
(Figure 3), reflecting differences in germination rate and percentage. Overall, pre-heating improved germination quality in both species, confirming thermal treatment as effective for breaking dormancy in indigenous seeds. *Amaranthus* showed a notably higher germination value than *Cleome*, which corresponded with more vigorous early root development. This aligns with findings by Moatshe-Mashika et al. (2024), who reported enhanced seedling growth and vigour following a 90-s pre-heat. Thermal treatments also improved drought resilience and nutrient uptake, boosting biomass and yield (Rhaman, 2025; Taylorson & Hendricks 1969). Temperatures above 20 °C deactivate phytochrome, accelerating dormancy break, whereas pre-chilling delays the process (Taylorson & Hendricks 1969). Pre-chilled seeds reduced germination effectiveness, particularly in *Amaranthus*, suggesting cold-induced stress, as supported by Chen et al. (2021) who advised against extended pre-chill treatment for this species because of stress-induced suppression of germination.

Seed pre-treatment methods significantly ($p < 0.05$) influenced imbibition (Figure 4) and germination period (Figure 5) in both *A. thurnbergii* and *C. gynandra*.



Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 4: Effect of pre-treatment methods on imbibition period of *Amaranthus thurnbergii* and *Cleome gynandra*.



Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 5: Effect of pre-treatment germination methods on the period of *Amaranthus thurnbergii* and *Cleome gynandra*.

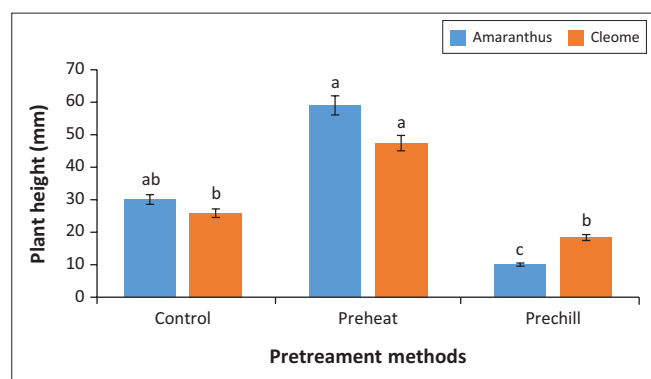
Pre-chilling notably extended imbibition time doubling it compared to the control while pre-heating reduced the imbibition time (Figure 4). However, the pre-heated seeds did not significantly differ from the control, indicating accelerated seed hydration. Across all treatments, *Amaranthus* imbibed water faster than *Cleome*, with the shortest duration occurring under pre-heating (3 days) and the longest under pre-chilling (6 days). This pattern suggests that cold-induced dormancy may hinder water uptake, especially in *Cleome*. Seed hydration, a vital trigger for physiological and biochemical changes leading to germination, was further delayed by pre-chilling, particularly in *Cleome*, which required up to 10 days to complete imbibition. According to Zembele and Ngulumbé (2022) and Taylorson and Hendricks (1969), cold stress suppresses germination by stabilising the far-red absorbing form of phytochrome, which prolongs dormancy despite water availability.

Following imbibition, *Amaranthus* consistently demonstrated faster germination across treatments

although differences were not statistically significant (Figure 5). This may indicate lower innate dormancy in *Amaranthus*, reducing dependence on pre-treatment. Notably, pre-heated *Cleome* seeds showed a 30% improvement in germination period over the control, implying that thermal pre-treatment may help overcome physiological dormancy and activate early metabolic processes.

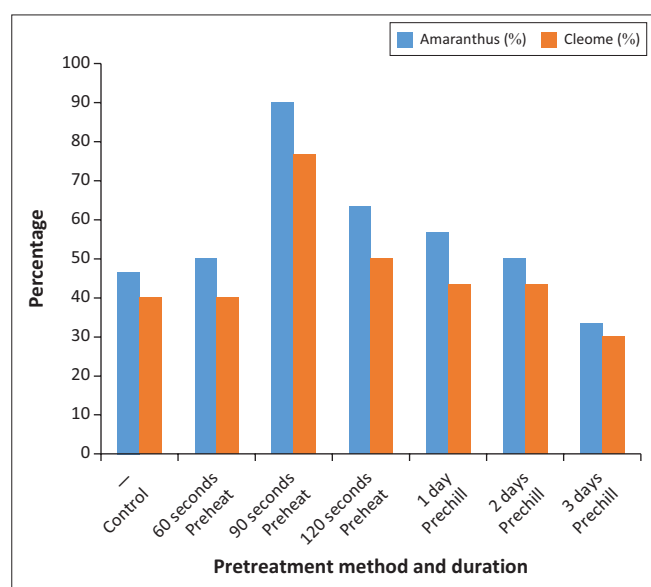
Overall, thermal treatments, particularly pre-heating, enhanced seed responsiveness and GS more effectively than pre-chilling, with *Amaranthus* showing greater receptiveness to heat-based stimulation. Dos Rois (2023) and Moatshe-Mashiq et al. (2024), who observed similar benefits in pre-heated *Amaranthus* accessions, support these findings while highlighting the limited effectiveness of chilling for species with physiological dormancy such as *Cleome*.

In terms of the impact of seed pre-treatment methods on seedling height of *Amaranthus* and *Cleome*, pre-heating



Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 6: Effect of seed pre-treatment methods on plant height of *Amaranthus thunbergii* and *Cleome gynandra*.



Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

FIGURE 7: Effect of pre-treatment method and duration on germination percentage of *Amaranthus* and *Cleome*.

resulted in a 46% – 49% increase in plant height compared to the control ($p < 0.05$), while pre-chilling reduced height by 39% – 67% across both species (Figure 6). The enhanced growth was attributed to the improved germination index, speed and value, reduced imbibition time when seeds were exposed to pre-heating compared to pre-chilling (Figure 1 to Figure 5). This suggests that thermal pre-treatment stimulates early growth through enhanced enzyme activity and hormonal signalling, particularly gibberellins, while cold exposure inhibits metabolic functions, inducing stress (Zembele & Ngulumbé 2022).

These findings reflect the ecological adaptation of *Amaranthus* and *Cleome* as warm-season plants native to tropical and subtropical Southern Africa. Seeds from such climates are naturally responsive to heat cues, which signal favourable growing conditions. Pre-heating emulates soil warming at the onset of the season, triggering enzymatic and metabolic pathways that facilitate germination (Reed, Bradford & Khanday 2022). Specifically, it activates amylase for mobilising stored nutrients and promotes gibberellin synthesis for cell elongation (Chen et al. 2021; Reed et al. 2022). Additionally, warmer temperatures soften the seed coat, improving water uptake and radicle emergence. At 90 s of pre-heating, *Amaranthus* reached a germination peak of 90% and showed marked increases in seedling height indicating optimal thermal responsiveness. However, prolonged exposure beyond this threshold diminished germination, pointing to potential heat-induced stress.

Germination percentage was significantly ($p < 0.05$) affected by the interaction among pre-treatment methods and duration (Figure 7). Pre-heating seeds for 90 s resulted in the highest germination rate, *Amaranthus* reached 85%; while *Cleome* achieved 75%, highlighting the efficacy of thermal stimulation in seed activation. This is attributed to heat-induced modifications to the seed coat, such as wax removal and surface cracking, which improve gaseous exchange and water uptake (Olatunji et al. 2013). However, the results demonstrate that the effectiveness of heat treatment is duration dependent. Extended exposure can damage the embryo, leading to reduced germination (Amusa 2011; Fredrick et al. 2017; Makuvara et al. 2022). The current study corroborates these findings, showing a slight decline in germination under prolonged heat, likely because of thermal stress. Similarly, prolonged chilling led to a 10% – 13% decrease in germination from control levels for both species, indicating low tolerance to cold-induced dormancy. As also shown in Figure 4 and Figure 5, chilling slowed imbibition and germination, further supporting this conclusion.

There was a significant interaction ($p < 0.05$) between pre-treatment methods and their duration on key germination metrics (Table 2). *Cleome* recorded the lowest germination value following a 72-h pre-chill, whereas *Amaranthus* achieved the highest value with a 90-s pre-heat. A similar trend was observed in plant height, where the shortest and tallest seedlings emerged from *Amaranthus* pre-chilled for 24 h and pre-heated for 90 s, respectively.

TABLE 2: Interactive effect of seed pre-treatment method and concentration on germination value and seedling height of *Amaranthus* and *Cleome*.

Pre-treatment	Duration Seconds/h	Amaranthus		Cleome	
		Germination value	Plant height (mm)	Germination value	Plant height (mm)
Control	-	2.13 de	10.00 e	3.27 abcd	47.3 bcd
Pre-heat In seconds	60	3.07 bcd	50.00 bc	2.47 cd	62.7 ab
	90	5.00 ab	76.00 a	5.29 a	66.00 ab
	120	4.53 abc	60.00 ab	1.20 de	60.00 ab
Pre-chill In hours	24	2.77 cd	20.7 e	3.0 bcd	30.00 cde
	48	2.97 bcd	48.3 bcd	2.90 bcd	50.7 bc
	72	3.00 bcd	28.3 cde	1.00 de	24.00 ed
Significance	-	*	***	*	***
LSD	-	0.47	5.2	0.47	5.2

Note: Mean separation by least significant difference at $p < 0.05$; Means of the same letter are not significantly different from each other.

LSD, least significant difference.

*, 0.05; ***, 0.0001.

These results highlight that short-duration pre-heating substantially boosts germination and seedling vigour, while prolonged pre-chilling suppresses development, suggesting low cold tolerance in both species, particularly *Cleome*. *Amaranthus* showed greater responsiveness and resilience to thermal treatments, tolerating longer exposure with improved outcomes. This is likely because of its reduced imbibition period, which shortened germination time and enhanced germination index, value and vigour (Figure 2 to Figure 5). Baatuuwie et al. (2019) proved the findings by reporting a positive correlation between germination rate and seedling growth explaining that the pre-treatment contributes not only to germination but also to the survival and establishment of seedlings.

Overall, pre-chilling consistently reduced germination performance, particularly in *C. gynandra*, suggesting that warm-season species native to tropical regions are more responsive to thermal cues than to cold stress.

According to Schmidt (2000), seed coat-imposed dormancy develops during seed maturation and drying, often delaying germination. The presence of an impermeable seed coat poses a key challenge to germination in indigenous plants, which can be mitigated through thermal pre-treatment. Pre-heating seeds has proven to be an effective pre-sowing strategy, enhancing germination and establishment by removing the cuticle and part of the palisade layer, thereby breaking dormancy (Zembele & Ngulumbé 2022).

The study highlights the importance of evaluating seed quality prior to sowing, with particular attention to species-specific dormancy levels, optimal temperature ranges and effective pre-treatment methods. Thermal stimulation, notably pre-heating for short durations, proved to be the most effective approach for enhancing germination rates, seedling vigour and early growth, especially in *Amaranthus*. Conversely, prolonged chilling consistently hindered performance, highlighting the sensitivity of these warm-season species to cold-induced dormancy. The findings reinforce that selecting appropriate pre-treatment strategies

tailored to the ecological background of each species can significantly improve propagation success. Furthermore, seed storage conditions and shelf life play a critical role in conservation programmes, ensuring seed viability and quality from harvest through to sowing. Integrating thermal pre-treatment into seed handling protocols can contribute to improved seedling establishment and resource efficiency, laying the foundation for robust production and sustainable plant development.

Conclusion

The results of this study carry broader implications for strengthening seed systems and advancing the propagation of indigenous crops. By establishing species-specific protocols, research can generate practical knowledge that elevates underutilised crops into mainstream agricultural practice. Policy frameworks that recognise and support indigenous species within national seed standards will ensure their inclusion in formal seed systems, while development initiatives can leverage these findings to promote community seed banks, farmer training and local seed enterprises. Such integration enhances food system resilience, diversifies dietary options and safeguards biodiversity. Ultimately, the adoption of scientifically validated pre-treatment methods, particularly thermal stimulation, can serve as a catalyst for more efficient seed systems, bridging conservation and production goals. Aligning these practices with research agendas, supportive policies and farmer-centred development programmes will not only improve propagation success but also empower communities, strengthen local economies and contribute to sustainable agricultural transformation.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

CRedit authorship contribution

Onkgolotse G. Moatshe-Mashiq: Conceptualisation, Methodology, Investigation, Writing – original draft, Visualisation, Project administration, Data curation, Resources, Funding acquisition. Patrick K. Mashiq: Conceptualisation, Methodology, Formal analysis, Visualisation, Project administration, Software, Validation, Data curation, Resources, Writing – review & editing, Funding acquisition. All authors reviewed the article, contributed to the discussion of results, approved the final version for submission and publication and take responsibility for the integrity of its findings.

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Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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