INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 24, No. 3, p. 56-64, 2024
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Germination and survival of *Syzygium polycephaloides* (C. B. Rob.) Merr. (Myrtaceae) under varying seed storage duration

**Jan Orville P. Bautista, Novelyn D. Buhong***

*School of Forestry and Environmental Sciences, Aurora State College of Technology, Zabali, Baler, Aurora, Philippines*

Article published on March 09, 2024

**Key words:** *Syzygium polycephaloides,* Germinative energy, Percent germination, Percent survival, Seed storage

## Abstract

*S. polycephaloides* or lipote is native in the Philippines that needs protection and conservation due to its usefulness and is considered as vulnerable and endangered. However, no studies were conducted about the effect of seed storage in germination and survival of *S. polycephaloides*. The study aimed to address this gap and study the impact of different duration of seed storage on the percent germination, percent germinative energy and percent survival of *S. polycephaloides*. Single mother tree of lipote served as seed source and collected four times with 10 days interval (T0 - 0 day storage), (T1 - 10 days storage) (T2 - 20 days storage) (T3 - 30 days storage) and sown it simultaneously. One-way ANOVA and Duncan Multiple Range Test post hoc analysis were used to assess the difference among treatments in terms of germination and percent survival indicators. Analysis on the seed storage revealed that there is a significant difference among treatments applied to *S. polycephaloides* seeds in terms of percent germination and percent survival. Specifically, *S. polycephaloides* seeds under T2 (20 days of storage) had the highest germination percentage of 93.12% followed by T3 (30 days storage) with 92.5%, T1 (10 days storage) with 81.8%, and T0 (control) with 78.13%. *S. polycephaloides* seeds under T3 (30 days storage) obtained the highest percent survival of 98.57% compared to T2 (20 days storage), T0 (control), and T1 (10 days storage) obtained 97.27%, 89.65%, and 87.04%, respectively. Both T2 and T3 are significantly higher as compared to the control (T0) ($p<.049$). Longer storage of seeds appeared to improved germination of *S. polycephaloides*. The results can be served as basis for future reforestation project and future researches aiming to improve the seed physiological condition of *S. polycephaloides* under seed storage.

***Corresponding Author:** Novelyn D. Buhong ✉ bautistajanorville29@gmail.com

## Introduction

Today, the demand of functional food has increase significantly in recent years. Functional foods are ones that offer advantages to health beyond merely meeting nutritional needs. They contain physiologically active ingredients that aid in lowering chronic conditions like cardiovascular disease, hypertension, cancer, diabetes, and other illnesses.

*Syzygium polycephaloides* is indigenous to the Philippines. Its berry can be juiced and turned into wine or eaten ripe and raw. The antioxidant content of *S. polycephaloides* is similar to vitamin E (Santiago *et al.*, 2007). In local communities, indigenous fruit trees are important because it serves as food, nutrition and income. However, out of 300 edible fruit tree species in the Philippines only few are cultivated commercially and many others are still remaining underutilized (Dulay *et al.*, 2022).

Storage of seeds helps to preserve its viability because there is a period of time between planting and harvesting. Some of the farmers, researchers, plantation owners used seed storage to recalcitrant, intermediate and orthodox seeds for the purpose of maintaining the seed in good physical and physiological condition from the time they are harvested until the time they are planted. Many *Syzygium* species like *S. cuminii*, *S. jambos* and *S. polycephaloides* are considered recalcitrant to intermediate seeds wherein these seeds are sensitive to drying and can be kept for several months in low temperature (Abbas *et al.*, 2003).

According to Sultana *et al.* (2016), there are some various elements that influence seed quality that includes temperature, insects, and all other biotic and abiotic components. Seed stored in low temperature germinate higher compared to the seeds stored in high temperature since high temperature increase the respiration rate and enzymes activity resulting the overhaul of food reserves before the seeds germinate that leads to seed decrease vigor and physical quality seed (Mbofung, 2012). Lack of availability of quality seeds leads to a decline in production due to low percent germination, poor development of seedlings and reduce adaptation in the field (Jyoti and Malik, 2013). Therefore, this study was conducted to determine the percent germination, percent germinative energy and percent survival of *S. polycephaloides* as affected by different seed storage duration.

## Materials and Methods

*Location of the study*

A nursery experiment was performed at the Aurora State College of Technology- School of Forestry and Environmental Sciences.

*Materials*

Six hundred forty (640) seeds of *S. polycephaloides* were collected for the experiment (Fig. 1). A fine wire mesh was used as a strainer for seeds while subjecting them in running water to remove impurities and fungi associated. Other materials used were glass containers for seed storage, refrigerator for cold room storage of seeds, sterilized forest soil and compost soil with a ratio of 2:1 as potting media, black polyethylene bags with size of 2.5 x 2.5 x 5 inches, ball pen, record book for daily observation, and camera for documentation.

**Fig. 1.** Sample of *S. polycephaloides* mother tree

*Preparation of seed and media*

A mixture of forest soil and carbonized compost soil in 2:1 ratio by volume was used. The soil media were sterilized by cooking for at least three (3) hours (Tacloy *et al.*, 2022) to eliminate fungi contaminants. After sterilization, the mixture was placed in polyethene bags. Fungicide was also applied to the planting bags and seeds to reduce the occurrence of fungal infection.

*Seed collection and seed storage*

Seed collection was done in four visits, each with a 10-day interval. The latest seeds collected (0 day before planting) was used as control treatment (To). Seeds collected 10 days before planting was used in T1, while seeds collected 20 and 30 days before planting were used in treatment 2 (T2) and treatment 3 (T3), respectively. To ensure that seed are mature and viable, the collection was done by climbing the *S. polycephaloides* mother tree. Collection from the ground was avoided. The selected good mother trees were characterized by their gregarious fruiting, fast growth, branchiness, and healthy trunk (Musngi and Aquino, 2021). Collection of seeds for storage was based on fruit color (black) and softened pericarp which is an indication that the fruit is already mature, and the seeds are likely viable (Putri *et al.*, 2020).

After collection, the fruits were washed with distilled water to remove impurities that can cause seed deterioration. The seeds were separated from the pericarp and were air dried for at least one (1) hour. Afterwards, the seeds were kept in glass containers labeled according to treatments and were stored in a refrigerator calibrated at 5 °C Devi *et al.*, (2016) to avoid rapid loss of seed moisture (Quang *et al.*, 2022). The first collection was stored in a refrigerator for thirty (30) days (T3), the second collection was stored for twenty (20) days (T2), the third collection was stored for ten (10) days (T1), while the fourth collection was not stored that served as the control (To). Then, they were planted simultaneously. Monitoring of seed germination was done daily by counting and recording the germinants per treatment per replication. Termination of the study was conducted after 50 days of observation when there was no longer significant increase in the number of germinants for five days.

*Treatment of data*

The study on seed storage consisted of four treatments (including control) and four replications arranged in a completely randomized design (CRD). In each treatment, forty (40) seeds were collected and used, with a total of 640 seeds for the three (3) treatments and the control. All the seeds collected were sown at the same time. The seed storage treatments (Table 1) and experimental layout (Fig. 2) are as follows:

**Table 1.** Seed storage treatment of *S. polycephaloides*

| Treatment number | Description |
|---|---|
| To | 0 day of seed storage |
| T1 | 10 days of seed storage |
| T2 | 20 days of seed storage |
| T3 | 30 days of seed storage |

| | | | |
|---|---|---|---|
| To | T1 | T2 | T3 |
| T1 | To | T1 | T3 |
| T1 | T3 | T3 | T2 |
| To | To | T2 | T2 |

**Fig. 2.** Experimental layout on seed storage of *S. polycephaloides*

The seed germination responses in all treatments were monitored and documented. Parameters were computed based on the following formula:

Percentage germination (PG) was determined by recording the total number of seeds that germinated at the end of the test divided by the total number of seeds sown and was multiplied by 100 to get the percentage (Maguire, 1962).

$$PG = \frac{\text{Total number of seeds that germinate}}{\text{Total number of seed sown}} \times 100$$

Percentage germinative energy was determined by recording the daily number of seeds that germinated up to the peak period divided by the total number of seeds sown multiplied by 100 to get the percentage. Peak period is the period with the greatest number of seeds that germinate (Ruan *et al.*, 2002).

% GE= {(Number of seeds that germinated up to peak period)/(Total number of seed sown)}×100

Percent survival (PS) was determined by counting the number of seedlings survived divided by the total number of seeds germinated multiplied by 100 (Bhardwaj, 2014).

$$PS = \frac{\text{Total number of seeds survived}}{\text{Total number of seed germinated}} \times 100$$

*Data analysis*

All the data gathered were statistically assessed using One-way Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) post hoc analysis was used to measure the specific differences between pairs of means.

## Results

*Percent germination*

Table 2 shows the percent germination of *S. polycephaloides* seed storage treatments. The highest rate of germination was recorded under T2 (20 days storage) with 93.13%. On the other hand, the germination rate for T3 (30 days storage) is 92.5%, followed by T1 (10 days storage) at 81.88% and To control (0-day storage) at 78.13%. Analysis of Variance revealed that T2 and T3 (20 days and 30 days storage) seeds of *S. polycephaloides* had a significant difference with To (Control) ($P \leq 0.05$). Specifically, *S. polycephaloides* under seeds stored for 20 days had the highest germination of 93.13% and is similar to seeds stored in 30 days with 92.5%.

*Percent germinative energy*

Seeds stored for 30 days (T3) and 20 days (T2), first emerged at day 14. Meanwhile, seeds stored for 10 days (T1) and seeds directly sown (To) emerged from day 17 and 18 to 40 days. T3 and T2 both showed early peak period (at 9 days and 20 days, respectively), followed by To and T1 (at 19 days and 21 days, respectively) (Fig. 3) The highest rate of germinative energy was obtained under T3 (30 days of storage) with 53%. On the other hand, the germinative energy for T2 (20 days of storage) is 45.62% followed by control (To) with 33.12% and 10 days of storage (T1) with 26.87%. Based on the results of the analysis (Table 3) there is no significant difference on the percent germinative energy of *S. polycephaloides* seeds among treatments ($P \leq 0.064$). T2 and T3 obtained the same peak period within (14 days), compared to To (24 days) and T1 (23 days). Only T3 had significant difference in percent germinative energy to T1 ($P \leq 0.017$).

**Table 2.** Percent germination of seed storage treatments on *S. polycephaloides*

| Treatments | Mean | FVAL | PVAL | C.V (%) |
|---|---|---|---|---|
| To – 0 day stored/ control | 78.125a | 3.531* | 0.049 | 7.58% |
| T1 – 10 days stored | 81.875ab | | | |
| T2 – 20 days stored | 93.125b | | | |
| T3 – 30 days stored | 92.5b | | | |

**Table 3.** Percent survival of *S. polycephaloides* seeds stored at different periods

| Treatments | Mean | FVAL | PVAL | C.V (%) |
|---|---|---|---|---|
| To – 0 day stored/control | $33.125^{ab}$ | 3.157ns | 0.064 | 25.93% |
| T1 – 10 days stored | $26.875^{a}$ | | | |
| T2 – 20 days stored | $45.625^{ab}$ | | | |
| T3 – 30 days stored | $53.00^{b}$ | | | |

**Table 4.** Percent survival of *S. polycephaloides* seeds stored at different periods

| Treatments | Mean average Survival (%) | $F_{VAL}$ | $P_{VAL}$ |
|---|---|---|---|
| Seed Storage | | | |
| To – Control | $89.645^{ab}$ | 3.564* | 0.047 |
| T1 – 10 days storage | $87.042^{a}$ | | |
| T2 – 20 days storage | $97.277^{b}$ | | |
| T3 – 30 days storage | $98.572^{b}$ | | |

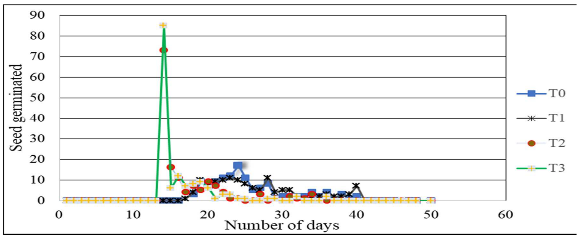


**Fig. 3.** Daily germination of *S. polycephaloides* seeds as affected by duration of seed storage

*Percent survival*

The highest rate of percent survival was obtained under T3 (30 days storage) with 98.57% ($p<0.05$). Compared to To, T1 (10 days) and T2 (20 days storage) at 97.27% followed by To (control) with 89.65% and T1 (10 days storage) with 87.04%. Based on the results of the analysis (Table 4), T2 and T3 (20 days and 30 days of storage) seeds of *S. polycephaloides* had significant difference with T1 (10 days of storage) ($p \leq 0.05$). This shows that the *S. polycephaloides* that had been stored longer had higher percent survival. However, T3 obtained the lowest number of mortalities with only 1.4% of the total seeds sown, T2 at 2.7%, followed by To at 10.4%, and T1 at 13%.

**Discussion**

*Effect of seed storage on percent germination*

In this study, there is lack of uniformity in the period of germination. Both T3 and T2 had early germination peaks, an indication that the seeds have more stored food to use for its growth that gives seedlings an advantage against environmental stresses. Another reason for the better seed germination for those stored longer is the presence of after seed-ripening type of dormancy. An example of plant that exhibits after-ripening seed dormancy is coconut which does not germinate even if they were harvested at its maturity. It still needs at least six months before germination occurs. After seed ripening, dormancy can cause delay of germination due to morphopyhsiological dormancy (MPD). This means that there is an incomplete development of embryo. Also, the early germinated seeds will likely be healthier and will have higher seedling survival since they are more resistant to attacks of fungi, pathogens and other biological agents. Longer period of germination makes the seedlings weak due to decline in stored food after sometime and this makes them susceptible to environment stresses. Further, there are some factors that affect early germination in stored seeds. Tong (2012) found out that external environment is an important factor on seed lifespan, which is dependent on seed moisture content, and the existing temperature and humidity. In seed germination, stored seeds for 20 days to 30 days in *S. polycephaloides* will be an advantage to use considering that percent germination of some seeds depends on their seed age by reducing seed germinability and performance, such as the speed of germination and seedling growth (Murdoch and Ellis, 1992). Also, there could be a gradual decrease in the proportion of hard seeds by storage time and in increase germination compared to fresh seeds zero day stored. Seed storage under T2 and T3 yielded slightly similar results in percent germination rate with 93% and 92%, respectively. This study showed that treatments with 20 days and 30 days of seed storage significantly increased the percent germination of *S. polycephaloides* since it extended the period of germination by providing enough time for the natural release from after-ripening dormancy. In addition, proper management of temperature during seed storage can help maintain seed viability for a longer period. This result is supported by the findings of Anandalakshmi *et al.* (2005) where the seeds of *S. cuminii* stored at 20℃ showed good germination and maximum germination even after 75 days of storage. However, this contrasts with the study of Yang *et al.* (2019), in which seeds of *S. jambos* stored at 3℃ for two months showed higher germination compared to seeds stored for 4, 6, 8 and 10 months. Rajjou and Debeaujon (2008) also reported that when seeds deteriorate during storage, they lose vigor, become more sensitive to stress during germination, and are unable to germinate. Fruit ripeness had a possible effect on seed viability, percent germination, and desiccation during post-harvest. According to the study of Tsan and Awang (2021), post-harvest of fully ripened fruit of *S. myrtifolium* obtained 100 percent germination rate which attained more physiological and morphological maturity. Hence, it is more favorable to use them in planting compared to semi ripened fruit that yield 93 % germination rate. Semi ripe fruits could ripen with warm and moist conditions and may germinate. This is rather similar to the after ripening process to overcome morphological dormancy in seed, through which cell development events continue and ultimately allow protrusion of the radicle (Pollock and Roos, 1972). The results of this study contrast with the study of Bohra *et al.* (2020) where half-ripe seed of *Musa indamdamensis* showed significantly higher (91.67%) germination. These are freshly extracted seeds that were sown immediately after harvest. This result did not differ significantly to 45 days of storage (89.58%), 90 days and 210 days (53%) and (21%). The result of this study is corroborated by the study of Yallesh *et al.* (2018), where increasing storage period decrease the viability of Jamun seeds (*S. cuminii*). As mentioned, fresh seed sown (0 day) had 93.40%, 3 days had 100%, 6 days had 98% and 9 days had 96% germination compared to seeds stored within 24 days which had 80.44% germination. Moreover, Walters (2005) reported that the length of storage time is strongly influenced by environmental and genetic factors such as storage temperature and seed moisture content. This could partly explain the variation per species.

*Effect of seed storage of percent germinative energy*

Seeds those were stored longer under refrigerated condition exhibited shorter germination time. This can either be due to indistinct mature color of the fruit or fruit quality at initial collection, lowering the viability for the earlier borne fruits than the mid and later period borne fruits and the preserving role of refrigeration on seed viability (Abbas *et al.*, 2003). This shows that the *S. polycephaloides* seeds under the various treatments had uneven percent germinative energy. Considering *S. polycephaloides* likely had an after ripening dormancy and is also affected by different seed sizes. It is imperative that only completely developed fruits with uniform seed size should be collected for seed-sowing purposes. According to Hartman *et al.* (2002), early germination is observed in *S. malaccense* compared to *S. jambos* due to bigger size seeds which present a large amount of reserved food that can be reflected in its germination speed. Sharma *et al.* (2008) stated that a prolonged duration of germination makes the seeds prone to degradation and infection by fungus and other agents. This is unlikely in this study considering that the germination is administered aseptically and lasted only 50 days. According to Taiz and Zeiger (2017), faster breakdown and mobilization of reserved food (cotyledons) cause the increase in germination speed and reduce infection by microorganism. Microorganisms can reduce the speed of metabolic reaction by fostering contamination leading to embryo deterioration (Bodrone *et al.,* 2017). According to Domin *et al.* (2019), an important indicator of seed quality is germinative energy. In addition, seed viability of most vigorous seedlings is defined by denoting germination energy and capacity. Furthermore, this implies that seedlings formed from seeds that germinate earlier have superior features and had higher resilience against harmful agents in unfavorable site conditions. In this study, 39 to 40% of sown seeds germinated up to peak period indicating that the remaining 60% of seeds collected are of lower quality. Thus, most of the *S. polycephaloides* seeds that were used in this study had weak germinative energy. Abbas *et al.* (2003) concluded that stored seeds of *S. cuminii* produce early and speed up germination rate during storage. The same is the result with the study of Anandalakshmi *et al.* (2005), wherein they stated that *S. cuminii* stored at 20°C with 11% to 35% moisture content shows higher peak germination. Such results contrast with the result of this study wherein T3 (30 days of storage) obtained strong germinative energy as compared to the rest of the treatments (0, 10, 20 days of storage).

*Effect of seed storage on percent survival*

The seeds stored for longer period exhibited best results in percent survival. Meanwhile, the seeds were not stored including the seeds stored for 10 days produce lower percent of survival. Marshal (1986) reported that seed size had a clear effect on rate of seedlings survival, the seeds with large size produces highest seedlings height and survival rather than small seeds that can result to lower survival rate, short lifespan and stunted height of seedlings. Also, environmental factor contributed to the percent mortality of *S. polycephaloides* seedlings, as many stunted seedlings died due to drought stress and high temperature in the pre-emergence until post emergence stage of *S. polycephaloides*. According to Eshetie *et al.* (2020), seedling size and climatic events may also cause differing mortality. Larger seeds can establish better due to greater nutrient and carbohydrate stored and less mortality rather than smaller seedlings, particularly in stressful environment. According to Waiboonya and Elliott (2020), seed storage had no significant effect on seedling survival. Success of seedlings development for survival depends on the percent germination capacity from damping off or any environmental stresses (Missanjo *et al.*, 2014). In this study, Treatment 3 obtained almost 100% survival rate with earliest seed emergence and high homogenous heights, as well as percent germination (92.5%). The early emergence increased the chance of seedling to overcome stressful environment and likely pathogen. The outcome of this study is supported by Chaudhari *et al.* (2022) where early emergence had better percent germination, higher survival and produced healthy and more vigorous seedlings. In this study, 93.13% of seeds sown were able to germinate and the overall survival with the quality of seeds collected. Similar result was also obtained by Prajapati *et al.* (2017) in kagzilime, Mahasin and Mustafa (2015) in mango and Merlin and Palanisamy (2000) in jackfruit where good quality seeds got the highest survival. Thus, propagation by seeds will be more appropriate for *S. polycephaloides*.

**Conclusion**

In the different duration of seed storage, it can be concluded that seeds stored for 20 days had the highest percent germination results when compared to 30 days, 10 days and 0-day seed storage, while 30 days of seed storage gained the highest percent survival as compared to all of the treatments. Both results had significant differences among treatments and thus mean storing seeds of *S. polycephaloides* from 20-30 days can further enhance germination.

**Recommendation(s)**

It is recommended that *S. polycephaloides* can be effectively propagated using seeds and also, seed storage for 20 and 30 days under refrigerated condition can be undertaken to improved percent germination and percent survival. For future researcher, it is recommended to investigate on increase seed storage duration for more than 30 days.

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