

Effect of Type of Seed Storage on Seed Coat Structure and Dormancy Changes During Seed Storage Condition of Acacia Species

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Abstract: The seed storage is the most significant way to find suitable plant stands and maintain the seed in high, quality, healthy, and protection of seed, from disease, pest, and insects. Physical seed dormancy is communal in Acacia species, and it creates complications in seed testing and planting. Whereas, the objective of the current study was considerate of dormancy changes and seed coat structure during storage condition. Seeds of four A. species were stored at room temperature ($20-25^{\circ}C$) and cold-stored at ($4^{\circ}C$) for a period of 0, 4, 8, 12, 16, 20 24, 28, 32 and 36 months. Moisture content was determined and recorded before and after the storage. The results revealed that the degree of dormancy variation among the species, they were 81% for A. nilotica, 74% for A. seyal, 15% for A. mellifera, and 5% for A. senegal. Whereas, room storage temperature presented significantly declined in germinability. Further, the cold storage conditions indicated an effective method for the preservation of A. seeds and maintenance of seed viability for 36 months. However, there were very few differences in response to breaking dormancy treatments before and after 36 months. As the storage period advances, A. seyal, A. mellifera, and A. senegal, there were progressively reduction in hard seed percentage from the beginning of the four months of cold storage. As well as, A. nilotica was increased in the first four months and then declined to advance during at the end of cold storage. The different responses may be reasonable by the reduction of the seed degradation in breaking dormancy and germination behavior into the room, and cold storage conditions can be utilized how these species might respond to environmental change, and also, used for the formation of artificial seed banks of Acacia species. Therefore, the seed coats feature of the four A. species displayed a few changed in the overall structure before and after room and cold storage conditions: Palisade layer, epidermis layer, hypodermis, linked with light line arrow and sclerified parenchyma layers.

Keywords: Acacia, storage, seed coat, structure, anatomy, room, cold, germination, dormancy

1. INTRODUCTION

Acacia species are growing natively in Savanna regions, there are more critical sources recorded to be useful for timber, forage, gum, food, fuel, firewood, fiber, tannins, medicine, ornamentals, shade, shelter, domestic utensils, handicrafts, nitrogen-fixing, soil stabilization, Agroforestry system, as well as environmental protection. Acacia seeds storage perhaps advantageous for good quality seed as well as can be harmful if the seed is of less quality, seeds will probably store for more extended periods without deterioration if saved at low temperatures. The main objective of natural condition seed storage is to secure the support of good quality seed for planting and restoration programmers whenever requested. Several species produce seeds at long intervals, limiting from a few years to many years. Declare a seed source between two crop seeds; a seed stock must be established (Wang, 1975). Moisture content and temperature are notably more critical factors affecting the longevity of seeds and influencing the rate of deterioration (Spano and Buselli, 2007). The literature about the studies on the effect of seed storage of *Acacias* is reviewed. There seems to be an association between plant ecology and seed storage behavior, and it is evident that species that show recalcitrant seed storage behavior do not occur naturally in habitats in desert and savanna. In some species, part of the seed with physical stored at room temperatures for several months or years becomes permeable (Govender et al., 2008; Alamgir and Hossain, 2005). Shields held at 20°C retain viability and remain soft for up to five years (Coaldrake.,1971). The evidence suggests that cold storage of 5°C is necessary to arrest the set of seed coat dormancy (Isikawa., 1960).

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Seed conservation is one of the most effective methods for preserving the useful germplasm of seed plants (Takayanagi, 1988). Therefore, the long-term dry storage at room temperatures may promote the breaking of physical dormancy (Meisert., 2002). In these environments, most of the plant species indicated orthodox seed storage behavior, while a few indicated intermediate seed storage behavior (Alamgir and Hossain., 2005). The advantages of treating seeds before to storage it seems that, little to indicate the long period consequences of storing seeds for the non-dormant mean (Rowe 2010; Merritt and Dixon 2011). Seed conservation is ordinarily classified into two groups: ex-situ and in situ (Draper et al. 2004). Ex-situ protection preserves the vegetal material exterior on the natural environment (Beardmore et al. 2014). Ex-situ conservation way consists of seed banks, in vivo banks, and in vitro banks (Assis et al. 2011), ensuring its preservation in a viable condition, also saving it genetically ultimately for long term (Hay and Probert 2013). On the other hand, in situ methods conserve biological diversity when the management and preservation of species in the natural environment (Beardmore et al. 2014; Teixido et al. 2017). Further, the parameters defined as three models to get in the quantity of germination response of the seed population to temperature. Furthermore, the benefit of knowing about the ecological relationships within plant species. Seed storage response to the temperature of several species, ecosystems have been resolved independently (Huang et al., 2009; Zeng et al., 2010; Wang et al., 2011; Hu et al., 2013). Whereas, shorter storage time, prevent overall seed storage environmental and broad temperature range of seed store, leading to improved seed health and seed quality (Farooq et al., 2008). Besides, there are little data about the effect of the type of seed storage condition and temperature on Acacia seeds (Benzing 2000).

The current paper describes and evaluates the influence of dormancy changes and seed coat structure during room and cold storage conditions in *Acacia* seeds.

2. MATERIALS AND METHODS

2.1. Seed Collection

Seeds of *Acacia seyal, A. nilotica, A. senegal,* and *A. mellifera*, were collected from Western Sudan and imported to the laboratory of the College of Pastoral Agricultural Sciences and Technology, Lanzhou University, China. During September 2016, Seeds were selected by sorting out the healthy, uninfected seeds of almost uniform size. These seeds were stored in plastic boxes at 5°C, and relative humidity 35-40%, then was used for evaluating different treatment.

2.2. Seed Storage Treatment

- Room, storage condition treatment, the selected seeds of *A. seyal, A. nilotica, A. senegal,* and *A. mellifera* were stored at the room, storage temperature at 20-25°C for 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 months.
- Cold storage condition treatment, the selected seeds of *A. seyal, A. nilotica, A. senegal,* and *A. mellifera* were stored in cold storage; seeds were stored at cold storage temperature at 4°C since 0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 months.

2.3. Seed Coat Structure Change During Seed Storage Conditions Treatments

Changes in the seed coat features were evaluated using scanning electron microscopy (SEM). Room and cold-stored seed were tested before and after stored for 0, and 36 months. Three seeds were chosen at random from each treatment and the control. All seeds were coated with gold and observed with a (JSM-6380LV (JEOL, Japan) scanning electron microscope at 20 kV. From all the examined seeds, two regions of the seed coat could distinctly identify: (1) a hilum and (2) the lens.

2.4. Seed Germination Test

Germination was carried out before and after the final of the storage experiment incubated at 20°C in incubator lighting for 12 hours a period. Samples of four replicates of 50 seeds were used for each treatment in the experiment and placed on top of 2 layers of filter paper in 90 mm Petri dishes. A seed was considered to have germinated when the radical extension was at least 0.5 cm. Seeds were evaluated according to the ISTA Rules (ISTA, 2016). The experiments were laid out in a completely randomized design.

2.5. Statistical Analysis

Differences in the effect of water potential and temperature on germination rate $(1/T_{50})$ and germination percentage of every species were analyzed using SPSS 20.0 software by using one-way ANOVA (P < 0.05).

3. RESULTS

3.1. Effect of Room and Cold Storage on Seed Germination and Hard Seed Performance

Germination percentage affected by *A*. species differed significantly over time of storage. In most of *A*. *senegal*, *A*. *mellifera*, and *A*. *seyal*, the germination percentage improved during cold storage, treatment to the advancement of storage time up to 4, and eight months of storage, which later decreased at the end of storage time 36 months (figure1). However, in *A*. *nilotica* the germination percentage decreased within the first four months of cold storage after that developed in progress among the cooling storage period.

In contrast, the germination percentage remarkable gradual declined in the whole of *A. senegal*, *A. mellifera*, *A. seyal*, and *A. nilotica* at the room, storage time (figure 1). Before starting storage, treatment, the seed of *A. seyal* and *A. nilotica* indicated no significant differences in seed viability. This may indicate that the seed species were highly viable. Further, hard seed percentage as impacted by *Acacia*. Species differed significantly during storage time. As the storage period advances, there was progressively reduction in hard seed percentage from the beginning of the four months of cold storage seed of *A. senegal*, *A. seyal*, and *A. mellifera*. As well as, the hard seed percentage of *A. nilotica* was increased in the first four months and then declined advanced during at the end of cold storage (figure 1). The death seed percentage of those *A.* species affected by differed significantly above the period of 36 months of cold and room storage. Since, storage time advances, the significant increase in the dead seed percentage recorded in *A. senegal* and *A. mellifera* exhibited decline.

Furthermore, the dead seed percentage was highest at the room storage as compared to cold storage at the end of 36 months of storage time (figure1). The abnormal seed percentage of those *A*. species influenced by differed significantly during 36 months of cold and room storage. At the storage time advances, there was a significant increase in the abnormal seed percentage of *A*. *senegal* and *A*. *mellifera*. Even more, the abnormal seed percentage was decreased at cold storage for *A*. *senegal*, *A*. *nilotica*, and *A*. *mellifera* among the end of the 36 months of storage time (figure1a, b, c, d).



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Figure1a, b, c, d. Effect of room and cold storage condition on seed germination during 36 months of four Acacia species.

3.2. Seed Coat Structure Change During Seed Storage Conditions Treatments

The seed coat structures were observed the hilum located on the opposite side of the lens in A. seyal, and A. nilotica (Figures 2a and 3a). Besides, the hilum and lens were placed in the middle of micropyle in A. mellifera and A. senegal (Figures 3a and 4a). In the inspected control seeds, the lens and hilum remained complete with visible cracks below 200 magnifications (Figure 2,3,4,5a). Whereas, the hilar groove regions displayed seemed expansion and cracked in the hilum and micropyle region more than in the controls. However, the seed cracks were gated clearly with eroded regions visible in the micropyle and the hilum area increased and opened the water gap after 36 months of the room and cold storage condition. Furthermore, the seed coats feature displayed a few changed in the overall structure after 36 months of room and cold storage conditions in Epidermis layer, hypodermis, linked with light line arrow, sclerified parenchyma layers. Further, the palisade layer presented smaller tissue and destroyed the parenchyma regions after 36 months of room and cold storage conditions for all Acacia species. Regarding the different cellular features between those species, the palisade epidermal layer showed denser, and thickness tissue had been penetrated in A. nilotica, and A. seyal (Figure 2def and 3def). In contrast, the palisade epidermal layer displayed small and thin tissue, seed coats with a narrower sclerified parenchyma in A. mellifera and A. senegal (Figure 4def and 5def). Therefore, after room and cold storage for 36 months, the palisade epidermal layer presented smaller and destroyed the parenchyma regions (Figure 2f, 3ef, 4ef, and 5ef).



Figure2. Scanning electron micrographs of seed coat structure change and Cross-sections of the seed coat anatomy during seed storage conditions treatments of A. nilotica. (a) Control, 0 months (b) room store treated for 36 months (c) cold store treated for 36 months, and (d) Control, 0 months (e) room store treated for 36 months (f) cold store treated for 36 months, respectively. H: hilum, L: lens open without outer permeable cell layers, M: micropyle open, and PL = palisade layer; HY = hypodermis; CU = cuticle; LL = light line arrow; SP = sclerified parenchyma layers.



Figure3. Scanning electron micrographs of seeds coat structure change and Cross-sections of the seed coat anatomy during seed storage condition treatments of the A. seyal. (a) Control, 0 months (b) room store treated for 36 months (c) cold store treated for 36 months, and (d) Control, 0 months (e) room store treated for 36 months (f) cold store treated for 36 months, respectively. H: hilum, L: lens open without outer permeable cell layers, M: micropyle open, and PL = palisade layer; HY = hypodermis; CU = cuticle; LL = light line arrow; SP = sclerified parenchyma layers.



Figure4. Scanning electron micrographs of seed coat structure change and Cross-sections of the seed coat anatomy during seed storage conditions treatments of the A. senegal. (a) Control, 0 months (b) room store treated for 36 months, (c) cold store treated for 36 months, and (d) Control, 0 months (e) room store treated for 36 months (f) cold store treated for 36 months, respectively. H: hilum, L: lens open without outer permeable cell layers, M: micropyle open, and PL = palisade layer; HY = hypodermis; CU = cuticle; LL = light line arrow; SP = sclerified parenchyma layers.



Figure5. Scanning electron micrographs of seed coat structure change and Cross-sections of the seed coat anatomy during seed storage condition treatments of A. mellifera. (a) Control, 0 months (b) room store treated for 36 months (c) cold store treated for 36 months and (d) Control, 0 months (e) room store treated for 36 months (f) cold store treated for 36 months, respectively. H: hilum, L: lens open without outer permeable cell layers, M: micropyle open, and PL = palisade layer; HY = hypodermis; CU = cuticle; LL = light line arrow; SP = sclerified parenchyma layers.

4. DISCUSSION

For all species evaluated in this study, whole preserved high germination under the different condition's storage tested, and germination after 36 months storage. The recorded, evidently performance, the existence of room and cold storage condition correlated variation in the seed germination behavior of those A. species and a significant influence of the period of room and cold storage on germination. Dormancy has broken within the room, and cold storage exposed two main groups of species, indicating different germination behavior is decreased or increased germinability. On the whole, two comparisons depicted that the storage condition may strongly influence seed germination. The distribution of seed germination percentages revealed that the cold storage is the more effective condition for dormancy broken compared to the room storage condition duration of seed storage. Seeds by deep and nondeep physical dormancy were stored below room and cold store conditions, the timing of sowing, there were three stages to consider. Firstly, dormancy loss can exist room and cold storage, but it cannot be complete, some of the seeds can change non-dormant and remain dormant. Secondly, the rate of dormancy broken becomes different between the species. Thirdly, several seeds can lose viability within the room and cold storage, also again, we had shown that could vary among the species and collection area (Baskin et al. 2006: Wang et al., 2010). Whereas, orthodox seeds are long-lived seeds and able successfully dried to moisture contents below 5% without injury and are can be to tolerate freezing, also, survived freezing or drying among ex-situ preservation, as opposite to recalcitrant seeds is remarkably short-lived which cannot be dried to moisture content under 20-30% lacking of injury and are unable to tolerate freezing, or which will do not survive drying and freezing during ex-situ maintenance (King and Roberts, 1980; Walters et al., 2004, Vozzo, 2002). Therefore, seed survival during the period of longevity varies greatly within species. It can also vary between accessions among a species due to differences in genotype and location. Affected by the area in potential longevity recorded from the environment during seed harvesting, maturation, duration of drying, time of seed harvest, pre-storage environment, and the period of seed storage (Hong and Ellis, 1996; Verdu, 2006; Bu et al., 2007, 2008). Besides, studies about the long-term viability are requested for the storability of those Acacia seeds in the gene bank. The different storage conditions response may have affected by many factors, including seed structure, intrinsic physiological characters of each species, and environmental factors such as temperature and humidity (Davies et al., 2016; Meiado et al., 2016). The temperature responses of the plants to view the environments in which they are adaptable. Even more, differences within the species have substantial ecological significance (Liu et al., 2011; Wang et al., 2011).

Thus, researches supported our found in the variation within cold and room storage may be due to differences in the storage conditions, temperature, and moisture content. Whereas, there were very few differences in response to breaking dormancy treatments before and after 36 months of storage in *A. nilotica* and *A. seyal*. However, the cold conditions storage for *A. nilotica*, *A. senegal*, *A. mellifera*, and *A. seyal* were remained completely viable among 36 months of storage. contrast, the room, condition

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was gradually declined seed viability during 36 months of storage and did not maintain seed viability. According to these findings, storing a permeable seed's dormancy of A. senegal and A. mellifera, revealed does not change germination, and this fascinates significant interest to the current seed-based restoration programs and promoted seed use effectiveness. Besides, the temperature and relative humidity are severe parameters that control the nature of the responses existing among the seed storage. However, dormancy evolution represented of seeds stored in the seed bank facing changing environmental conditions of humidity and temperature. Besides, seed storage below agricultural conditions generally affects controlled dry environments (Basbouss and Leymarie, 2016). Even more, ecological factors practiced plants among seed maturation needed light, soil nitrate, and the temperature was affected on the degree of dormancy (Chiang et al. 2011; Kendall et al. 2011). Whereas, the temperature might promote seed dormancy degree once treated the plant before flowering, demonstrating the reality of retention mechanism (Chen et al. 2014). Therefore, ecological variation between seed maturation chiefs to diverse degrees of dormancy during individual seeds (Simons 2006). Further, seeds stored on the soil below normal conditions display their ecology to regulate their dormancy degree. Such as, dormancy might lose in imbibed seeds after a rapid exposure for low temperatures (Finch and Savage 2006). As well, there are several studies referred to scanning electron microscopy (SEM), seed coat features changes, treated by following sulfuric acid and hot water, such as Dichrostachys cinerea (Kelly et al. 1992), A. ancistrocarpa, (Erickson et al. 2016), A. kempeana (Hanna 1984), Stylosanthes scabra (Serrato Valenti, et al. 1989), Australian legumes (Morrison et al. 1992), Rhus aromatica (Li et al. 1999) Schizolobium parahyba (Graziela, 2017).

In conclusion, the potential of dormancy broken to gated among cold and room storage must be considered when examined on initial seed dormancy were conducted, maybe when seeds from different locations were stored before to propagating plants. It is to suggest that fresh seeds must be used after collection before proceeding for experimentation and following seed storage for the long term. In our study raised the most important trait of the types of seeds storage conditions responses and anatomical seed coat efficiency in *Acacia* species. Therefore, the author studied new methods and innovations of overcoming seed dormancy and anatomical seed coat changing by selected *Acacia* species. These outcomes will be resulting in the future to promote livelihood and sustainable agriculture and seed storage programs.

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