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Seed viability assessment of *Campolay* fruits (*Lucuma campechiana*) across varying weights and storage periods

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Abstract. *Widjaya AH, Latifah D, Primananda E, Zulkarnaen RN, Rahayu A. 2024. Seed viability assessment of* Campolay *fruits (*Lucuma campechiana*) across varying weights and storage periods. Nusantara Bioscience 16: 23-28.* *Campolay* (*Lucuma campechiana* Kunth), a locally cultivated fruit in West Java, Indonesia, has become popular in gardens and yards, although its trading activity remains relatively limited. The methods used for propagating this fruit include seeds, grafting, and layering. However, hard and impermeable seed coats present a challenge in germination. This research aims to investigate the impact of seed weight and storage duration on the viability of *Campolay* seeds. This will be done by employing a factorial, completely randomized design. The study will examine various variables related to germination, including total germination, germination rate, simultaneity, time to achieve 50% germination, time to first germination, and time to final germination. Although these factors do not significantly affect total germination, it was observed that seeds stored for longer periods, up to 6 weeks, exhibited higher germination rates and faster germination times. Interestingly, the seeds' weight did not impact germination, and there was no interaction between the duration of storage and the seed weight. The overall capacity for seed germination ranged from 92% to 97%. These findings indicate that storing *Campolay* seeds in moist sawdust under dark conditions can delay germination, which ensures a more consistent and uniform sprouting process. Moreover, this approach can also aid in the transportation of the seeds and support programs aimed at conserving plant germplasm.

Keywords: *Campolay*, germination, seed viability, seed weight, storage periods

Abbreviations: BBG: Bogor Botanical Gardens; DAS: Day After Sowing; DMRT: Duncan Multiple Range Test; GT: Germination Total; ISTA: International Seed Testing Association; MC: Moisture Content; P: Periode; P50: The number of day required for 50% of the seed to germinate after sowing; RHS: Royal Horticultural Society; RH: Relative Humidity; STAR: Statistical Tool for Agricultural Research; W: Weight

INTRODUCTION

Campolay (*Lucuma campechiana* Kunth) is a tropical fruit that traces its roots back to the Sapotaceae family (POWO 2023). *Campolay* is a plant originating from Mexico. This exotic fruit reached the Philippines in 1915, later making its mark across Southeast Asian countries, including Indonesia. In the archipelago, it goes by many names, such as *Sawo Mentega, Sawo Ubi, Alkesa, Kanistel*, and *Sawo Belanda*. Across the seas in Taiwan, locals fondly refer to it as *Xiantao*, a poetic moniker translating to the "peach of the immortals". The nomenclature "*Campolay*" itself pays homage to the Mexican city of Campeche, and in English-speaking regions, it is recognized as canistel, egg fruit, or yellow sapote (Amalia et al. 2021; Pertiwi et al. 2022).

The allure of *Campolay* lies not only in its diverse nomenclature but also in its nutritional richness. This tropical gem is laden with high carbohydrates and calories, making it a substantial dietary component, as *Vatica venulosa* Blume contains 42.5% carbohydrates (Widjaya et al. 2021a). Further analysis reveals its mineral composition, boasting notable amounts of iron (Fe), vitamin B3 (niacin), and carotene (pro-vitamin A), which imparts the fruit its striking yellow hue. Beyond being savored as a standalone delicacy, *Campolay* finds its way into culinary creativity, contributing to snack noodles when combined with ingredients such as guar gum and mocaf (Lim 2013; Karsinah and Rebin 2013).

The scientific community has delved into unraveling the intricacies of *Campolay*, exploring its nutritional content, taxonomy, and processed products. Studies have unveiled a treasure trove of essential elements within the fruit, including vitamin C, total carotene, total sugar content, and an array of minerals, including calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) (Pertiwi et al. 2022; Do et al. 2023). These findings highlight Campolay's gastronomic appeal and its potential as a health-enhancing dietary component (Elsayed et al. 2016; Pai and Shenoy 2020).

Campolay's propagation, a vital aspect of its cultivation, predominantly relies on seeds. These seeds, with taproots that fortify adult plants, contribute to the plant's resilience, even though its fruiting age is relatively prolonged, spanning 3-6 years. However, the journey from seed to plant is not without challenges. The seeds are endowed with hard, impermeable seed coats, endowing them with dormancy properties. Breaking this dormancy is essential for the germination process. Without the requisite treatment, germination may be delayed, occurring 2-3 months after planting (Crane and Carlos 2013). These seeds, classified as recalcitrant, exhibit short viability, with storage at room temperature (20°C) maintaining their viability for up to six months (Hong et al. 1996; Malavert et al. 2017).

Cultivating *Campolay* is not without its hurdles. The germination stage, marked by dormancy properties and variations in seed weight, presents challenges (Crane and Carlos 2013). The hard seed coats, impermeable to water, require specialized treatment for dormancy breaking (Zulkarnaen et al. 2015; Latifah et al. 2020). Moreover, disparities in seed weight can influence the overall viability and vigor of *Campolay* seeds. These challenges underscore the need for a nuanced understanding of the interplay between seed weight and viability, particularly in germination and seedling health (Baskin and Baskin 2014; Bian et al. 2018).

This directs attention to the principal objective of the contemporary research initiative: a systematic inquiry into the correlation between seed weight and the viability of *Campolay* seeds. This entails a comprehensive scrutiny encompassing germination rates and the overall vigor of ensuing seedlings. Concurrently, the study attempts to elucidate the recalcitrant attributes of *Campolay* seeds across diverse storage durations. This factor is important for seed banking initiatives and long-term cultivation planning (Zulkarnaen et al. 2020; Mueller et al. 2021). The study aspires to offer practical solutions for *Campolay* growers, aiding them in optimizing seed weight for enhanced viability and robust seedling health. In addition, the insights gained into the recalcitrant nature of *Campolay* seeds will contribute valuable data to the broader understanding of tropical fruit propagation and horticultural practices (Pertiwi et al. 2020).

Therefore, In the current global context, marked by a growing interest in diverse and exotic fruits, it is essential to invest in research to understand and improve *Campolay* cultivation. Such research not only benefits *Campolay* growers but also contributes significantly to our knowledge of tropical fruit cultivation, promoting sustainable agricultural practices and playing a key role in biodiversity conservation within scholarly discourse.

MATERIALS AND METHODS

Study area

Campolay fruit comes from the Bogor Botanical Gardens (BBG) in Indonesia, with the catalog number Vak IV.D. 182-182a (Figure 1) (Ariati et al. 2019). It was originally from Cuba and was planted in the gardens on March 12, 1990. The fruit was harvested on December 11, 2014, when its skin turned yellowish, which typically happens 60 to 90 days after the flowers bloom. We used tools like digital calipers and scales to study the fruit and its seeds. We also matched the colors we observed with a color chart from the Royal Horticultural Society (RHS 2007).

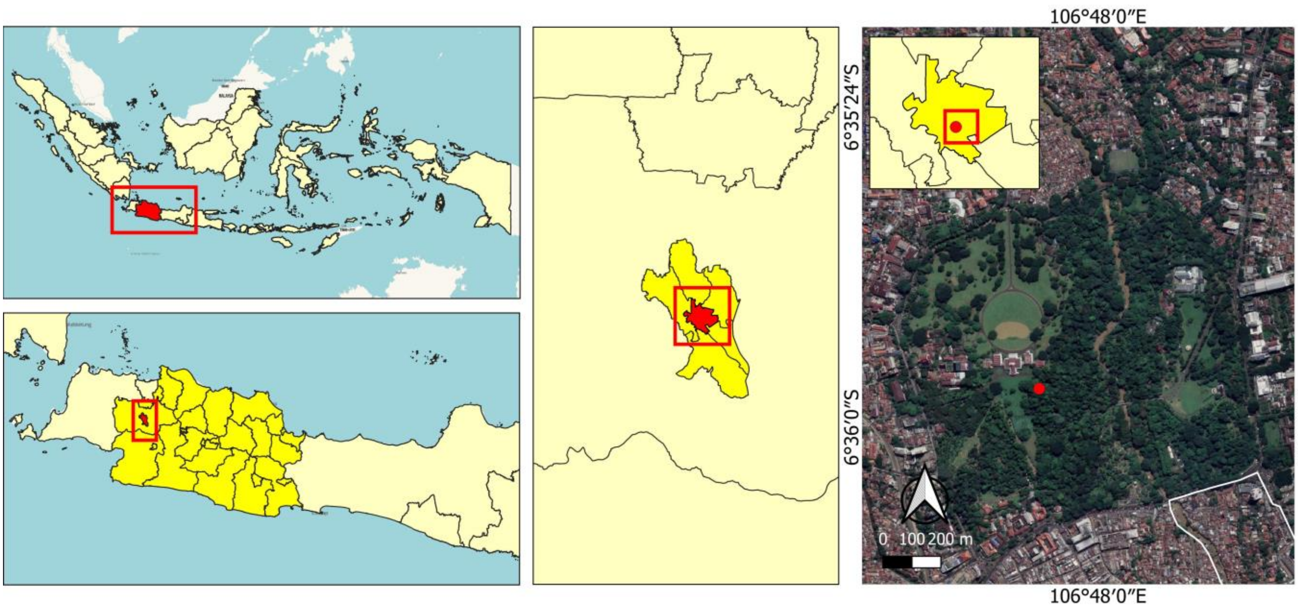

Figure 1. Map of research location in the Bogor Botanic Garden, West Java, Indonesia

Procedures

Seed weight class and seed Moisture Content (MC)

Classification of seeds based on the seed weight with analytical balance, directly separated between heavy seeds (>11.01 g), medium (9.01-11.00 g), and lightweight seeds (<9.00 g).

Seed moisture content was evaluated using a 105°C constant temperature oven for 17 hours (ISTA 2015), with the formula:

$$MC = \frac{(M2-M3)}{(M2-M1)} \times 100\%$$

M1 is the weight of the container used, M2 is the weight of the seed and the container before the oven, and M3 is the weight of the seed and the container after the oven. The initial moisture content of *Campolay* seeds was 46.66% (3 replicates, $F8.11 = 0.12$, $p < 0.05$, coefficient of variations 3.43%).

During the 0-6 weeks storage period, the moisture content of seeds was maintained in the range of 45.98%-47.08%. The range of seed moisture content showed that *Campolay* seeds had recalcitrant characteristics.

Seed germination

Germination research was conducted at the Glasshouse and Seed Bank-BBG from December 2014 to June 2015. The materials used were sand, insecticides, fungicide, a polybag 25 cm x 35 cm, a storage box, sawdust, black plastic and 100ºC hot water to sterilize media. The research design used was a factorial, completely randomized design, namely seed weight (W) and seed storage period time (P). The weight of the seeds to be tested in this study consisted of heavy (>11.01 g), medium (9.01-11.00 g), and lightweight (<9.00 g). seeds were tested at 0, 2, 4 and 6 weeks of storage. Thus, there were 12 treatment combinations with three replications of 36 experimental units (polybags). Each experimental unit consisted of 10 *Campolay* seeds, with 360 observation units. The linear design model used is:

$$Y_{ijk} = \mu \pm \alpha_i \pm \beta_j \pm (\alpha\beta)_{ij} \pm \varepsilon_{ijk}$$

Where: Yijk represents the response variable, μ stands for the overall mean, αi represents the effect of the first factor (i), βj represents the effect of the second factor (αβ)ij signifies the interaction between the first and second factors, and εijk denotes the random error. This formula is utilized in statistical analysis to describe the relationships between various factors in an experiment or study.

The germination medium used was sand in a glasshouse (microclimate conditions: average temperature 26.25±1.29°C, average humidity (RH) 78.39±4.67% and sunlight intensity 686.86±420.94 lux). The media was sterilized using hot water at 100°C, then sprinkled with about 2.5g/polybag of insecticide. *Campolay* seeds/polybags were planted after the media had cooled. Observations were made every two days, and the observed variables were

Germination total, calculated by the formula:

$$GT = \frac{n}{N} \times 100\%$$

Where: GT: Germination Total, n: seed germinated, N: seed sowing.

Germination rate, calculated by the formula:

$$x = \frac{\Sigma n}{\Sigma(txn)} \times 100$$

Where: x: Germination rate, n: Seed germinated, t: The day when the seeds germinate

Simultaneity, calculated by the formula:

$$\frac{\Sigma n}{\Sigma\{(T-t)2\,x\,n)\}} \times 100 \; ; \; T = \frac{\Sigma(txn)}{\Sigma n}$$

Where: Σn: The total number of seeds to germinate, t x n: n seeds were germinated on day t

P50 is the number of days required for 50% of the seed to germinate Day After Sowing (DAS),

The initial germination assessment is calculated from the emergence of the first sprout following the sowing of the seeds (indicating the day on which the first seed begins to germinate).

The final germination assessment is calculated from the emergence of the last sprout after seed sowing (indicating the day on which the last seed germinates).

Seed storage

Seeds were stored in plastic boxes (box containers) containing moist sawdust covered with newsprint. The storage box was tightly closed using a container box lid and closed again using black plastic to stimulate dark storage conditions so that the recalcitrant seeds could survive without germination (Latifah et al. 2014). The boxes were Stored in a room with an AC temperature of 20±2°C and Relative Humidity (RH) of 50-60%.

Data analysis

STAR (Statistical Tool for Agricultural Research) software was used for data analysis. We Analyzed of Variance (ANOVA) with the F-test at a 5% significance level to determine if the treatments had significant effects. When significant effects were observed, we applied the Duncan Multiple Range Test (DMRT) at the same 5% level to identify which treatments differed significantly (Widjaya et al. 2021b).

RESULTS AND DISCUSSION

The germination performance of *Campolay* seeds was examined, considering various factors, including seed weight and storage duration. It was determined that there were no statistically significant variations in seed germination among different seed weights and storage periods. However, it is crucial to note that although germination rates remained consistent, there were notable differences in the coefficient of germination rate and concurrent seedling growth across various storage durations (Table 1).

The germination rates of *Campolay* seeds exhibited disparities depending on the duration of storage. Seeds stored for 4 and 6 weeks demonstrated considerably faster germination than those stored for 0 and 2 weeks. This observation is particularly interesting as it suggests that the duration of storage has a noticeable impact on the germination of *Campolay* seeds. It is important to highlight that this variation was more pronounced in seeds with longer storage durations.

This difference in germination rates is attributed to the inherent properties of *Campolay* seeds, particularly the presence of a hard seed coat that renders them impermeable to water. The statistical analysis ($F_{24.35} = 55.04$, $p < 0.05$, coefficient of variation 12.43%) emphasizes the significance of this finding. Germination rate, a crucial parameter in evaluating seed vigor (Sadjad et al. 1999), reflects the seed's ability to germinate rapidly under different conditions. Seeds with higher vigor demonstrate a faster germination rate, and this characteristic is essential for the consistent and uniform emergence of seedlings, especially under diverse field conditions (Schmidt 2000).

The germination coefficient of *Campolay* seeds exhibited notable distinctions compared to a storage period of 6 weeks ($F_{24.35} = 6.16$, $p < 0.05$, coefficient of variance 100.01%; data not transformed). However, no significant differences were observed at storage durations of 0, 2, and 4 weeks, nor were there significant variations concerning different seed weights, as outlined in Table 1. After 6 weeks of storage, the seed coat may soften, allowing the seeds to absorb water and promoting germination.

The observed variations in germination coefficient at different storage durations, particularly the significant change at the 6-week mark, provide insights into the dynamic nature of *Campolay* seed dormancy and germination, contributing valuable knowledge to seed physiology and agricultural science. These findings emphasize the complex interaction between the seed coat's properties, the storage duration, and the potential for germination of *Campolay* seeds (Baskin and Baskin 2014).

Other studies have also investigated the effect of storage duration on seed germination. Nguyen et al. (2015) studied the variability in light, gibberellin, and nitrate requirement of *Arabidopsis thaliana* (L.) Heynh. seeds due to harvest time and dry storage conditions. Yilmaz and Tonguc (2013) investigated the effects of temperature on the germination of *Fraxinus ornus* subsp. *cilicica* (Lingelsh.) Yalt. seeds. Guo et al. (2020) studied the effect of temperature, light, and storage time on the seed germination of *Pinus bungeana* Zucc. ex Endl. Another study by Koutsika-Sotiriou et al. (2022) investigated the seed germination of *Silybum marianum* (L.) Gaertn. populations of Greek origin and the effects of temperature, duration, and storage conditions. For *Campolay* seeds, the observed faster germination rate after longer storage periods may reflect increased seed vigor. This could be essential for uniform and consistent seedling emergence, especially in variable field conditions. The findings emphasize the complex interaction between the properties of the seed coat, the duration of storage, and the potential for germination of seeds.

Seed size is an essential factor in seed biology, with its implications reaching the concept of seed vigor. Vigour is a critical measure of a seed's potential to thrive in unfavorable conditions, making it an essential attribute in agricultural and ecological contexts. Seeds with high vigor not only have the potential to develop into normal and healthy plants under less-than-optimal environmental conditions but also exhibit enhanced performance in ideal settings, leading to robust and productive plants. Moreover, seeds possessing vigor can endure extended periods of storage under less-than-ideal conditions while maintaining their ability to germinate and flourish when provided with favorable conditions. This is evidenced by the practices at the Royal Botanic Gardens, Kew, and the Millennium Seed Bank, where seeds are carefully dried and frozen to extend their viability, exemplifying the crucial role of seed vigor and proper storage in seed longevity (Pagano et al. 2023). Seed longevity is acquired during late maturation, accompanied by the degreening and de-differentiation of chloroplasts into non-photosynthetic plastids called eoplasts.

In contrast, seed weight is often considered a significant measurement associated with germination and viability. It provides valuable insights into a seed's capacity to initiate germination, with heavier seeds often showing more favorable germination traits. Besides, the weight of the seed functions as an indicator of the seed's long-term viability, demonstrating its potential to stay alive and maintain its germination capacity over prolonged periods (Schmidt 2000). The correlation between seed weight and these attributes is crucial in guiding seed selection, storage, and successful crop establishment, thus significantly impacting agricultural practices and ecological dynamics.

The study has revealed a strong correlation between the seed shelf life and the intricate dynamics of seed germination. Our research demonstrates a substantial increase in both the speed of germination ($F_{24.35} = 23.34$, $p < 0.05$, coefficient of variance 23.32%; data not transformed) and the earlier culmination of the germination process ($F_{24.35} = 27.38$, $p < 0.05$, coefficient of variance 14.02%; data not transformed) in seeds subjected to prolonged storage durations. This intriguing finding suggests that the longer seeds are stored, the quicker they germinate. An interesting observation is that this acceleration of germination appears to be independent of variations in seed weight (Table 2).

Moreover, the study highlights a substantial increase in the germination speed and the earlier culmination of the germination process in seeds subjected to prolonged storage durations. This effect, which significantly influences the timing of germination, remains consistent regardless of seed weight (Table 2). These findings carry significant implications for the practical implementation of seed management and conservation practices. It is suggested that the strategic use of *Campolay* seed storage can be beneficial when transporting seeds over long distances, from parent trees to production planting areas, which often takes several weeks.

The storage of *Campolay* seeds in a moist sawdust medium significantly influences the preservation of seed viability. This method plays a crucial role in maintaining the viability of the seeds (Table 2). The impact of this storage technique on shortening both the first and last day of germination indicates its effectiveness in sustaining seed viability. This discovery validates the observed phenomenon of untreated *Campolay* seeds, which typically require a significantly longer period of 2-3 months to initiate germination (Crane and Carlos 2013).

Interestingly, the weight of *Campolay* seeds does not seem to have a substantial influence on their germination capacity. The duration these seeds are stored before being sown has a significant impact. *Campolay* seeds, when stored for extended periods of up to 6 weeks, tend to germinate more efficiently and with greater synchrony. Consequently, many seeds sprout simultaneously, typically denoting favorable plant development conditions.

Additionally, the seed achieves crucial germination milestones such as the P50 (the time required for 50% of seeds to germinate), the initial and final day of germination more quickly. *Campolay* seeds demonstrate a remarkably high success rate in germination, ranging from 92% to 97%. This indicates that one can securely store these seeds for up to 6 weeks without concern about their germination capacity diminishing. In certain cases, their germination performance may even improve under extended storage conditions.

Table 1. Germination total, germination rate, and simultaneity

	Germination total (%)	Germination rate (%/day)	Simultaneity (%/day)
Storage			
0 week	92.22	1.34 [d]	0.0168 [b]
2 week	97.78	1.61 [c]	0.0241 [b]
4 week	93.33	2.05 [b]	0.0379 [b]
6 week	92.22	2.70 [a]	0.0968 [a]
Seed weight			
Weight	95.84	1.92	0.0367
Medium	92.50	1.88	0.0350
Lightweight	93.33	1.98	0.0599

Note: The same letters in the same column showed no significant differences according to DMRT at the level of 5%

Table 2. The time when 50% germination occurred, the time to first germination and the time to final germination

	Time when 50% germination occurred (day)	Time to first germination (day)	Time to final germination (day)
Storage			
0 week	74.67[a]	47.89[a]	108.67[a]
2 week	61.00[b]	33.11[b]	93.44[b]
4 week	46.00[c]	26.78[bc]	79.00[c]
6 week	37.00[d]	19.79[c]	59.78[d]
Seed weight			
Weight	54.25	33.83	81.67
Medium	58.25	32.75	85.75
Lightweight	51.50	29.08	88.25

Note: The same letters in the same column showed no significant differences according to DMRT at the level of 5%

Moreover, the study discovered that the effects of storage time on germination remain consistent across seeds of varying weights. Therefore, regardless of whether the seeds are heavy or light, the impact of storage time on germination remains largely unchanged. These findings enhance our understanding of Campolay seed biology and provide valuable practical insights for conservation efforts and agricultural practices (Sukarya and Witono 2017; Latifah et al. 2020).

In conclusion, the study provides interesting insights into the viability and germination behavior of *Campolay* seeds. The influence of seed weight on germination capacity is not statistically significant, whereas the duration of seed storage emerges as a critical determinant. Prolonged storage periods of up to 6 weeks result in highly efficient and synchronized germination, characterized by significantly higher germination rates and accelerated attainment of key milestones. Indeed, the overall success of germination remains consistently high, ranging from 92% to 97%, even after extended storage, highlighting the resilience of *Campolay* seeds. Importantly, these advantages of prolonged storage are observed regardless of the weight of the seeds. Moreover, using a moist sawdust medium for storing *Campolay* seeds appears to be an efficacious approach for expediting germination and maintaining seed viability. These findings have practical implications for seed management, germplasm conservation, and agricultural practices, thereby contributing to our comprehension of the biology of *Campolay* seeds.

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