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Research Article

Storage Conditions and Period Effects on Quality of *Pinus kesiya* Seeds from Malawi

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Abstract: Long-term storage of *Pinus kesiya* Royle ex Gordon seeds is essential not only for continuous seedling production, but also for genetic conservation. In Dedza, Malawi *Pinus kesiya* seeds are collected in good production years, stored in sealed black polythene tubes at (4±1) °C and used thereafter for seedling production. However, information about the effect of conditions and period of storage on seed quality is scarce. Therefore, the objective of this study was to evaluate the germination percentage (GP) and germination energy (GE) of *Pinus kesiya* seeds recently collected and after four and ten months after storage in sealed black polythene bags at the temperature of (4±1) °C, dried at 20%, 14%, 7% and 3% moisture content levels. The results shows there were significant (P<0.001) differences on both GP and GE among different moisture content levels. GP and GE increased with a decrease of moisture content up to 7%, then decline at 3% moisture content. Consequently, there were significant (P<0.001) differences on both GP and GE among storage period at 3% moisture content. GP and GE decreased with an increase of storage period. In contrast, there were no significant (P>0.05) differences on GP among the storage period at 20%, 14% and 7% moisture content levels. Similarly, no significant (P>0.05) differences were observed on GE during the first four months of storage at 20%, 14% and 7% moisture content levels. However, GE significantly declined by the tenth month. Highest GP of (96.6±2.1) %, (95.8±2.6) %, (95.2±2.8) % and GE of (78.3±2.2) %, (75.9±2.7) %, (60.2±2.7) % at 7% moisture content were obtained for 0, 4 and 10 months of storage respectively. Therefore, the present study recommends storage of *Pinus kesiya* seeds in sealed black polythene bags with a moisture content of 7% at (4±1) °C temperature in order to maintain the seed viability for a long period. Further studies are required to determine the pre-chilling period in order to maintain the high germination energy.

Keywords: germination percentage, germination energy, seed viability, moisture content, temperature, pre-chilling

INTRODUCTION

Seed storage is the preservation of viable seed from the time of collection until it is required for sowing [1]. The reason for this is that it is rare that the best time for seed collection coincides with the best time for sowing. Bonner [2] stated that long term storage of seed is often essential not only for continuous seedling production but also for gene conservation via seed banking in coniferous forest tree species as abundant seed production does not happen every year. The viability of seed is the average number of live seeds per unit weight as determined by germination tests [3]. Viability varies with species, place and time of collection as well as length of storage [3,4].

Seeds of most species (unless they are placed in conditions favourable for germination or sufficiently dried and kept at extremely low temperature) will suffer changes and will eventually lose viability, consequently reduced germination rate. There are some seeds however, which will lose ability to germinate within few weeks, no matter how good the collection, extraction and storage techniques were [3]. The storability of a seed lot is primarily determined by the vigor of the seed at maturity and level of deterioration at the time it enters storage. A reduction in storage potential is one of the specific consequences of seed deterioration, which in turn is governed by the genetic constitution, environmental factors during seed development and storage conditions. Seed deterioration occurs as a result of physiological and biochemical perturbations, such as impairment of energy synthesis mechanisms, reduction in respiration and biosynthesis activities, chromosomal aberration and deoxyribonucleic acid (DNA) degradation and membrane alteration, which eventually reduces seed vigor, storability, germination capacity, and emergency potential [5].

Improper storage conditions and biochemical and physiological changes in seeds have been shown to reduce germination capacity of conifer seeds [2,3,5]. A number of physiological changes in cell tissues may be associated with physiological aging in seeds, hence reduced germination viability. They include: Loss of food reserves caused by respiration, accumulation of toxic or growth-inhibiting by-products of respiration, loss of activity of enzyme systems, deterioration of semi-permeable cell membranes [5]. It is still uncertain to what extent these various effects are the causes or only the symptoms of deterioration of seeds in storage. Among the storage conditions storage temperature and moisture content of the seeds are shown to be most influential [6]. For most pine species storage at 4°C with 6-8% moisture content is recommended [2]. In as much as the seeds are orthodox in nature, they can be dried to low moisture content and stored cool. Under these conditions the seed retain high viability for more than 10 years. However, under highland conditions even with relatively high moisture content of 7-8%, high viability can be maintained for at least up to two years under ambient temperature [6].

Pinus kesiya Royle Ex. Gordon of the Pinaceae family is one of the mostly distributed pines in Asia [5]. It is an important plantation species across the world, including in southern Africa and Malawi. *Pinus kesiya* is a tree reaching up to 30 – 35 m tall with a straight, cylindrical trunk. The bark is thick, dark brown, with deep longitudinal fissures [7 – 10]. The branches are robust, red brown from the second year, the branchlets horizontal to drooping. The leaves are needle-like, dark green, usually 3 per fascicle, 15 – 20 cm long, the fascicle sheath 1 – 2 cm long and persistent [3]. *Pinus kesiya* produces soft and light timber which is intensely commercially used for a wide range of applications, including construction, furniture manufacturing, paper pulp, and temporary electric poles. The species also produces high quality fuelwood for homestead energy requirements [7 – 10]. When tapped, *Pinus kesiya* yields some resin, which is distilled to give turpentine, and rosin which is used in, for example, paint industries [11]. Pine-leaf oil is sometimes used for medicinal baths, and the seeds may be consumed locally. In addition, the species plays a significant role in providing ecosystem services of controlling floods [11, 12]. In Dedza, Malawi *Pinus kesiya* seeds are collected in good production years, stored in sealed black polythene tubes at (4±1) °C and used thereafter for seedling production. However, information about the effect of conditions and period of storage on seed quality is scarce. Therefore, the objective of this study was to evaluate the germination percentage and germination energy of *Pinus kesiya* seeds recently collected and after four and ten months after storage in sealed black polythene bags at the temperature of (4±1) °C, dried at 20%, 14%, 7% and 3% moisture content levels.

MATERIALS AND METHODS

Study site and tree sampling

Open pollinated seeds were collected from 15 *Pinus kesiya* trees at tree breeding block 74/2/3 of provenance trial seed source in the Chongoni Arboretum, Dedza, Malawi located at altitude 1600m, longitude 34°16'E and latitude 14°19'S. The area receives 1200 mm to 1800 mm rainfall per annum, with a mean annual temperature of 14°C. It is situated about 85 km south east of Lilongwe the capital. The *Pinus kesiya* trees in the Arboretum was planted in 1974 at a spacing of 3m x 3m with seed source from Phillipines. Sampled trees were systematically selected in order to minimize rate of self-pollinationand were measured for total height and diameter at breast height (dbh). Suunto clinometer and calliper were used to measured total height and dbh respectively. The characteristics of the growth data are presented in Table 1.Healthy cones from the last year's crop were collected from the middle part of the crowns. Cone collection and seed extraction were performed between Mayand July2012.Cones were brought to the laboratory, air dried until almost opened.

Experimental design and data collection

Seeds were extracted from the cones and subjected to four moisture content levels: 20%, 14%, 7% and 3%. Dried seeds were then stored in sealed black polythene bags at (4±1) °C until use. Seed moisture content (based on fresh weight) was determined by oven-drying seed samples (at 103±2 ºC during 17±1 hour, ISTA [13]) right after collection, repeating it after four and ten months of storage. Three separate, germination tests were conducted in the nursery with a total of 3000 seeds for each experiment. Thus, 50 seeds from each tree were used and the experiment was completely randomized in four replicates, hence making a total of 200 seeds for each tree and experiment. The germination tests were conducted soon after the seed extraction in July 2012, and after four months and ten months of storage in December 2012 and June 2013 respectively. Seeds were sown on the seed bed using the procedure as outlined by Ingram and Chipomphа [14]. Regular watering was done as per requirement to maintain adequate moisture necessary for germination.

Germination energy and germination percentage were defined as the percentage of seeds germinated at 7 and 21 days after sowing, respectively [15].The seed was considered germinated by a visible protrusion of split seed coat with the cotyledons, hypocotyls, and epicotyl on the surface of the soil.

Statistical analysis

Data obtained were tested for normality and homogeneity with Kolmogorov-Smirnov D and normal probability plot tests using Statistical Analysis of Systems software version 9.1.3 [16]. After the two criteria were met the data were subjected to analysis of variance (ANOVA) using the same Statistical Analysis of Systems software and means were separated with

Fischer's least significant difference (LSD) at the 0.05 level.

Table-1:Characteristics of the growth data set

Variable	Mean± S.E.	Standard deviation	CV%
Height (m)	29.5±0.9	1.42	4.8
dbh (cm)	38.4±0.5	2.03	5.3

S.E. is standard error; CV is coefficient of variation

RESULTS AND DISCUSSION

Effect of seed moisture content and storage period on germination percentage

Moisture content subjected to the seeds at the beginning of the storage remained unchanged until 10 months. It appears that the sealed black polythene bags used in this study allowed maintaining seed moisture content at (4±1) °C without modifications throughout all the storage period. However, reduction in storage moisture content had significant (*P*<0.001) effect on the germination percentage. Even though there were no significant (*P*>0.05) differences between 20% and 14% moisture content levels, in general germination percentage improved with decrease in moisture content up to 7% but further reduction to 3% moisture content resulted in decrease in germination percentage. The results further shows that germination percentage was not significantly (*P*>0.05) affected by storage period at 20%, 14% and 7% moisture content levels. In contrast, the germination percentage significantly (*P*<0.001) declined with an increase of storage period at 3% moisture content level (Table 2).

Seed moisture content and storage temperature are regarded as the main influential factors of orthodox seeds durability, in which *Pinus kesiya* seeds belong [17, 18]. Orthodox seeds can be preserved for many years by ensuring that their moisture content is below certain threshold values during storage at low temperatures [19, 20]. The present results are in agreement to those in literature [17, 20-22]. Kamotho et al. [21] demonstrated that drying to extremely low water contents may shorten seed longevity and that there is an optimum moisture level for storage at which longevity is maximized and below that seeds are damaged, while Walters [17] and Probert [22] reported that deterioration of orthodox seeds in storage increases with increase in seed moisture.

On the other hand, the present results are different from those reported by [6, 21]. Temel et al. [6] reported a significant reduction in germination percentage of *Pinus nigra* after 10 years of storage, while Kamotho et al. [21] reported a significant increase in germination percentage of *Cleome gynandra* after 6 months of storage. The differences may arise due to genetic control and environmental conditions of seed sources [20] and also that in the study of *Pinus nigra* seeds, the moisture content of the stored seeds was not known, and moisture content of stored seeds has direct influence in seed viability [6, 17, 22].

Research has shown that although polyethylene is not suitable for long-term storage of orthodox seeds, it can be used for short-term or medium-term storage [21, 23]. In this study, seeds were stored in a sealed black polythene bags for a period of 10 months, which could be referred to as short-term. Therefore, the present study recommends storage of *Pinus kesiya* seeds in sealed black polythene bags with a moisture content of 7% at (4±1) °C temperature in order to maintain the seed viability for a long period.

Table-2: Effect of moisture content and storage period on the germination percentage of *Pinus kesiya* seeds

Storage period (months)	Germination percentage at different moisture content (%)			
	20	14	7	3
0	$65.4±2.9^{bx}$	$67.8±2.7^{bx}$	$96.6±2.1^{ax}$	$54.1±2.8^{cx}$
4	$64.1±2.3^{bx}$	$65.5±2.6^{bx}$	$95.8±2.6^{ax}$	$46.6±2.4^{cy}$
10	$62.7±2.8^{bx}$	$64.3±2.4^{bx}$	$95.2±2.8^{ax}$	$40.4±2.5^{cz}$
CV%	8.3	6.8	5.1	6.9

Note: Means with different subscript (a,b,c) within a row and (x,y,z) within a column significantly differ (*P*<0.001)

Effect of seed moisture content and storage period on germination energy

Summary of the results on the influence of seed moisture content and storage period on germination energy is presented in Table 3. The results shows that there were significant (*P*<0.001) differences on germination energy among different moisture content levels. Germination energy increased with a decrease of moisture content up to 7%, then decline at 3% moisture content. Consequently, there were significant (*P*<0.001) differences on germination energy among storage period at 3% moisture content. Germination energy decreased with an increase of storage period. In contrast, there were no significant (*P*>0.05) differences on germination energy during the first four months of storage at 20%, 14% and 7% moisture content levels. Germination energy, however, significantly (*P*<0.001) declined by the tenth month.

In agreement with the present results, Temel et al. [6] and Pasquini and Defossé[20] reported that germination energy declined during storage period. Other studies have demonstrated that high germination energy can be maintained in storage by lengthening the pre-chilling period [20, 24].However, germination energy is known to be dependent on genetic control that may vary among seed lots. Therefore, there may be considerable variability in the response to pre-chilling both among and within pine species [20, 25].It is recommended, therefore, that optimal time for pre-chilling should be determined.

Table- 3:**Effect of moisture content and storage period on the germination energy of *Pinus kesiya*seeds**

Storage period (months)	Germination energy (%) at different moisture content (%)			
	20	14	7	3
0	39.9±2.7[bx]	40.6±2.9[bx]	78.3±2.2[ax]	28.5±2.6[cx]
4	35.8±2.8[bx]	37.4±2.5[bx]	75.9±2.7[ax]	20.6±2.5[cy]
10	24.6±2.1[by]	26.1±2.6[by]	60.2±2.7[ay]	14.2±2.8[cz]
CV%	5.7	9.4	8.9	7.5

Note: Means with different subscript (a,b,c) within a row and (x,y,z) within a column significantly differ (P<0.001)

CONCLUSION

The study has revealed the beneficial effects of drying *Pinus kesiya* seeds to low moisture content and storing at low temperature on maintaining seed viability for long period. Germination percentage and energy increased with a decrease of moisture content. In contrast, there were no significant differences on germination percentage among the storage period. Similarly, no significant differences were observed on germination energy during the first four months of storage. However, germination energy significantly declined by the tenth month. Therefore, the present study recommends storage of *Pinus kesiya* seeds in sealed black polythene bags with a moisture content of 7% at (4±1) °C temperature in order to maintain the seed viability for a long period. Further studies are required to determine the pre-chilling period in order to maintain the high germination energy.

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