Vol. 14, No. 5 (2025): 1726 - 1737 https://doi.org/10.23960/jtepl.v14i5.1726-1737

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Effect of Storage Duration and Seed Type on the Viability and Growth of Agave (*Agave sisalana* L. Perrine) from In Vitro Culture

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Article History:

Received : 11 June 2025
Revised : 22 July 2025
Accepted : 28 August 2025

Keywords:

Agave,
Duration,
In vitro culture,
Seeds,
Storage.

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ABSTRACT

Agave seeds have a relatively short lifespan, thus require appropriate storage methods to maintain their viability and germination capacity. This study aimed to identify the optimal combination of storage duration and seedling treatments to preserve seedling quality and growth performance of agave. The experiment was conducted in a factorial randomized complete design with three replications. Each experimental unit consisted of 10 plants. The first factor was storage duration (0, 21, and 35 days). The second factor was seedling treatments involving seeds with: (1) complete leaves and roots, (2) complete leaves and half roots, (3) top leaves and half roots, (4) complete leaves without roots, and (5) top leaves and no roots. The seedlings were stored in a greenhouse with average temperature 25.67 °C and relative humidity 82.92%. The observed parameters included weight loss, germination percentage, seedling height, number of leaves, leaf length and width, number and length of roots, and fresh weight after 45 days of planting. The results showed that storage duration and root or leaf trimming significantly affected seed viability and seedling growth. Storage for 21 days combined with full leaves and half roots produced the highest germination percentage, growth performance, and fresh weight. Agave seeds can be stored for up to 35 days while maintaining high viability, and different seed types stored for 35 days still exhibited 100% germination.

1. INTRODUCTION

Agave is a versatile strategic commodity widely utilized as a source of fiber, raw material for beverages, bioenergy, and other derivative products. The global demand for agave fiber has reached approximately 319,000 tons per year (Parnidi *et al*., 2016; Parnidi & Setyo Budi, 2016; Santoso & Cholid, 2019). However, worldwide production accounts for only 281,800 tons annually, indicating that global fiber demand remains unmet. This gap presents a promising opportunity for further expansion of agave cultivation.

The production of uniform, pathogen-free planting material is crucial for the success of large-scale agave cultivation. Due to the slow pace of conventional propagation, tissue culture techniques have become the primary method for mass production of elite clones as well as for germplasm conservation (Bautista-Montes *et al*., 2022; Cruz-Cruz *et al*., 2013). Despite significant advances in micropropagation, major challenges persist during acclimatization and subsequent distribution stages.

During the transition from in vitro to ex vitro conditions, agave plantlets often experience high vulnerability due to abrupt environmental changes, imbalanced shoot-to-root ratios, and physiological disorders that trigger transplant shock (Morales-Ramos *et al*., 2024; Puente-Garza *et al*., 2015). Several strategies, such as media optimization, humidity regulation, and the application of arbuscular mycorrhizal fungi (AMF), have proven effective in enhancing survival rates and nutrient uptake of agave plantlets during acclimatization (Aguilar *et al*., 2016; Morales-Ramos *et al*., 2024). However, most studies have concentrated on the early acclimatization stage, while handling practices for seedlings post-acclimatization up to distribution remain underexplored.

Efficient short-term storage methods are essential to maintain the viability and quality of planting materials during distribution. Packaging technologies and controlled storage conditions have been demonstrated to effectively preserve the viability and quality of horticultural propagules. Chauhan *et al*. (2019) reported that a microplate system combined with vacuum packaging and slow-growth storage at 10 °C in darkness successfully maintained the viability of potato and ginger micro-cuttings at 81–100%. Similarly, the application of Modified Humidity Packaging (MHP) in potted roses under simulated transportation and room-temperature storage conditions significantly reduced water loss without compromising quality (Luca *et al*., 2021). In cut orchids (*Dendrobium*), Modified Atmosphere Packaging (MAP) at 13 °C and 95% relative humidity effectively reduced respiration and ethylene production, thereby extending vase life compared to normal atmospheric conditions (Poonsri, 2021). In contrast, specific studies addressing storage and distribution of agave seedlings derived from tissue culture, particularly after acclimatization, are still limited (de Rosario *et al*., 2025).

In several plant species, root trimming and partial defoliation have been applied to facilitate packaging, reduce transpiration, and minimize mechanical damage during transportation (Li Jun-Nan *et al*., 2014; Hua *et al*., 2012). Root pruning can stimulate the regeneration of fine roots, whereas leaf reduction can temporarily decrease water loss. Nevertheless, their long-term effects on plant growth vary among species and have not been empirically tested in post-acclimatized agave seedlings.

This knowledge gap is particularly critical in Indonesia, where seedling production sites are often located far from cultivation areas, cold-chain facilities are limited, and distribution typically occurs at ambient temperature using simple packaging methods. General recommendations for bare-root seedling storage in forestry species such as low temperature and high humidity (Burdett, 1990; Li Jun-Nan *et al*., 2014) have yet to be validated for tissue-culture-derived agave seedlings aged three months post-acclimatization. Therefore, this study aimed to identify the optimal combination of storage duration and root or leaf trimming treatments to preserve the quality and growth performance of agave seedlings.

2. METHOD

2.1. Time and Research Materials

The study was conducted from January to September 2022 in the greenhouse of the Indonesian Sweetener and Fiber Crops Research Institute. The plant material consisted of *in vitro*-derived agave seedlings that had undergone a three-month acclimatization period. Uniform, healthy seedlings with an initial fresh weight of 80–100 g were selected as experimental materials. Additional materials included trays, newspaper, plastic bags, an analytical balance, a vernier caliper, and other standard research tools. The morphological conditions of the agave seedlings used in this study are presented in Figure 1.

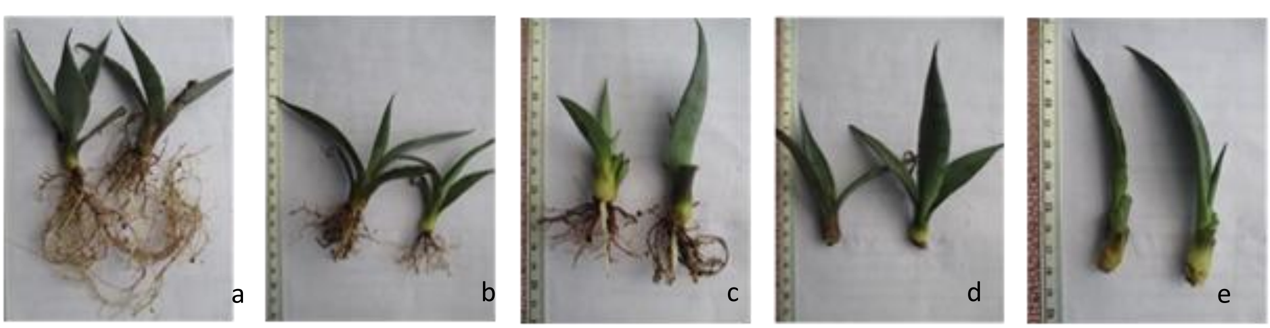

Figure 1. Condition and shape of agave seeds before storage: (a) S1, (b) S2, (c) S3, (d) S4, and (5) S5

2.2. Research Design

The experiment was arranged in a two-factor factorial design using a Randomized Complete Design (RCD) with three replications. Each experimental unit consisted of 10 seedlings. Factor 1 is storage duration consisted of K0 = Control (no storage), K1 = Storage for 21 days, and K2 = Storage for 35 days. Factor 2 is seedling types (root and leaf trimming treatments) involved: S1 (seeds with complete leaves and complete roots), S2 (seeds with complete leaves and half roots), S3 (seeds with top leaves and half roots), S4 (seeds with complete leaves without roots), and S5 (seeds with top leaves and no roots). Figure 1 described seed used in this experiment. In total, 15 treatment combinations (3 × 5) were tested, involving 450 seedlings.

2.3. Research Implementation

2.3.1. Seedling Storage Duration

Agave seedlings were weighed to determine their initial fresh weight (FW_0). Root and/or leaf trimming was carried out according to the treatment. The seedlings were then wrapped in newspaper, placed in white plastic bags, and stored in a greenhouse at an average temperature of 25.67 °C and relative humidity of 82.92% for the designated storage periods (0, 21, or 35 days). Storage was conducted under non-aseptic environmental conditions. The parameters observed during storage included:

1. Reduction in fresh weight after storage (%) was calculated from initial fresh weight (FW_0) and fresh weight after storage (FW_s):

$$FW\ reduction\ (\%) = \frac{FW_0 - FW_S}{FW_0} \times 100 \quad (1)$$

2. Qualitative observations of leaf and root conditions after storage in each treatment.

2.3.2. Post-Storage Seedling Evaluation

Stored seedlings were transplanted into polybags (10 cm × 12.5 cm) filled with a soil : sand : compost mixture (3:1:1). Seedlings were maintained for 45 days following standard agave cultivation practices, including watering and fertilization. The growth parameters observed were as follows:

1. Post-storage viability (%): A seedling was considered viable if it survived and exhibited new growth (≥ 1 new leaf or ≥ 1 cm root elongation) on day 15 after planting, with reconfirmation on day 45. Viability was calculated as:

$$Viability\ (\%) = \frac{Number\ of\ viable\ seedlings}{Total\ seedlings} \times 100 \quad (2)$$

2. Plant height (cm): Measured from the media surface to the tip of the longest upright leaf on day 45.
3. Leaf length (cm): Measured on the longest leaf on day 45.
4. Leaf width (cm): Measured at the widest section of the widest leaf on day 45.
5. Number of roots: Counted as the total number of primary roots that developed or persisted on day 45 after seedlings were removed from the polybags and roots were gently washed.
6. Root length (cm): Measured from the base to the tip of the longest root on day 45.
7. Increase in fresh weight after 45 days (%):

$$FW\ increase\ (\%) = \frac{FW_{45} - FW_S}{FW_S} \times 100 \quad (3)$$

where FW_s = fresh weight after storage, and FW_{45} = total fresh weight (shoots + roots) on day 45 after carefully washing off media and air-drying the plant surface for 10 minutes.

2.4. Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) with PKBT STAT 3.2 software. When significant differences were detected, post-hoc comparison was performed using the Honestly Significant Difference (HSD) test at a 95% confidence level.

3. RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed that both seedling type and storage duration had a highly significant effect on the reduction of fresh weight after storage. Storage duration significantly affected plant height, number of leaves, and fresh weight after replanting. Seedling type had a significant effect on leaf width. In contrast, storage duration, seedling type, and their interaction did not significantly influence the number or length of roots after replanting.

Table 1. Summary of analysis of variance for the effects of storage duration and seedling type on agave seedlings.

Parameters	Storage duration	Seed types	Storage duration x Seed types	CV (%)
Reduction in Fresh Weight After Storage (%)	**	**	**	5.76
Viability (%)				0
Plant Height (cm)	*	tn	tn	12.81
Leaf Width (cm)	**	tn	tn	12.52
Number of Leaves	tn	**	tn	15.67
Number of Roots	tn	tn	tn	20.9
Root Length (cm)	tn	tn	tn	17.09
Increase in Seedling Fresh Weight After Replanting (%)	*	tn	tn	21.76

3.1. Effect of Storage Duration and Seedling Type on the Viability and Growth of Agave Seedlings

The analysis of variance indicated that prolonged storage led to a greater reduction in the fresh weight of agave seedlings (Figure 2 and 3). Treatments involving organ reduction (root and/or leaf trimming) significantly influenced the rate of fresh weight loss during storage. Seedlings with complete organs (intact leaves and roots) exhibited the highest reduction in fresh weight, whereas seedlings with only top leaves and without roots showed the smallest reduction (Table 2). This phenomenon can be explained by differences in tissue structure: root organs contain relatively more vascular tissues, which facilitate water loss during storage, while agave leaves possess a thick waxy cuticle that reduces transpiration, thereby minimizing water loss (Taiz *et al.*, 2015).

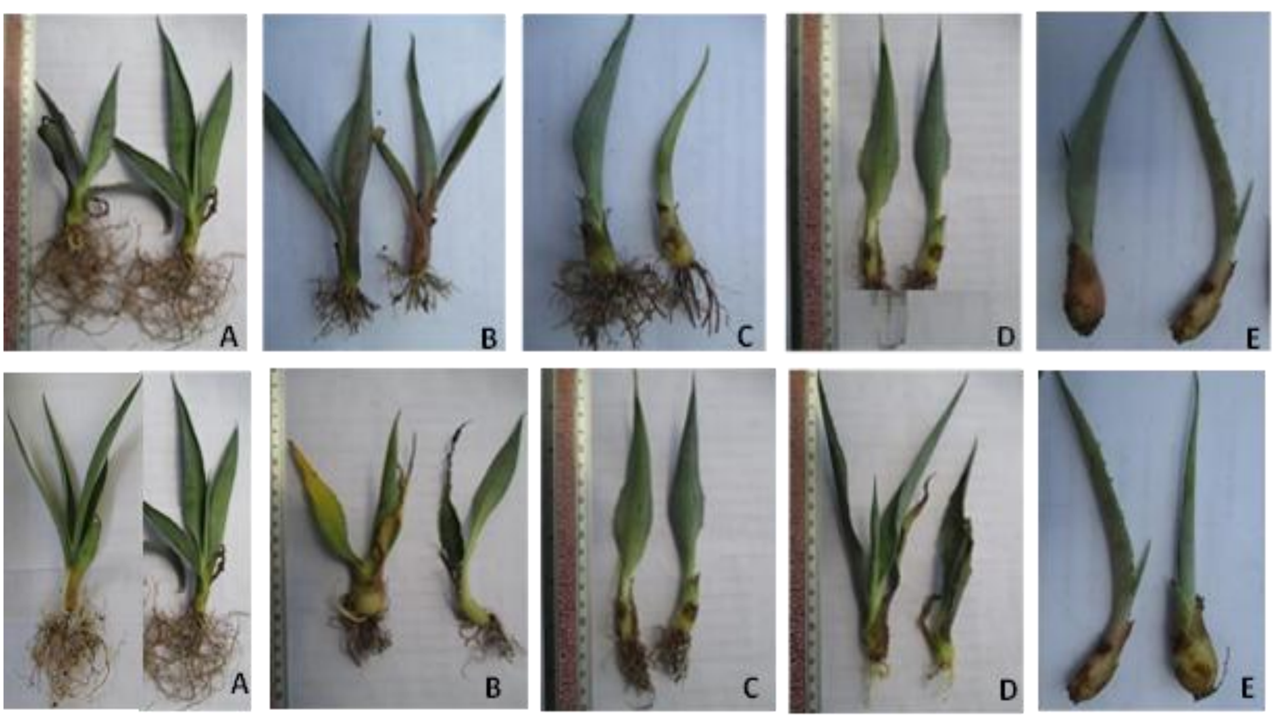

Figure 3. Various types of agave seeds after storage for 21 days (top), and 35 days (bottom)

Table 2. Effect of storage duration and seedling type on fresh weight reduction of agave seedlings.

Seed types	Storage duration (days)			Average of Seed types
	0	21	35	
Complete leaves and roots	0.00a	60.57a	39.32a	33.30a
Complete leaves and half roots	0.00a	58.15ab	36.01ab	31.39ab
Top leaves and half roots	0.00a	48.71c	31.62cd	26.78c
Complete leaves without roots	0.00a	56.12b	34.76bc	30.29b
Top leaves and no roots	0.00a	47.47c	29.06d	25.51c
HSD 5%	4.12	4.12	4.12	2.38
Average of Storage duration	0.00c	54.20a	34.15b	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

Agave seedlings can be classified as having recalcitrant seed characteristics due to their hight water content, which makes them less tolerant to long-term storage. This finding is consistent with Ariyani *et al*. (2023), who reported that both seeds and seedlings tend to lose weight during storage as a result of water loss. In the present study, the smallest reduction in fresh weight after 21 days of storage was observed in seedlings with top leaves and no roots (32.12%), whereas the largest reduction occurred in seedlings with intact leaves and roots (37.66%). A similar trend was observed after 35 days of storage, with the lowest reduction recorded in seedlings with top leaves and no roots (22.26%) and the highest in seedlings with intact leaves and roots (27.96%). In addition to fresh weight reduction, storage for up to 35 days also induced elongation of young leaves and the formation of new roots. Leaf elongation was likely an etiolation response caused by low-light storage conditions (Taiz *et al*., 2015). Meanwhile, new root formation may be considered a positive response, as roots play an essential role in water and nutrient absorption following transplanting. Alwani et al. (2019) reported that seedlings with a more developed root system are better able to withstand field conditions compared to seedlings without roots.

Interestingly, the results demonstrated that neither storage duration nor seedling type had a significant effect on germination capacity, as all treatments showed a 100% germination rate (Table 3). This contrasts with the general seed viability theory, which states that prolonged storage typically reduces seed viability due to the decline in food reserves and water content (Alwaniet al., 2019; Hairani *et al*., 2020; Ariyani *et al*., 2023). Similarly, Annisa et al. (2015) emphasized that extended storage without special treatment results in reduced physiological function, ultimately lowering seed vigor and viability. However, the findings of this study revealed that agave seedling viability remained stable despite fresh weight reduction. This could be attributed to the biological differences between vegetatively grown seedlings and true seeds. Seedlings possess active vegetative organs (leaves and roots), and their resilience is determined more by the physiological status of these organs than by internal food reserves, as is the case in seeds (Pirredda *et al.*, 2023). Therefore, agave seedlings can be stored for up to 35 days without compromising their growth potential, although a reduction in fresh weight does occur.

Table 3. Effect of storage duration and seedling type on viability of agave seedlings.

Seed types	Storage duration (days)			Average of Seed types
	0	21	35	
Complete leaves and roots	100	100	100	100
Complete leaves and half roots	100	100	100	100
Top leaves and half roots	100	100	100	100
Complete leaves without roots	100	100	100	100
Top leaves and no roots	100	100	100	100
HSD 5%	-	-	-	-
Average of Storage duration	100	100	100	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

3.2. Effect of Storage Duration and Seedling Type on the Seedling Growth

3.2.1. Plant Height

The results showed that storage duration had a significant effect on agave plant height, whereas seedling type and the interaction between the two factors were not significant (Table 4). The shortest plants were observed after 35 days of storage, followed by 21 days of storage, while the tallest plants were recorded in the control (no storage). This outcome aligns with the general physiological principle of seedling storage prior to transplanting. Delayed planting tends to induce drought stress due to exposed root systems and limited substrate, thereby reducing post-transplant shoot growth (McKay, 1997). Similarly, bare-root nursery practices have shown that delayed transplanting increases root desiccation and reduces vigor and early growth compared to immediate planting (Goyette *et al*., 2023).

Table 4. Effect of storage duration and seedling type on agave plant height.

Seed types	**Storage duration**			**Average of Seed types**
	0	**21**	**35**	
Complete leaves and roots	20.93	19.05	18.15	19.38
Complete leaves and half roots	21.38	20.38	17.6	19.79
Top leaves and half roots	20.03	18.15	16.57	18.25
Complete leaves without roots	21.7	20.43	17.1	19.74
Top leaves and no roots	21.1	19.92	14.7	18.57
HSD 5%	-	-	-	-
Average of Storage duration	21.03a	19.59ab	16.82b	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

From a hormonal perspective, prolonged storage periods may increase the accumulation of growth-inhibitory signals, particularly abscisic acid (ABA), and alter the ABA-to-ethylene ratio. This hormonal balance has been shown to regulate shoot growth sensitivity and suppress cell elongation during the early stages of seedling development (Muller, 2021; Hua *et al*., 2012). Crosstalk among phytohormones, including ABA, ethylene, and jasmonates, also contributes to the inhibition of cell elongation under stress conditions (Alonso *et al*., 2024).

In addition to hormonal regulation, carbohydrate availability plays a decisive role. Longer storage increases maintenance respiration, which in turn reduces non-structural carbohydrate reserves essential for the growth of new leaves and the elongation of young tissues (Liu *et al*., 2020). For seedlings, the depletion of such metabolic reserves significantly compromises initial vigor (Hernández-Cuevas *et al*., 2023).

Specifically in agave, recent studies have emphasized the importance of ex vitro seedling quality, including root-to-shoot balance, metabolite reserves, and water status, as critical determinants of post-transplant performance (Delgado-Aceves *et al*., 2025). Seedlings transplanted immediately after removal from culture or nursery conditions exhibit superior growth performance compared to those subjected to delayed planting (Sanchez *et al*., 2020). Consistent with these findings, our study demonstrates a reduction in plant height as storage duration increases. This trend parallels observations in other crops. In sugarcane, for instance, storage of planting materials beyond a few days results in reduced sprouting capacity and delayed early growth due to decreased water availability and energy reserves (Parnidi & Hamida, 2021). The overarching mechanism involves the combined effects of stress-induced metabolic depletion and water loss, ultimately constraining vegetative growth, including plant height.

3.2.2. Leaf Width and Number of Leaves

The results showed that leaf width was significantly affected by storage duration, while the number of leaves was mainly influenced by seedling type. Seedlings planted immediately after removal (0 days storage) exhibited greater average leaf width compared to those stored for 21 and 35 days (Table 5). The reduction in leaf width can be attributed to the decreased availability of photosynthates as a result of storage-induced stress. Drought stress or water deficit occurring during storage suppresses cell expansion by lowering turgor pressure, thereby limiting cell wall loosening and expansion. Consequently, leaf blade widening is restricted, resulting in narrower leaves (Scheres & van der Putten, 2020; Cosgrove, 2024).

This physiological mechanism is further reinforced by source–sink dynamics, whereby under stress conditions photosynthates are preferentially allocated to maintenance respiration and osmotic regulation rather than to the formation of new organs. As a result, leaf development is constrained and expansion is reduced (Fernie *et al*., 2020).

Meanwhile, the number of leaves was significantly influenced by seedling type. Seedlings with rolled leaves combined with either half roots or no roots produced a greater number of leaves compared to other types. This response is likely due to the adaptive role of leaf rolling, which reduces water loss through transpiration, thereby maintaining relatively higher tissue water content. Leaf rolling also helps preserve membrane integrity and sustain physiological activity in the shoot apex, enabling continued initiation of new leaves even under suboptimal storage conditions (Nar *et al*., 2009). Consequently, although leaf size tends to be smaller, leaf number can still be maintained.

Table 5. Effect of storage period and seed type on leaf width and number of leaves

Seed types	Leaf Width (days)			Average of Seed types	Leaves Number (days)			Average of Seed types
	0	21	35		0	21	35	
Complete leaves and roots	2.87	2.37	1.97	2.4	3.17	3.17	2.83	3.06b
Complete leaves and half roots	2.45	2.53	2.4	2.46	3	3.67	3	3.22ab
Top leaves and half roots	2.83	2.47	1.98	2.43	4.33	3.83	3.83	4.00a
Complete leaves without roots	2.6	2.33	2.28	2.41	4.33	3.67	3.17	3.72ab
Top leaves and no roots	2.77	2.9	2.13	2.6	4.67	4	3.33	4.00a
HSD 5%	-	-	-	-	-	-	-	0.79
Average of Storage duration	2.70a	2.52a	2.15b		3.9	3.67	3.23	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

Root reduction in certain seedling types also influenced shoot growth responses. Root pruning is known to trigger compensatory growth through the initiation of new roots, while simultaneously redirecting assimilates toward shoot development. This mechanism may explain why seedlings with half roots or no roots produced a greater number of leaves, even though their early growth was temporarily delayed due to physiological adjustment (Kiwai *et al*, 2022; Feng *et al*., 2022; Jimenez-Torres *et al*., 2022). The quality of agave seedlings is strongly dependent on water status, metabolite reserves, and root system integrity. Our findings confirmed that seedlings transplanted immediately after storage exhibited higher vigor compared with those subjected to delayed planting, since prolonged storage stress often leads to reduced leaf size and suppressed growth (Delgado-Aceves *et al*., 2025). Collectively, these results emphasize the importance of minimizing storage duration to preserve seedling quality.

3.2.3. Root Number and Length

The results indicated that neither storage duration nor seedling type had a significant effect on root number or root length, although seedlings with complete organs tended to show relatively better root growth (Table 6). This can be explained by the highly responsive nature of root systems to environmental conditions, including water availability, oxygen levels, and energy reserves (Młodzińska, 2023). Seedlings with intact organs are more likely to sustain physiological continuity, which supports stable adventitious root formation and root elongation compared with other seedling types.

Table 6. Effect of storage duration and seed type on the number and length of agave roots

Seed types	Root Number (days)			Average of Seed types	Root Length (days)			Average of Seed types
	0	21	35		0	21	35	
Complete leaves and roots	13.83	13.83	11.33	13	22.05	19.1	18	19.72
Complete leaves and half roots	12	11.67	11.17	11.61	21.13	17.7	19.13	19.32
Top leaves and half roots	13	12.83	10.17	12	19.47	17.57	17.2	18.08
Complete leaves without roots	11	13	7.5	10.5	19.94	20.12	18.03	19.36
Top leaves and no roots	11	10.17	9.33	10.17	17.03	21.35	18.12	18.83
HSD 5%	-	-	-	-	-	-	-	-
Average of Storage duration	12.17	12.3	9.9		19.92	19.17	18.1	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

An intriguing finding of this study was the formation of new roots in seedlings whose roots had been completely removed. This response represents a physiological adaptation to the loss of root tissue, in which the plant triggers regeneration through the activation of new root meristems or the differentiation of parenchyma cells at the stem base. The ability to produce new roots reflects a form of morphological plasticity that ensures plant survival, and in many species, rapid root regeneration is a key determinant of successful adaptation following stress or root manipulation (Chauhan *et al*., 2019; Tian *et al*., 2022).

In vegetative propagation, the capacity for new root formation is a critical trait because roots serve as the primary channels for water, nutrient, and mineral uptake. The faster new roots are established, the more effectively seedlings can overcome the stagnation phase of growth and restore metabolic activity. Recent studies in various horticultural and forestry species have demonstrated that root pruning can activate hormonal signaling particularly auxin, cytokinin, and ethylene that accelerates adventitious root initiation (Lakehal & Bellini, 2019). Thus, although root excision initially removes the main absorptive organ, seedlings retain an adaptive capacity to regenerate roots during storage. Agave seedlings with stronger root regeneration potential are able to adapt more rapidly to new environments, even after undergoing root removal treatments (Delgado-Aceves *et al*., 2025). This aligns with the present findings, where new roots were consistently observed in the complete-root removal treatment, enabling seedlings to continue growth despite the initial loss of roots.

3.2.4. Fresh Weight

The results showed that fresh weight was significantly affected by both storage duration and seedling type, but not by their interaction (Table 7). The highest fresh weight was obtained in seedlings that were transplanted immediately without storage, whereas storage up to 35 days resulted in a substantial decline. This reduction can be attributed to decreased tissue water content and reduced biomass accumulation during the storage period. Such conditions are consistent with earlier reports (Sedigheh *et al*., 2011; Anjum *et al*., 2011), which highlight that the longer plants are subjected to stress without an adequate supply of nutrients and light, the greater the risk of water loss through respiration and transpiration, ultimately leading to reduced total biomass.

Table 7. Effect of storage duration and seed type on the fresh weight of agave seedlings

Seed types	**Storage duration (days)**			**Average of Seed types**
	0	**21**	**35**	
Complete leaves and roots	187.89	91.6	62.72	114.07
Complete leaves and half roots	180.23	128.51	36.11	114.95
Top leaves and half roots	183.07	105.62	61.12	116.6
Complete leaves without roots	202.03	120.76	46.58	123.13
Top leaves and no roots	179.66	124.18	54.92	119.59
HSD 5%	-	-	-	-
Average of Storage duration	186.58a	114.13ab	52.29b	

Values followed by the same letter within the same column are not significantly different according to HSD test at the 5% level.

Seedling type also had a significant effect on fresh weight. Seedlings with intact leaves but without roots exhibited the highest fresh weight compared to other seedling types, whereas the lowest fresh weight was recorded in seedlings with only upper leaves and no roots. This finding highlights the critical role of leaves in sustaining photosynthesis, even in the absence of a complete root system. Conversely, reducing leaf area can diminish photosynthetic capacity, although it may simultaneously reduce the rate of transpiration. As noted by Ariyani *et al.* (2023), a smaller leaf surface area reduces the number of active stomata, thereby lowering water loss through transpiration and extending seedling viability during storage. Thus, a trade-off exists between the function of leaves as photosynthate producers and their role as organs that potentially increase water loss.

Fresh weight can also serve as an indicator of metabolite accumulation. The primary factors influencing fresh weight are the number of roots, which support water and nutrient uptake, and the leaf area, which determines photosynthetic capacity. The greater the amount of photosynthate produced and distributed into plant tissues, the higher the fresh weight. These results are consistent with previous findings showing that the presence of both roots and leaves in optimal amounts enhances biomass accumulation through improved photosynthetic activity and nutrient absorption (Juprianto *et al*., 2018; Oktaviana *et al*., 2015). More recent studies further emphasize that fresh biomass is often used as an important parameter to evaluate seedling quality, as it is strongly correlated with plant vigor following transplantation (Delgado-Aceves *et al*., 2025).

From a practical perspective, these findings provide several important implications: (i) minimizing the storage duration after acclimatization to preserve leaf turgor and expansion,(ii) maintaining temperature and humidity during unavoidable storage to reduce water loss and maintenance respiration, (iii) selecting seedling types with rolled leaves that help retain leaf number during storage, even if the leaf size tends to be smaller, and (iv) carefully managing root pruning to avoid reducing initial vigor while still allowing compensatory growth.

4. CONCLUSION

In vitro derived agave seedlings exhibit a relatively long storage potential, with storage durations of 21 to 35 days maintaining high viability. Seedling treatment involving the reduction of leaf organs (leaving leaves in a rolled condition), complete removal of root organs, and subsequent storage for 35 days resulted in 100% germination capacity and successfully induced new root formation. For long distance distribution, reducing leaf and root organs was also effective in decreasing seedling volume and weight, thereby lowering transportation costs. These findings highlight that seedling management through vegetative organ modification and optimized storage duration represents a practical strategy not only to preserve the quality and viability of agave seedlings but also to enhance distribution efficiency and broaden their utilization potential.

ACKNOWLEDGMENTS

Our gratitude goes to the Head of the Source Seed Procurement Unit (UPBS) of the Fiber Crops Research Center for permission to use research materials in the form of agave seeds from in vitro culture. We would like to thank the Head of the Karangploso Experimental Garden of the Sweetener and Fiber Crops Research Center (Balittas) for permission to use the greenhouse during the activity.

AUTHOR CONTRIBUTIONS

M. Murianingrum, Parnidi, Moch. Machfud, and Marjani: conceptualization, design methodology, and data analysis. Fatkhur Rochman, Mala Murianingrum, and Parnidi: data curation. Mala Murianingrum, Parnidi: writing the manuscript draft. Marjani and Fatkhur Rochman: visualization and editing the manuscript. Bambang Heliyanto, Rully Dyah Purwati; supervision, review the manuscript. All the authors contributed equally to this research and the drafting of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they do not have any commercial or associative interest that represents a confict of interest in connection with the work submitted.

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