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Research Article

Seed Longevity of Tagasaste (*Chamaecytisus palmensis*) after Seed Treatment Using Boiling Water as Affected by Storage Durations

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Storage duration and seed treatments before storage are important factors responsible for retaining seed longevity. Hence, germination of tagasaste seed in the laboratory or nursery has been difficult and problematic without seed treatment, and yet, there is no information about the tagasaste seed longevity after treatment. Thus, this research was carried out to investigate the longevity of tagasaste seed after seed treatment for optimum germination percentage. The tagasaste seed was collected from the field of Holetta Agricultural Research Centre and treated monthly from March 2016 through February 2017. The germination test for the treated seed was done in the month of March for three consecutive years (2017, 2018, and 2019). The experiment was conducted in a randomized complete block design with three replications. The data were analyzed using the SAS software, and the least significant difference was employed for mean separation. The germination percentage was not significantly ($P > 0.05$) different within the months of the first, second, and third years of storage durations. While, the germination percentage of the seed stored after treatment for one, two, and three years was nonsignificant ($P > 0.05$). Therefore, the treated seed of tagasaste can be stored under the ambient conditions of a tropical highland environment for about three years without significant loss in germination percentage. However, the study should be conducted for more years to know the effect of storage duration on seed viability of tagasaste after seed treatment.

1. Introduction

Tagasaste (*Chamaecytisus palmensis*) or tree lucerne is a leguminous perennial shrub or small tree of the Fabaceae family which grows up to a height of 5-6 m [1, 2]. This leguminous tree is being used more extensively throughout the world in various production systems. This tree is capable of enhancing crop production, through land recovery and soil fertility maintenance, and livestock production, through increased availability of high-quality feed. Tagasaste can be established from cuttings, direct seeding, and transplanted seedlings. The seedlings are raised in March or April in a plastic pot or bare land using irrigation, and field transplanting is done on the onset of the main rains from June to early July. The seed has a hard coat, which reduces the germination ability of seeds. According to Assefa [3], soaking the seed of tagasaste in boiling water for 9–11 minutes is used to decrease the hardness of the seed coat, thereby, increasing the germination percentage from 3% (untreated seed) to 75%.

Seed longevity is highly variable and dependent on various factors [4]. The longevity of seeds stored under normal conditions is primarily determined by the seed moisture content and storage temperature, with their lifespan increasing predictably as the temperature and moisture content decrease [5]. There are, however, significant differences in seed longevity among species. Ex situ seed storage is a critical step in preserving plant genetic resources for the long term. To preserve the genetic integrity of the stored samples, seed viability must be maintained for longer periods. To optimize the physiological aging and to control insect pests, the seed can be maintained by managing storage conditions and determining the best storage period. The term "storage period" implies that natural seed aging occurs

over a long period of time. If the storage period is extended, the natural seed aging process is accelerated and the chemical changes occur, resulting in seed deterioration. Seed deterioration is the loss of viability, quality, and vigour as a result of natural aging or adverse environmental factors such as high temperature, high humidity, and moisture, among others [6, 7]. Storing seeds for longer than the recommended time can reduce germination potential, seedling establishment, and final seed production [8].

Seed deterioration and loss of viability is a normal occurrence during storage [9, 10], and using the wrong storage medium, such as room temperature storage [11, 12], has led to low seed germination. The duration of seed storage can be one of the factors that contribute to seed deterioration when a natural seed aging event occurs [13]. Temperature, seed nature, seed moisture content, and relative humidity are all factors that affect seed longevity during storage [14–16]. Proper storage conditions can help seeds maintain a high level of viability for an extended period [14, 15, 17]. The seeds that have been stored for longer time, particularly in unfavorable conditions, have lost their viability. The severity of this physiological harm varies. The seed coat, which is of maternal origin and serves as a physical and chemical shield, as well as the embryo are all vulnerable to physiological harm [18].

Different end-users, such as farmers and industries, assess the seed quality. Farmers, for example, expect to receive high-quality seeds that germinate and produce normal seedlings in field conditions [19]. Germination is the process by which seeds begin to absorb water, followed by embryo elongation and radical penetration through the endosperm and seed coat [20]. The germination rate is influenced by seed viability, dormancy, and environmental effects that impact the seed and seedling, whereas the germination ability is measured by germination percentage. Seed dormancy is a natural occurrence that allows species to survive in their natural environment [21]. Germination ability is the proportion of seeds that germinate from seeds subjected to the right conditions for development, while the germination rate is the speed at which the seeds germinate and is affected by seed viability, dormancy, and environmental effects that impact the seed and seedling [22]. Different plant species' seeds lose viability in different ways. Hence, currently, farmers perceived improved forage production as a feasible option for improving feed supply. This requires an adequate supply of quality forage seeds, but the unavailability of forage seed associated with unaffordable prices and low-quality seed are the major constraints for the fodder production.

Indeed, the germination of tagasaste seeds in the laboratory or nursery has been difficult and problematic without treating the seeds. The seeds of tagasaste are treated in bulk by different users and utilize the seeds which can be stored for a longer time. However, the seeds lose their viability after storing the treated seed for a longer time. So, the length of storage duration after tagasaste seed treatment using boiling water is not well known and there is no information about the tagasaste seed longevity after treatment. Thus, this study was executed to evaluate the effect of storage duration on seed longevity of tagasaste after seed treatment using boiling water at Holetta Agricultural Research Centre.

2. Materials and Methods

2.1. Study Area. The study was carried out at Holetta Agricultural Research Centre from 2016/2017 to 2019/2020 (for four consecutive years). Holetta Agricultural Research Centre is located at 9°00′N latitude, 38°30′E longitude at an altitude of 2400 meters above the sea level. It lies 34 km to the west of Addis Ababa on the road to Ambo and is characterized by the long-term (30 years) average annual rainfall of 1055.0 mm, average relative humidity of 60.6%, and average maximum and minimum air temperature of 22.2°C and 6.1°C, respectively. The rainfall is bimodal, and about 70% of the precipitation falls in the period from June to September, while the remaining 30% falls in the period from March to May. The predominant soil type in the area is the red nitosol, which has an average organic matter content of 1.8%, total nitrogen of 0.17%, pH of 5.24, and usable phosphorus of 4.55 parts per million [23].

2.2. Seed Treatment. Every 30 days starting from March 2016 to February 2017, the seed of tagasaste was treated with boiling water for 11 minutes, and then, the seed was dried properly before storing (Table 1). The seed treatments were done for 12 months only, and the treated seed was stored for two consecutive years (2018 and 2019) to estimate their viability. Every month, 20 g of seeds were treated and properly stored in a separate bag at ambient storage conditions (room temperature).

2.3. Germination. The treated seeds were used to raise the seedlings using polyethylene plastic pots at Holetta forage nursery site in the month of March for three consecutive years (2017, 2018, and 2019). The germination test for the treated seed was then conducted every year in the month of March. A tagasaste seed of a variety MoA, which is dominantly grown in the study area, was used for the germination test. The germination experimental set-up was allowed to stand under an open environment at bench level. Up to emergence, watering was done daily using a watering-can sprayer, in the morning from 6:00 to 7:30 a.m. Following emergence, the water application was reduced to twice a week. The germinated seedlings were recorded daily for 30 days.

2.4. Experimental Design. The germination test was carried out in a randomized complete block design with three replications. Each cluster or plot had a total of 10 plastic bags each planted with two seeds. After germination, one seedling was thinned from each plastic bag, and then, the germination percentage was estimated.

2.5. Statistical Analysis. Analysis of variance (ANOVA) procedures of the SAS general linear model (GLM) was used to examine the data [24]. The LSD test at 5% significance was

Table 1: Treatments used to study the effect of storage durations on seed viability of tagasaste after seed treatment using boiling water.

SN	Month	Months of seed storage		
		1st year	2nd year	3rd year
1	March	12	24	36
2	April	11	23	35
3	May	10	22	34
4	June	9	21	33
5	July	8	20	32
6	August	7	19	31
7	September	6	18	30
8	October	5	17	29
9	November	4	16	28
10	December	3	15	27
11	January	2	14	26
12	February	1	13	25

used for comparison of means. The following model was used to analyze the data:

$$Yij = \mu + Si + Bj + eij, \quad (1)$$

where *Yijk* represent the dependent variables (germination percentage), μ represents the grand mean, *Si* represents the effect of storage duration *i*, *Bk* represents the effect of block *k*, and eij represent the random error effect of storage *i*, location *j*, and block.

3. Results and Discussion

3.1. Germination Percentage. The effect of storage durations on the germination percentage of tagasaste seed after seed treatment is given in Table 2. The result of the analysis showed that the germination performance of the tagasaste seed was not significantly ($P > 0.05$) different among the months of the first, second, and third years of storage durations. This finding is in agreement with the result of [25] who reported that the common vetch seed can be stored under the ambient conditions of a tropical highland environment for about two years without significant loss in germination percentage. However, this observation is inconsistent with the work results provided by [13, 26, 27] who reported that sunflower, maize, and *Pinus bungeana* Zucc. ex Endl. seed germination declined significantly after one year of storage, respectively. Also, the result of this finding disagrees with [28–30] who demonstrated that seed germination, emergence rate, and seedling establishment decreased with an increase in the seed storage period. The seed stored for one and two months after treatment numerically gave a better germination percentage during the first year of storage. While, the seed stored for seventeen and twenty-five months numerically inferred better germination percentage among the months of second and third year storage duration, respectively.

The germination percentage of the treated tagasaste seed stored for 1–36 months ranges from 10% to 40%. Numerically, the maximum and minimum germination percentage was obtained from the seed stored for 25 and 30 months, respectively. Generally, the seed germination ability was low because the overall germination percentage was less than 50%. The seed that did not germinate indicates that they had lost viability due to any other related factors other than the storage durations that were possibly not favorable. These factors might be storage conditions/seed moisture content and storage temperature, seed treatment, and the inherent germination performance of the variety [15, 16, 31, 32], and a further study will be needed to identify why the germination percentage of this forage species has not exceeded 40%.

The germination dynamics of the treated tagasaste seed under different storage durations are given in Table 3. The mean germination percentage of the treated tagasaste seed was not significantly ($P > 0.05$) different among the storage durations of the first, second, and third years. While, mean germination percentages of the treated tagasaste seed were not significantly ($P > 0.05$) affected by storage durations at six and three-month interval. Numerically, a better germination percentage was recorded for the treated seed stored for 2 years, 1–6, and 1–3 months in comparison to the germination test at 12, 6, and 3-month intervals, respectively. The mean germination percentage (27.43) result obtained in this study was slightly lower than the result (40.02%) reported by Assefa [3] for the tagasaste seed treated indifferent boiling water times, and the germination test was conducted on soil media. The variation in this report might be due to the difference between the tested tagasaste varieties and the management (watering frequency and amount) of the experimental unit (pot) and climatic conditions.

The germination percentage trend of the treated tagasaste seed stored for 1–12, 13–24, and 25–36 months is given in Table 3. The results showed that the germination percentage was stable for the first two months and then considerably decrease with the storage durations from 2 to 5 months. During the fifth to sixth months of storage durations, the germination percentage increased with the storage duration period and was stable from 6 to 9 months' time. After the ninth month of storage duration, the germination percentage decreased and highly fluctuated with the increase in storage durations. This might be due to the moisture content variation of the treated seed.

The germination percentage showed a decrement for the treated seed stored for 13-14 months. The germination percentage was maintained for seed treated and stored for 14–16 months of storage durations and rise and fall was observed for the seed stored for 16–18 months. During 18–21 months of storage durations, the germination percentage showed a slight increase. However, after 21 months of storage duration (from 21 to 24), the germination percentage was slightly decreased.

The germination percentage of treated tagasaste seed was decreased if stored for 25–30 months after treatment. From 30 to 34 months of storage durations, the germination percentage showed great variations. The germination percentage was maintained from 34 months to 36 months of storage durations.

The result showed that the germination percentage of the treated tagasaste seed was better in the months of the

Table 2: Germination performance (%) of tagasaste (*Chamaecytisus palmensis*) seed stored for one, two, and three years.

Parameter	Storage duration up to one years (months)												Mean	CV	*P* value
Months	1	2	3	4	5	6	7	8	9	10	11	12			
Germination	33	33	28	22	12	25	25	28	27	23	30	20	28	34.7	0.24
	Storage duration from one to two years (months)														
Months	13	14	15	16	17	18	19	20	21	22	23	24			
Germination	30	27	28	28	33	25	28	30	33	32	30	25	29	38.9	0.99
	Storage duration from two to three years (months)														
Months	25	26	27	28	29	30	31	32	33	34	35	36			
Germination	40	23	22	31	10	13	28	26	37	23	23	23	25	59.3	0.57

CV, coefficient of variation.

Table 3: Germination performance of tagasaste (*Chamaecytisus palmensis*) seed stored at different storage duration intervals.

Storage duration (one year interval)	Germination %	Storage duration (six-month interval)	Germination %	Storage duration (three-month interval)	Germination %
1	28.00	1–6	31.12	1–3	31.67
				4–6	30.56
		7–12	25.56	7–9	26.67
				10–12	24.45
2	29.00	13–18	28.61	13–15	28.33
				16–18	28.89
		19–24	29.72	19–21	30.55
				22–24	28.89
3	24.79	25–30	22.64	25–27	28.33
				28–30	16.94
		31–36	26.94	31–33	30.55
				34–36	23.33
Mean	27.43		27.43		27.43
CV	15.02		11.68		28.61
P value	0.35		0.26		0.61

CV, coefficient of variation.

third year than in the second and first year of storage durations. The minimum germination percentage was recorded during the month (30) of the third year. This might be related to the dormancy period of the seed. In agreement with the result of this study were Yuhan Tang, Keliang Zhang, Yin Zhang, and Jun Tao' Dormancy-Breaking and Germination Requirements for Seed of *Sorbus alnifolia* (Siebold and Zucc.) K.Koch (Rosaceae), a Mesic Forest Tree with High Ornamental Potential, and Herranz J.M., Ferrandis P., and Martínez-Duro E.' Seed germination ecology of the threatened endemic Iberian. *Delphinium fissum* subsp. *sordidum* (Ranunculaceae). Plant Ecology. 2010, 211, 89–106, reported that the physiological dormancy has been identified as the vital type of seed dormancy among plants; cold stratification for 26–180 days is necessary for the seed of various species to break dormancy, depending on the species involved. A better germination percentage was obtained from the seed treated and stored for 25 months followed by the seed stored for 33 months. During the second year storage duration, a better germination percentage was recorded at 17 and 21 months. The maximum germination percentage was recorded for the seed treated and stored for 1-2 months when the seed was stored for a year.

4. Conclusion

This study has shown that the longevity of treated tagasaste seed until three years did not vary significantly for seed germination percentage. Thus, from this study, it can be concluded that the treated seed of tagasaste can be stored under the ambient conditions of a tropical highland environment for about three years without significant loss in germination percentage. Hence, we conclude convincingly that the storage period does not affect the seed germination percentage in tagasaste for three years. However, the study should be conducted for more years to know the effect of storage duration on seed viability of tagasaste after seed treatment.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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