ORIGINAL ARTICLE

Effect of storage temperature and duration on *Pouteria adolfi-friederici (Engl.) Baehni* seed longevity and influence of soil mixture on seedlings growth performance

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ABSTRACT

Pouteria adolfi-friederici (Engl.) Baehni (*Kerero*) is an indigenous tree that is overexploited for high-quality timber production. However, there is little/no documented information on its seed longevity and seedling quality for successful field establishment. Therefore, the present study aimed to investigate seed longevity and evaluate the effect of soil mixtures on seedlings' growth performance. Seeds were collected from 20 selected mother trees in Gera District. Seeds were stored in cold room at +5 and 21/22^{0}c and tested their germination ability at 5, 180 and 360 days after storage. The growth performance of seedlings in Greenhouse was further evaluated with three different soil mixtures (2 part forest soil & 1 part sand, i.e., 2:1 Ratio (SM1); 1 part forest soil & 2 part sand, i.e., 1:2 Ratio (SM2) and 2 parts forest soil alone (SM3)). From 1 kg (360 seeds) pure seeds of 95% purity, 257 seedlings were estimated to be raised at the Laboratory. Seeds stored at 21/22^{0}c for 5 days had 75% germination potential. The highest seedlings survival rate (100%) was counted in soil mixtures with 2:1 ratios (2 parts forest soil: 1 part sand). The highest mean value of height and root length was measured in 2 parts forest soil alone. The largest root collar diameter (RCD) and root dry weight were recorded in 2:1 Ratio. Number of leaves and root fresh weight had the highest record in 1:2 ratios (1 part forest soil and 2 parts sand). But, no significant variation ($p<0.05$) was observed among the three soil mixtures. Unlike root-to-shoot ratio, height and RCD had a significant correlation ($p<0.01$) with other morphological variables. The quality index confirmed that 2:1 ratios were the best soil mixture for raising *P. adolfi-friederici* seedlings at greenhouse. The best seed source provenance and vegetative propagation selection should be further considered for successful field establishment and plantation development.

***Key words**: Gera, seedling quality index, RCD*

INTRODUCTION

Pouteria adolfi-friederici (Engl.) Baehni subsp. *adolfi-friederici* is an indigenous multipurpose tree species belonging to the family *Sapotaceae* (Fichtl and Adi, 1994; Friis, 2003; Bekele, 2007). It is a very tall tree with a height of up to 45 (-50) m tall (Friis, 2003) with a clear straight bole nearly 16 m long (Maundu and Tengnäs, 2005; Bekele, 2007). The tree has a breast height diameter up to 4 m (Fichtl and Adi, 1994). Its local name is *Kerero* in Amharic and Afan Oromo languages. *Pouteria adolfi-friederici* is widely distributed in Shewa, Arsi, Welega, Ilubabor, Kefa, Gamo Gofa, Sidamo and Bale floral regions (Friis, 2003; Bekele, 2007) within altitudinal ranges of 1,350-2,450 m above sea level (Friis, 2003). It is extensively overexploited and depleted for its high-quality timber production in potential areas of Ethiopia for various wood and wood-based products. As a result, only a few *P. adolfi-friederici* old aged and very tall mother trees are observed in potential and native ranges in the remnant high forest areas of the southwestern and southeastern Ethiopia. The remaining patches or scattered trees are further distributed in the protected areas, church forests and graveyards, under agroforestry system and farm boundaries. Its highly suitable habitats have also shifted from the northern and central parts to the southern parts of Ethiopia (Tadesse et al., 2022). According to Friis (2003) investigation on species status, although classified as *not threatened* on a global basis, the species is overharvested and very little concern is given for conservation or reforestation of the species. With this, it is very hard to see its wood (Fichtl and Adi, 1994).

Furthermore, mother trees are extremely tall with a height of up to 50 m so that climbing and collection of mature and fresh seeds is also very challenging. Consequently, fallen seeds or fruits are collected under mother trees, which are not fresh, well matured or easily attacked by insects or infested by disease. This has a considerable effect on poor germination capacity of seeds and then the subsequent loss of seed viability in a short duration. Fallen seeds or fruits, in turn, are eaten by seed predators (e.g. birds, rodents, smaller animals, etc.), decayed or rapidly germinated due to higher moisture availability. Seedlings and saplings are further easily free-grazed by herbivores, and there is little probability of being a mature tree. Packing and loading of processed seeds or fruits, transportation, handling and processing are additional practical problems for the limited availability of fresh and mature seeds. This is because the temperature and the moisture content affect the seed viability and germination potential. On the other hand, various sources reported that seeds have recalcitrant nature, which is characterized by short seed shelf life. Hence, the seed germination capacity and rate of germination becomes declined with a subsequent loss of seed viability and vigor with the progress of storage duration (Bahru et al, 2015). Thus, seed emergence, germination percentage, rate of germination and seed viability are indicators of seed longevity. Overall, provision of high quality and fresh seeds is required for viable seedling production of *P. adolfi-friederici*. This is because planted seedlings are intended to provide the necessary product or service due to its high quality and viability of seeds (FAO, 1985). In addition, raising high quality and large number of seedlings with best morphological and physiological features are required under favorable site conditions.

Similarly, selection of appropriate soil mixture or soil media helps to enhance the survival rate and growth performance of seedlings at Greenhouse and nursery. This, in turn, contributes to successful seedlings field establishment and adaptation potential and eventually for small- and large-scale plantation development. This means the physical, chemical and biological characteristics of the soil determine seedling production and successful establishment. This is because it provides the necessary available nutrients and water, creates favorable soil aeration, optimum temperature and available moisture condition as well as soil fertility to plant growth and development. At the same time, the nature of soil property further determines the insect pest infestation and diseases infection and occurrence on plants. Suitable soil mixture also helps to form well-established root system in the soil and firmly supports the plants. Furthermore, there is no latest information or little efforts have been made to explore on effects of soil mixtures on early survival and growth performance of *P. adolfi-friederici* seedlings at Greenhouse.

In general, P. adolfi-friederici is a potential candidate species for tree domestication under agroforestry system or around homesteads due to its great economic contribution and the possibility of growing the species on farm. Likewise, an intraspecific morphological variation was identified using three main morphometric parameters (stem height, BDH and bole length) among the five populations in different natural forests of southwest Ethiopia (Seid and Mengesha, 2022). Such future research direction further helps to conserve the species genetic base, establish seed orchards, improve its genetic and breeding base, and improves productivity (fast growth and high timber quality and yield) and plantation expansion and development. Therefore, the objectives of the present study mainly focused on: 1) to investigate effects of storage temperature and duration on *P. adolfi-friederici* seed longevity at cold room, 2) to evaluate effects of

different soil mixtures on early survival and growth performance of *P. adolfi-friederici* seedlings at Greenhouse, and 3) to select an optimum soil mixture for high quality and large quantity seedling production at Green House for small- and large-scale successful seedling establishment in potential areas.

MATERIALS AND METHODS

Description of the study site

This experimental study (seed longevity test at the Laboratory and various soil mixtures on seedlings growth performance at Greenhouse) was conducted at Central Ethiopia Forestry Development Center (CEFDC), Ethiopian Forestry Development (EFD), Addis Ababa, Ethiopia. It is situated at Gurd Shola, Bole Sub-city. The Greenhouse is located in the Highland (*Dega*) Agro-ecology at the altitude of 2368 m a.s.l. between $37^{0}04'$E Longitude and $09^{0}96'$N Latitude. Addis Ababa has a mean annual rainfall of 1000 mm and a mean monthly temperature of 20^{0}c.

On the other hand, *P. adolfi-friederici* fruits and seeds were collected in April, 2016 at Gera District, Jimma Zone of Oromia National Regional State, southwestern Ethiopia (Fig.1). It is part of the Belete-Gera National Forest Priority areas. The seed collection site lies between Latitudes $08^{0}65'$N & $08^{0}66'$N and Longitudes $37^{0}01'$E & $37^{0}02'$E at elevation of 2248-2280 m a.s.l.

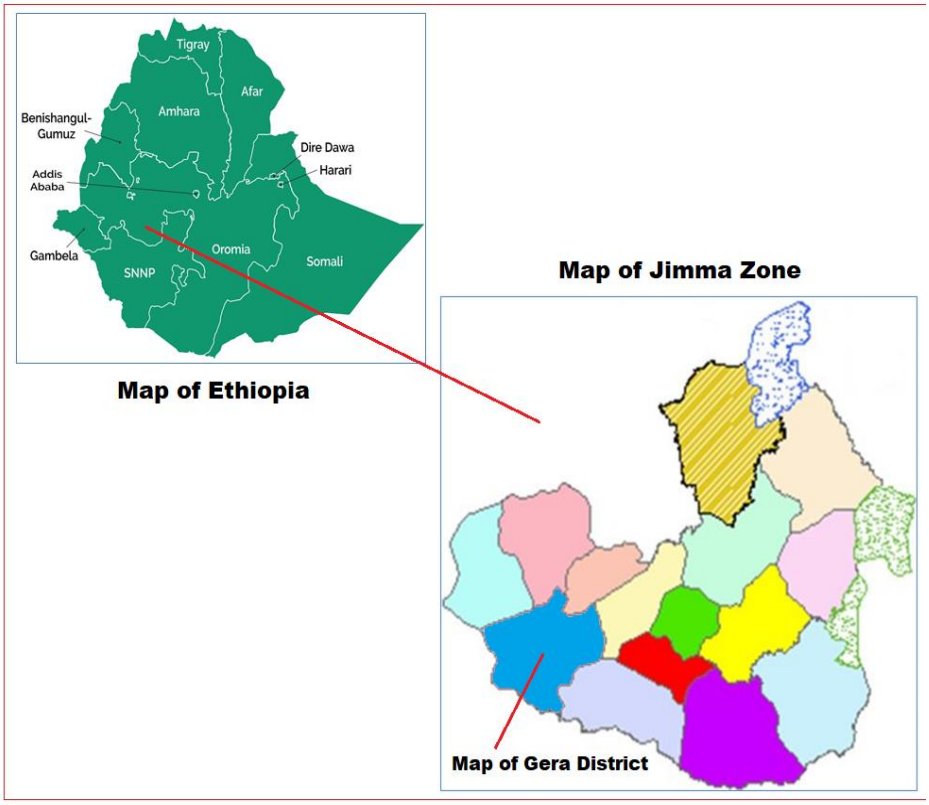

Fig. 1. Map of the study area (*P. adolfi-friederici* fruits and seeds collection area)

Experimental Design and Treatment Application

Seeds were collected from 20 selected mother trees stands at a distance of at least 100 m apart between them so as to ensure maximum genetic diversity (FAO, 1975). Then seeds were separated from fleshy collected fruits at field (Fig. 2a&b). There after a total of 15 kg collected seeds were put into perforated plastic bags and safely transported to CEFDC, EFD, Addis Ababa. Seeds which had been attacked by insects, physically damaged or decayed were excluded, extracted and cleaned at seed processing room following the procedures by FAO (1985). Following this, seeds were air-dried under shade at seed processing room, made ready for storage and stored at cold room until the seed test at Laboratory was conducted.

Fig. 2. Images of *Pouteria adolfi-friederici* fruits and seeds taken from Gera District: a) fleshy fruits fallen on the ground from mother trees, b) mature seeds after processed and separated from fruits.

Laboratory experiment: Once the freshly collected seeds were ready for Laboratory test, moisture content, purity percentage and seed weight per kg of *P. adolfi-friederici* seeds were determined at Laboratory, following the methods by FAO (1985). Initially, to determine moisture content, two samples of 5 gm seeds each were drawn from the total seed lot before the determination of purity analysis. Samples were weighed and dried in an oven for 17 hours at 103^0c. Finally, samples were placed in a desiccator, cooled for 30 minutes and then reweighed. The wet weight or fresh weight basis of moisture content was calculated as:

$$\text{Moisture content \%} = \frac{\text{Original weight} - \text{Oven dry weight}}{\text{Original weight}} \times 100$$

$$\text{Purity \%} = \frac{\text{Weight of pure seed}}{\text{Total weight of original sample}} \times 100$$

Hereafter, two samples of 35 gm *P. adolfi-friederici* seeds each were drawn from the total seed lot containing all the impurities and weighed. Following this, the pure seed was removed and reweighed separately. The percentage of pure seed was determined as follows:

Eventually, to determine seed weight of *P. adolfi-friederici,* 8 replicates of 100 pure seeds each were randomly drawn from the total seed lot and expressed as weight of whole seeds. Hence, the 1000 pure seed weight was converted to number of pure seeds per kg.

$$\text{Number of seeds per kg} = \frac{1000 \times 1000}{\text{weight in g of 1000 seeds}}$$

To measure its seed size, 100 seeds in 4 replicates of 25 seeds each were randomly drawn from the total seed lot. Following this, seed length and seed width was measured in centimeters (cm) using seed caliper (Bahru et al., 2015).

Finally, the number of estimated seedlings to be raised per kg of seeds in the Laboratory also calculated as follows:

Number of estimated seedlings raised per kg of seeds	**=**	***Number of seeds per kg***	***x***	***Percentage germination***	***x***	***percentage purity***

Following these tests, effects of various seed pre-sowing seed treatments on germination capacity of *P. adolfi-friederici* seeds were assessed. For this test, freshly collected seeds after cleaned and processed were used before storage at cold room. Hence, the pre-sowing seed treatment was tested with normal seeds and independent of either the seed longevity or the Greenhouse experiment. Accordingly, seeds were subjected to six seed pre-sowing treatments: nicked seeds (Treatment 1/T1); seed coat removed (T2); seeds were immersed in hot water (100^0c) for 1 minutes (T3); seeds were immersed in hot water (100^0c) for 5 minutes (T4); seeds soaked in cold water (19^0c) for 24 hours (T5) and control seeds (seeds without treatment) (T6). These seed pre-sowing treatments were adopted from previous works by Bahru et al. (2014&2015) to various tree seeds and Bamboo species. A small portion of the seed coat was carefully removed during nicking at the side of the hilum using sharp scissor until a small hole was remained for imbibition of water and oxygen. Great care was taken not to damage seed embryo and the emerging radicle as well. Likewise, proper care was

also given to remove the hard seed coat from the cotyledon so that not to separate the cotyledons and hence damage the embryo.

In line with this, effect of storage temperature and duration on *P. adolfi-friederici* seed longevity was further investigated. Accordingly, total seed lot was mixed thoroughly and divided equally into two seed samples using completely randomized design (CRD) (Gomez and Gomez, 1984) *P. adolfi-friederici* seed longevity test was evaluated in a two-factor experiment involving two storage temperature and three seed storage durations in 4 replications, each. Following this, seed samples were randomly drawn and placed separately into two locally used plastic water containers (*Roto* in Amharic), *i.e.*, treatments. The first container (treatment) was stored at cold room at $+5^0$c (Treatment I/TI), while the second container was stored at room temperature, *i.e.*, $21/22^0$c (Treatment II/TII) as also partially applied by Baskin and Baskin (2001). Storing seeds at low temperature enables to maintain the viability of seeds and prevent from insect attack by reducing seed moisture (Hartmann and Kester, 1983; FAO, 1985). Moreover, the rate of physiological changes of many seeds becomes slow down by storing them at low temperatures Baskin and Baskin (2001). On the other hand, the seed germination tests were conducted at three storage durations or time, *i.e.*, on 5 days (freshly collected seeds) (Duration 1/D1), 180 days (D2) and 360 days (D3) after storage at cold room. Hence, seeds were allowed to germinate at Laboratory shortly after the collection of seeds, *i.e.*, within a week (Baskin and Baskin, 2001). Finally, 100 sampled seeds were drawn randomly from each treatment (TI & TII) and tested for germination capacity at 5 (D1), 180 (D2) and 360 days (D3) interval since seed storage at cold room. The seed germination tests were carried out with nicked seeds, which had best germination capacity.

Finally, all germination tests were conducted at Laboratory in such a way that all treatments (six pre-sowing seed treatments and two treatments in each storage duration of the seed longevity test) had 100 seeds in 4 replicates of 25 seeds, each. Seeds were sowed on Laboratory plastic Trays (hereafter referred to as Trays), where sand served as germination medium. Following completion of sowing, Trays were covered with plastic cover so as to create optimum temperature for seed germination. The sand was kept moist with distilled water as much as possible throughout the entire experimental period. In order to moisten the sand, water was added until the sand was saturated. Water was added to sand if the water vapour was disappeared from the plastic cover. Seeds were inspected everyday starting from the second day after sowed. All germinated seeds were counted and removed out daily in order to avoid the double counting of seeds. A seed was considered as germinated or emerged when the radicle (primary root) was emerged from seed coat and clearly appeared visually (Fig. 3). The daily germination count was continued until no more seeds had been germinated (60 days). This method was adopted from Baskin and Baskin (2001) and Bahru et al. (2014 & 2015).

Fig. 3. Emerged radicle (a) and sprouted young shoot (b) from *Pouteria adolfi-friederici* seeds germination test at Laboratory

Greenhouse experiment: This experiment was independent of the Laboratory experiment except well-developed germinated seedlings were served as an input to the Greenhouse experiment instead of raising the required seedlings. With this, 100 polyethylene pots (hereafter referred to as pots) (20 cm height and 20 cm diameter size) per soil mixture (treatment) were prepared containing a mixture of forest soil and sand or forest soil alone in different ratios to raise the seedlings. Hence, the three soil mixtures or treatments (SM1-SM3) at Greenhouse were: 2 part forest soil & 1 part sand, *i.e.*, 2:1 ratios (SM1); 1 part forest soil & 2 part sand, *i.e.*, 1:2 ratios (SM2) and 2 part forest soil alone (SM3). In this experiment, SM3 served as control treatment. Accordingly, using a Completely Randomized Design (CRD), 100 pots in 5 replications of 20 pots, each were

established at Greenhouse. Following this, 100 *P. adolfi-friederici* seedlings having 2-4 leaves were uprooted from the Trays at Laboratory and transplanted to pots at Greenhouse on each treatment (*i.e.*, SM1, SM2 and SM3). Seedlings' survival ability at Greenhouse was supervised until two weeks and the dead seedlings were replaced by other normal seedlings. Watering was done daily early in the morning and late in the afternoon when it is necessary. Weeding, hoeing, inspection of pest and disease as well as other necessary silvicultural managements were further carried out regularly and whenever required.

Data collection and Statistical analysis

Collected germination data at Laboratory for each treatment (pre-sowing seed treatment and seed longevity) were first arcsine transformed before statistical analysis to fulfill the normality assumption (Gomez and Gomez, 1984; Baskin and Baskin, 2001). Thereafter, germination capacity or germination percentage and rate of germination were determined. Accordingly, germination capacity is the proportion of total number of germinated seeds at the end of germination period to the total number of seeds sown and expressed as in percentage. Similarly, the seed germination rate is the total number of seeds germinated on day D divided by the total number of days counted from the beginning of the test, following the method adopted by Gadissa et al. (2022). On the other hand, data collection at Greenhouse on survival rate and growth performance of *P. adolfi-friederici* seedlings was conducted at the age of 12 months after the completion of transplanting of seedlings on pots at Greenhouse. Accordingly, the survival count was conducted among the three treatments (SM1, SM2 and SM3). After that, the percentage survival capacity of each treatment was calculated as the number of *P. adolfi-friederici* seedlings survived at the age of 12 months divided by the total number of planted seedlings multiplied by 100. Similarly, the growth performance of *P. adolfi-friederici* seedlings for each treatment was recorded at Greenhouse. These parameters included: seedling height, Root Collar Diameter (RCD), number of branches and leaves for each seedling. Accordingly, seedling height was measured using ruler in centimeter (cm) from the soil surface (root collar) to the tip of the main seedling axis (apical bud). The RCD was measured using a digital caliper in centimeters (cm) near the soil surface. In line with this, the number of branches and leaves in each seedling was counted. Furthermore, to determine the seedlings' biomass estimation, 20 seedlings in each treatment were chosen and sampled. Then, each seedling was uprooted separately from each treatment using systematic random sampling method. Following this, each seedling's shoot (leaves, branches and stem) was cut and separated from the root at the soil surface using a sharp plant pruning scissor. During uprooting process, great care was taken to remove the soil from the root system. This is because fine roots particularly associated primary and secondary roots were cut and left into the soil so that it affected the root length and root fresh & dry weights. Root length was measured using ruler in cm from the soil surface to the tip of the root. Finally, each seedling's shoot and root were put separately into the coded Manila Envelope (*Kaki Wereket* (Amharic)). Fresh weight of each seedling's shoot and root were measured using sensitive analytical balance in gram (g) as soon as possible not to lose moisture and affect the weight. Thereafter, each shoot and root with their respective envelopes placed into the dry-oven at a temperature of 73 ± 2^0c for 24 hours (Bahru et al., 2014&2018). Finally, envelops were removed out from the dry-oven. Then, dry weight of each seedling's shoot and root was measured soon not to absorb moisture and affect the weight. The process was continued in the same way until insignificant weight difference was recorded between dry weights by measuring randomly drawn samples from small, medium and large weights.

Data collected from the laboratory and greenhouse were analyzed by using descriptive statistics such as percentages, figures and tables. In addition, statistical tests on seedlings early survival rate, growth performance and biomass estimation parameters were performed using SPSS statistical software package version 27.0 (SPSS inc., IBM, USA) and the results were reported as Mean±Standard Deviation (SD). The LSD test was used for post-hoc analyses to determine the statistical significance difference between treatments at $p<0.05$ confidence interval. A two-tailed Pearson correlation analysis (SPSS Inc., IBM, USA) was performed to determine the relationships between seedlings' height and diameter at breast height (DBH) with other morphological variables. On the other hand, using seedlings morphological features such as seedlings' height, RCD, shoot and root dry weights as well as shoot-to-root ratio, seedling quality is predicted at field (Dickson et al., 1960). Therefore, the seedling quality index (QI), which is ranged from 2 to 0, was calculated using the following formula (Dickson et al., 1960) and computed with the recorded *P. adolfi-friederici* treatments.

$$\text{Quality Index (QI)} = \frac{\text{Total seedling dry weight}}{\left(\left(\frac{\text{Seedling height}}{\text{Seedling RCD}}\right) + \left(\frac{\text{Shoot dry weight}}{\text{Root dry weight}}\right)\right)}$$

RESULTS AND DISCUSSION

Purity, moisture content and seed size and weight

During Laboratory test, the seed purity analysis was determined since a total of 15 kg bulk sample was collected and a lot of impurities were found as indicated in Fig. 2b. With this, purity analysis of *P.*

adolfi-friederici seeds at Laboratory indicated the presence of high purity level (95%). High purity level (95%) of *P. adolfi-friederici* seeds at Laboratory suggested that the seeds could be classified as pure seeds and hence contributed for better germination ability. Thus, such purity analysis of a given seed sample is the first test to be conducted so as to identify mature, pure and germinating seeds for subsequent tests (FAO, 1985). Its moisture content from the average of the two samples was found to be 24.3% before storage at cold room. Still after one year seed storage, *P. adolfi-friederici* seeds moisture content was found to be 38.9%. Its moisture content, in turn, rose from 24.3% (during initial storage) to 38.9% (at the end of one year) might be due to the absorption of moisture from the air (*i.e.*, hygroscopic nature of seeds) at cold room (+5^{0}c temperature). This higher amount of moisture content (>20%) in fresh and mature collected seeds indicated the seeds have recalcitrant seed storage behavior (FAO, 1985; Hong et al., 1996). However, the most important seed storage conditions that maintain seed viability (in the case of Orthodox seeds) are low moisture content and low storage temperature (Hartmann and Kester, 1983). This is because various storage problems arise with increasing seed moisture including insect and fungal attack, stimulating germination and heating effect. Collectively, FAO (1985) explained that moisture content has an impact on longevity of seeds during storage. Hence, proper management of the optimum moisture content during storage is recommended to maintain seeds longevity and viability. But in any case, to give a general conclusion on *P. adolfi-friederici* seed storage behavior further more comprehensive and detail research on different moisture and temperature ranges up to five years of storage should be suggested.

Similarly, the overall seed weight test after purity analysis indicated that there were 360 seeds contained in one kilogram (kg) of pure *P. adolfi-friederici* seeds. This, in turn, has a mean thousand seed weight of 2,777.8 g. With this, 360 *P. adolfi-friederici* seeds/kg in the present study as compared to 450 seeds/kg reported by Maundu and Tengnäs (2005), confirmed that seeds are larger in size and weight. FAO (1985) and Ghosh and Singh (2011) reported that heavy and larger seeds weigh more per seed than smaller seeds, and hence contain more food reserves. Consequently, they are more likely to have higher germination percentage by providing more energy and produce initially more vigorous seedlings (FAO, 1985; Ghosh and Singh, 2011). Therefore, from one kg (360 seeds) of pure *P. adolfi-friederici* seeds, 257 seedlings were estimated to be raised at Laboratory. The mean seed length and width were 3.10$\pm$0.36 cm and 1.49$\pm$0.24 cm, respectively. The seed length ranged from 1.30-3.80 cm, respectively. The lowest and highest seed width was also 1.00 and 3.10 cm, respectively. Therefore, seed size and weight are important characters for selection of well-adapted and highly productive seed sources for silvicultural practices. As a result, this improves seedling productivity and reducing nursery cost through selection of quality and the required amount of seeds (FAO, 1985; Ghosh and Singh, 2011).

Effect of pre-sowing seed treatment on germination capacity

Germination of *P. adolfi-friederici* seeds were significantly ($P<0.001$) varied among the six major pre-sowing seed treatments. Germination capacity at the end of the experiment, *i.e.*, 60 days was ranged between 0 and 75% among treatments at 24.3% of moisture content (Fig. 4). Likewise, the seed germination rate was ranged from 0 to 1.25. The result indicated that no germination was recorded on the 1st and 2nd weeks. However, only 5% germination in T1 and 11% germination in T2 was recorded at the beginning of 3rd week after seeds sowed at Laboratory. Most of the germination in T1 (55%) was observed in the 4th and 5th weeks after sowed, while 15% of germination was recorded at the end of the experiment. Germination in T1&T2 was fastest, uniform and completed from the 4th week after sowed to the end of germination (60 days). Such fastest and uniform germination capacity in nicked seeds (T1) and seed coat removal (T2), suggested that the removal of hard seed coat enables easier imbibition of water to seeds and thereby creates favorable condition for seed germination. On the other hand, rate of germination in control seeds (T6) was very slow and uneven in the entire germination period despite its better germination capacity (52%) compared to the remaining treatments (T2, T3-T5). This is because the hard seed coat prevents easier imbibition of water to seeds and hence it takes longer time to do so. Overall, germination test at Laboratory indicated that the highest germination capacity (75%) was recorded in nicked seeds (T1), followed by control seeds (T6), which accounted for 52%. Its germination rate was 1.25 and 0.87 in their respective order. Various previous research outputs for different species similarly indicated that nicked seeds are the best pre-sowing seed treatments (Bahru et al., 2014). But, sowing control seeds (T6) is more useful from the practical aspect to save human power, time and resource for large-scale seedling raising, successful establishment and plantation expansion. This is due to the fact that treating seeds (e.g. acid or mechanical scarification using file, scalpel, knife, etc.) is a very time-consuming and labour intensive method of making seeds permeable to water (Baskin and Baskin, 2001). In addition, application of chemicals (e.g. acid) is not environmentally sound due to the ever rising pollution effect across the globe. However, sowing nicked seeds (T1) is more practical in small-scale seedling raising, successful establishment and plantation expansion. The next germination capacity was recorded by T2, T4 and T5 that accounted

for 48, 33 and 26%, respectively. The computed seed germination rate, in turn, was 0.80, 0.55 and 0.43 in their respective orders. Consequently, higher germination ability in T4 compared to T3 suggested that the longer exposure of seeds to hot water treatment (100^0c) for 5 minutes in T4 resulted for cracking the hard seed coat and hence causes easier imbibition of water to seeds. Similarly, higher germination percentage in T4 compared to T5 might suggest that hard seed coat was easily cracked by hot water treatment (T4) instead of cold water (T5) and hence easier imbibition of water to seeds. Thus, a reduced germination in hot or boiling water treatments (T3 and T4) is an indication of possibly damaged seed embryos (Baskin and Baskin, 2001). Overall, application of appropriate pre-sowing seed treatment depending on the nature and type of seeds is strongly recommended to improve the germination capacity of seeds and reducing the resource and time cost. Moreover, our finding confirmed that germination capacity of seeds has strong impact on viable seedling production, seedling growth performance as well as morphological appearance (Hartmann and Kester, 1983).

Fig. 4. Pre-sowing seed treatments of *P. adolfi-friederici* seeds. The six seed pre-sowing treatments were: nicked seeds (T1); seed coat removed (T2); seeds were immersed in hot water (100^0c) for 1 minutes (T3); seeds were immersed in hot water (100^0c) for 5 minutes (T4); seeds soaked in cold water (19^0c) for 24 hours (T5) and control seeds (T6).

Effect of storage temperature and duration on seed longevity

Recorded Laboratory data after 360 days of seed storage revealed that germination was 4% in TII, whereas no germination was recorded under TI (Fig. 5). Consequently, seeds stored under cold room ($+5^0$c) or TI were severely attacked by fungus and the fungus covered the entire seed coat and eventually some seeds were decayed and remained dead. Therefore, poor germination capacity (0%) in TI at cold room ($+5^0$c) as compared to TII (4%) at $21/22^0$c most probably associated with higher moisture availability and hygroscopic nature of seeds under low temperature ($+5^0$c) during seed storage. This, in turn, causes seed decay and fungus attack and hence seeds lost their seed viability and vigour. With this, the result of the present study confirmed that storing seeds under optimum moisture content and temperature and storage duration improves or maintains the germination capacity of seeds for the required time. Previous studies by Mubvuma et al. (2013) on *Moringa* seeds, Sukesh and Chandrashekar (2013) on *Vatica chinensis* seeds and Guo et al. (2020) on *Pinus bungeana* seeds support the finding of the present research on *P. adolfi-friederici* seeds.

On the other hand, seed germination was dramatically reduced over storage duration for both treatments (TI & TII). Accordingly, the germination capacity of *P. adolfi-friederici* seeds was 75, 19 and 0% after 5, 180 and 360 days of storage duration at cold room, respectively. The seed germination rate also had 1.25, 0.27 and 0 values, respectively. With this, the fresh and mature collected seeds (5 days of storage) had the highest germination (75%), while seeds stored at cold room for 360 days had the least germination percentage (4%). This clearly indicated that storage duration had a negative impact on germination capacity, germination rate, seed longevity and viability. This is because as the seed storage duration increased, there is a gradual decline in germination capacity, germination rate or seed viability and/or an increase in seed dormancy. Similar earlier findings were also reported by Teketay (1994), Baskin and Baskin (2001), Ayana et al. (2012) and Bahru et al. (2015) for various seeds of plant species in relation to storage duration. Hence, *P. adolfi-friederici* seedlings should be raised from fresh and mature collected seeds to get the best quality and the required quantity of seedlings at nursery or seedling production sites. Finally, our result concluded that *P. adolfi-friederici* seeds are classified under recalcitrant seed storage

behavior due to the requirement of higher moisture content (>20%) and higher temperature (21/22^{o}c) for a short storage duration (a maximum of 360 days) after this particular Laboratory seed research and other related earlier studies.

Effect of soil mixture on survival and mortality rate of seedlings

Analysis of data on *P. adolfi-friederici* seedlings indicated that the highest survival rate (100%) was recorded in SM1 followed by SM2, which accounted for 97% (Fig. 6). Conversely, 10% mortality rate was recorded in SM3. As a result, the soil mixture in SM1 with more proportion of forest soil might supply more nutrients and water to seedlings than SM2. The highest survival rate (100%) in SM1 suggests the requirement of appropriate soil mixture (forest soil and sand) for successful survival of seedlings and hence the great care to be considered during seedling raising at Greenhouse and/or nursery. Thus, the present result confirmed that various soil mixtures had a considerable impact on successful survival rate and growth performance of seedlings at Greenhouse. This is because the availability of nutrients and water in a given soil mixture not only improves the survival potential of seedlings but also their growth performance (Salinas-Peba et al., 2014). Various earlier findings obtained by some authors (Mulugeta, 2014; Segaw et al., 2016) also support this research output. Therefore, at Greenhouse SM1 was recommended for raising large number of *P. adolfi-friederici* seedlings with the best survival potential.

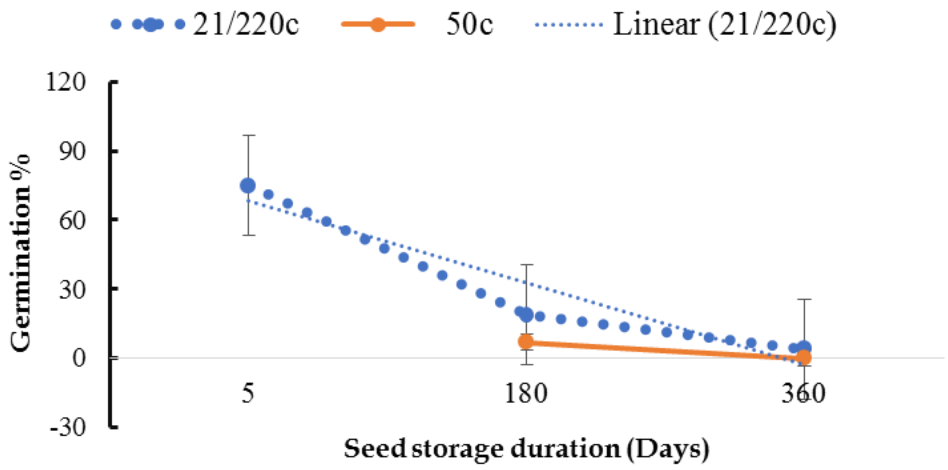

Fig. 5. Effect of seed storage temperature & duration on *P. adolfi-friederici* seed longevity. Seeds stored at cold room at +5^{0}c (TI), room temperature at 21/22^{0}c (TII) & its trendline on 5, 180 & 360 days of storage.

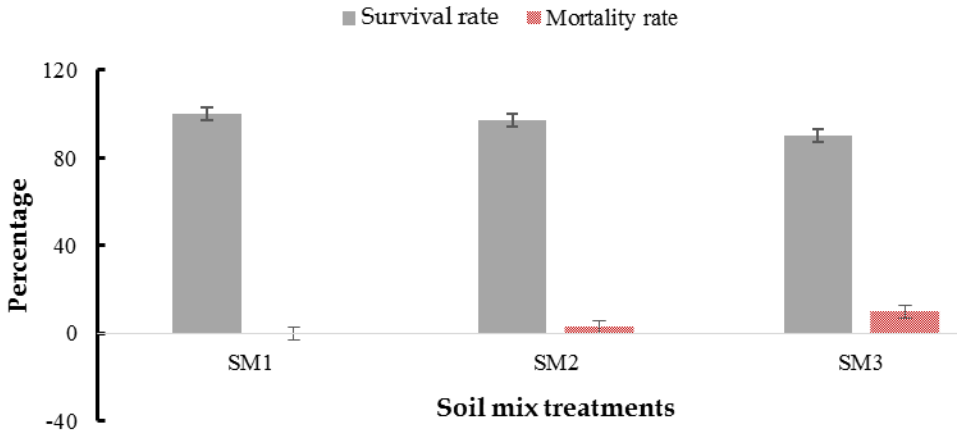

Fig. 6. Effect of soil mixture on seedlings' survival and mortality rate. The three soil mixtures include 2 part forest soil & 1 part sand, *i.e.*, 2:1 ratios (SM1); 1 part forest soil & 2 part sand, *i.e.*, 1:2 ratios (SM2) & 2 part forest soil alone (SM3).

Effect of soil mixture on growth performance of seedlings

The present result confirmed that the mean seedlings' height was 23.87±7.30 cm, while the mean RCD was 0.64±0.24 mm. The total mean height of *P. adolfi-friederici* seedlings (around 24 cm) in the present study assured that the seedlings are reached the optimum plantable height (15-30 cm) at field (Jaenicke, 1999). The required size of seedlings, in turn, attributed to the successful establishment, survival and growth performance of seedlings at field. The seedlings' height and RCD among the three treatments ranged from 6 to 46 cm and 0.2 to 1.30 mm, respectively. The tallest mean seedlings' height (24.98±8.43 cm) was recorded by SM3, while SM1 had the shortest mean height (23.31±6.80 cm) (Table 1).

The tallest seedlings size in SM3 compared to SM1 might be associated with the high light demand and hence more competition among seedlings or may be other factors not yet discovered. Otherwise, better height performance in SM3 may not be necessarily related to the soil mixture. Earlier study by Segaw et al. (2016) also confirmed that both *Cajanus cajan* and *Sesbania sesban* seedlings had less height performance despite the higher proportion of forest soil in the soil mix ratio. On the other hand, the largest (0.67±0.19 mm) and the smallest (0.61±0.29 mm) mean seedlings' RCD was recorded by SM1 and SM2, respectively. The largest RCD in SM1 most probably associated with the optimum provision of nutrients and availability of water by forest soil, while better soil aeration and thereby soil fertility by the addition of sand to the mixture. Consequently, these basic requirements in the soil mixture considerably improved the size of seedlings RCD in SM1. On the contrary, the smaller RCD in SM2 compared to SM1 might be related to inadequate supply of nutrients and water to seedlings despite more aeration in the mixture. The least RCD in SM3 probably suggests limited availability of nutrients and water to seedlings due to inadequate aeration and thereby soil compaction. Therefore, raising *P. adolfi-friederici* seedlings with SM1 is more helpful to produce seedlings with the required size of RCD and height within a short period of time. Unlike height of seedlings, the largest seedlings RCD growth performance in SM1 revealed that higher ratio of forest soil might be suitable soil mixture for *P. adolfi-friederici* seedlings. Overall, longer seedlings' height and larger RCD could be contributed for better physical support and field establishment as well as greater survival rate and higher growth performance with better adaptation capacity of the seedlings at field conditions. Some of the pervious works that support this result include Florence (1996), Takoutsing et al. (2013), Terefe et al. (2016) and Bahru et al. (2014).

Similarly, total number of leaves developed on *P. adolfi-friederici* seedlings at the age of one year further ranged from 2 to 30 among the treatments. The highest total mean number of leaves (11.22±4.05) was counted in SM2, while the lowest mean value was recorded in SM1 (10.46±3.06). Furthermore, the total number of branches ranged from 7 to 36 among the treatments. Of these, 20% of the seedlings developed branches, where each seedling had 1 to 5 numbers of branches. With this, the largest mean seedlings' number of branches was counted in SM3 (2.04$\pm$1.47), while the least was in SM1 (1.33$\pm$0.69). The highest total number of leaves recorded in SM2 might be related to better supply of nutrients and water by higher proportion of sand in the soil mix and this resulted in reduced root growth. Consequently, shoot growth is triggered to enhance the efficiency of photosynthesis within seedlings. The large number of leaves per seedlings is contributed for large surface area for light absorption (Lambers et al., 1998; Taiz and Zeiger, 2010). This further enhances higher efficiency of photosynthesis within leaves and resulted in higher growth rate of seedlings (Lambers et al., 1998; Taiz and Zeiger, 2010).

On the other hand, practical observation during uprooting the one-year-old seedlings indicated that the tap root is associated with dense primary and secondary roots and they formed well-developed root system around the soil inside pots. Some seedlings developed two or three tap roots instead of one and the root system was well-developed around the taproots. Collected Greenhouse data showed that seedlings tap root length among the three treatments was ranged from 7 and 36 cm, respectively. The longest mean seedlings' tap root length was measured in SM3 (22.85$\pm$4.42 cm), while the shortest was in SM1 (22.20$\pm$6.51 cm). The longest tap root length as well as associated roots in SM3 might suggest an adaptive response of the root system to the limited availability of nutrients and water due to soil compaction. As a result, the longest tap root length able to enhance the surface area for efficient absorption of water and nutrients from the surrounding soil and provide a firm physical support as well (Silvana, 1998). Furthermore, it serves as a link between fine roots and leaves through an elaborate system of larger transport roots, trunk, branches and twigs (Perry, 1982). By contrast, the shortest tap root length in SM1 most probably associated with more efficient absorption of water and nutrients nearby the root system due to good aeration. In general, all the seedlings' growth parameters (height, RCD, leaves & branches) showed there was numerical variation among the treatments (SM1, SM2 & SM3). However, the statistical test showed no significant variation ($P<0.05$) among the three treatments for all the recorded seedlings' growth parameters.

Finally, the greater fresh and dry root-to-shoot ratio in SM2 could be related with higher proportion of sand than forest soil. The ratio appears to be related with water uptake by the root and photosynthesis by the

shoot (Taiz and Zeiger, 2010). But, during severe water deficit more energy is shifted from the shoot and allocated to root system, where further root growth is supported into deeper soil (Lambers et al., 1998; Taiz and Zeiger, 2010). Thus, higher root-to-shoot ratio helps to firmly support the seedlings and enhances more access to water and nutrients from the soil (Takoutsing et al., 2013). Meanwhile, these authors further described that the ratio contributes high absorption and storage capacity of water in moisture stress areas.

Table 1. Mean values of seedlings' early growth performance parameters among *P. adolfi-friederici* treatments (n=20 or 100, mean±Standard deviation (SD))

Treatments	Shoot length (cm), n=100	RCD (mm), n=100	No of leaves, n=100	No of branches, n=100	Root length (cm), n=20	Root/Shoot length Ratio, n=20
SM1	23.31 ± 6.80	0.67 ± 0.19	10.46 ± 3.06	1.33 ± 0.69	22.20 ± 6.51	0.96 ± 0.22
SM2	23.41 ± 6.59	0.63 ± 0.21	11.22 ± 4.05	1.38 ± 0.74	22.40 ± 4.75	1.11 ± 0.35
SM3	24.98 ± 8.43	0.61 ± 0.29	11.14 ± 4.02	2.04 ± 1.47	22.85 ± 4.42	0.98 ± 0.24
P in ANOVA (f)	1.532	2.264	1.234	2.990	0.079	1.714
Sig.	0.218	0.106	0.293	0.058	0.924	0.189

Effect of soil mixture on biomass yield

Total seedlings' biomass yield estimation of *P. adolfi-friederici*, *i.e.*, the seedlings' shoot and root fresh & dry weight as well as root-to-shoot ratio was presented in Table 2. Shoot fresh biomass yield ranged from 0.48-31.5 g, while the shoot dry biomass was 0.19-14.2 g. The highest mean seedlings' shoot fresh weight (10.25±7.56 g) and mean shoot dry weight (4.40±3.47 g) was recorded in SM1, each. On the other hand, the total root fresh and dry *P. adolfi-friederici* seedlings biomass yield ranged between 0.16-14.6 g and 0.07-7.9 g, respectively. The highest mean root fresh biomass yield (4.45±3.28 g) was weighed in SM2, whereas the highest mean root dry weight (2.22±1.73 g) was estimated in SM1. Laboratory result further revealed that the largest seedlings' dry root-to-shoot ratio (0.60±0.21) was weighed by SM2, while the smallest ratio (0.50±0.16) was recorded by SM3. The root-to-shoot ratio of *P. adolfi-friederici* seedlings is expressed as the ratio of the dry belowground (root) weight to the dry aboveground (shoot) weight of seedlings. It is a quantitative measurement that measures the overall health, *i.e.*, the growth, development and yield of seedlings. From this result, the largest root-to-shoot ratio in SM2 compared with SM1 might suggest a relatively limited available nutrient and intense water stress that trigger root growth and reduce the shoot growth and the finding further confirmed by various studies (Silva et al., 2012; Mašková and Herben, 2018). Despite the numerical variation, significant differences ($P<0.05$) did not observe among the three soil mix treatments within the given measured biomass parameters, *i.e.*, shoot and root fresh & dry weights.

Table 2. Mean seedlings' biomass estimation among *P. adolfi-friederici* treatments (n=20 or 100, mean+Standard deviation (SD))

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Root/Shoot Ratio	Shoot dry weight (g)	Root dry weight (g)	Root/Shoot Ratio
SM1	10.25 ± 7.56	4.23± 3.04	0.45± 0.17	4.40 ± 3.47	2.22 ± 1.73	0.55 ± 0.19
SM2	9.20 ± 7.39	4.45± 3.28	0.52± 0.18	3.80 ± 2.96	2.21 ± 1.72	0.60 ± 0.21
SM3	9.99 ± 6.08	4.03± 2.55	0.43± 0.25	4.39 ± 2.75	2.20 ± 1.48	0.50 ± 0.16
P in ANOVA (f)	0.123	0.102	0.866	0.253	0.001	1.407
Sig.	0.885	0.904	0.426	0.777	0.999	0.253

Evaluation of seedlings' quality for out-planting performance at field

Raising high-quality seedlings at nurseries enables successful establishment, better survival rate and fast growth performance at field. This, in turn, provides maximum benefit to small scale farmers and private investors engaged on forestry development Jaenicke (1999). With this, both seedling height and RCD were highly correlated with different morphological attributes (Table 3). Accordingly, seedling height was strongly correlated with the different morphological variables ($p<0.01$), except for root-to-shoot ratio. Meanwhile, seedling RCD was strongly correlated with the different morphological variables ($p<0.01$), except for root-to-shoot ratio and height to RCD ratio. With this, the highest significant positive correlation was observed between shoot dry weight and total seedling dry weight ($r=0.990$; $p<0.01$). On the contrary, there was no significant correlation ($p<0.05$) between the ratio of root-to-shoot ratio and other morphological variables, except for root length and root dry weight. However, root-to-shoot ratio had significant negative correlation with seedling root length ($r=-0.291$; $p<0.05$) and

seedling root dry weight (r=-0.259; p<0.05). Generally, our result was closely consistent with Tian et al. (2017), who reported that seedling height and RCD has a strong significant positive correlation (p<0.01) with other morphological variables unlike shoot-to-root biomass. Therefore, such correlation of morphological variables might be used to evaluate seedling quality at Greenhouse and thereby forecast its performance at field.

Table 3. Correlation matrix of *P. adolfi-friederici* seedlings' morphological variables measured (Pearson coefficients, two-tailed, n = 60).

Variables	Height	RCD	Height to RCD Ratio	Root length	Shoot dry weight	Root dry weight	Total seedling dry weight	Root/Shoot Ratio
RCD	.786**							
Height/RCD Ratio	.391**	-.237						
Root length	.498**	.492**	.079					
Shoot dry weight	.725**	.646**	.178	.536**				
Root dry weight	.736**	.709**	.113	.555**	.919**			
Total seedling dry weight	.743**	.681**	.158	.553**	.990**	.965**		
Root/Shoot Ratio	-.121	-.166	-.005	-.291*	.022	-.259*	-.077	

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Based on this result, linear regression equations were developed for six main variables that strongly correlated to each other (Fig. 7). The equation was written as $y=a+bx$, where y is a dependent variable, x is a predictor variable for the linear regression model, a is the y-intercept at x=0 and b is the slope of the regression equation. Consequently, the highest values of coefficient of determination (R^2) was recorded in the developed linear regression models (R^2 = 0.54 to 0.98), which means at least 53% of the total variation in the dependent response variable was explained by the linear regression model and hence the strength of the model. Moreover, the larger correlation coefficient (R=0.54 to 0.98) and Beta values (0.73 to 0.99) further indicated the strong linear relationships between the growth (height and RCD) and biomass variables. The strongest linear correlation among the six pairs of variables was illustrated by seedling shoot dry weight with total seedling dry weight. At the same time, seedling root dry weight had a strong correlation with shoot and total seedling dry weight. In addition, seedling height had a relatively strong association with root and total seedling dry weight. Seedling RCD and height in turn, strongly correlated and revealed strong association with other variables. Therefore, the strong linear association of seedling height and RCD with all other variables assured that seedling quality at Greenhouse was evaluated only with height and RCD data without considering other variables, which is more cost effective and time saving process.

Furthermore, the highest seedling quality index was recorded in SM1, which scored (0.18 $\pm$ 0.14) (Table 4). This was followed by soil mixture treatments of SM3 and SM2. With this end, the *P. adolfi-friederici* seedlings quality index (0.16-0.18) reported in this study was consistent with the range of seedling quality index (2-0) for white pine and white spruce seedlings found by Dickson et al. (1960). Thus, the current finding might be helpful for measuring the seedling quality at Greenhouse and predicting the survival and performance of out-planting *P. adolfi-friederici* seedlings using the seedlings quality index as the case in nursery (Dickson et al., 1960).

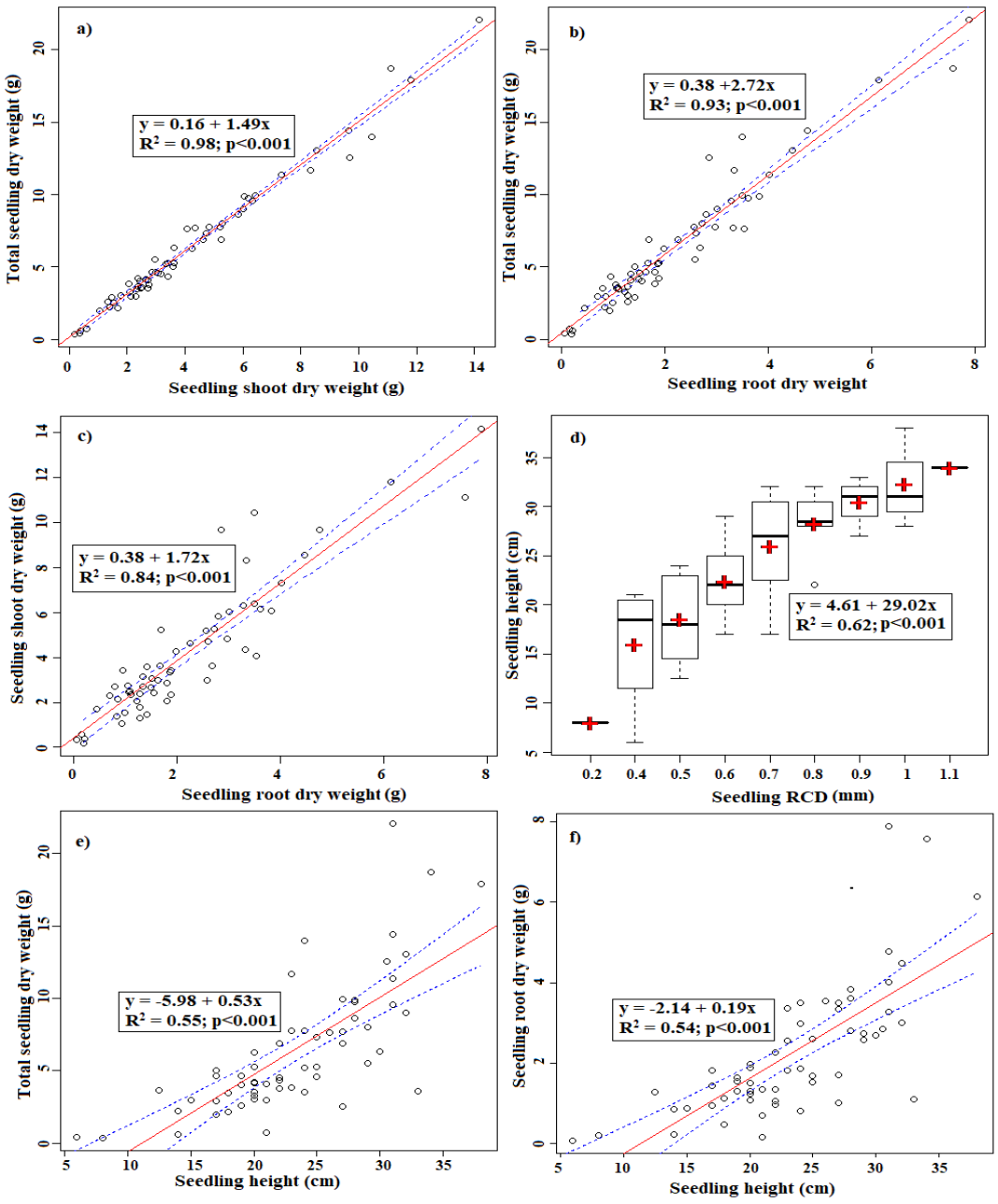

Fig. 7. Relationship between *P. adolfi-friederici* seedling growth and biomass variables and the developed linear regression equations and coefficient of determination (R^2) that indicated the strength of regression model.

Table 4. Evaluation of *P. adolfi-friederici* seedlings quality index among the three soil mixture treatments at Greenhouse (n=20, mean±Std)

Treatments	Shoot length (cm)	RCD (mm)	Total Dry weight (g)	Shoot-to-Root Ratio	Quality Index (QI)	Rank
SM1	23.25 ± 5.42	0.67 ± 0.19	6.63 ± 5.13	2.00 ± 0.64	0.18 ± 0.14	**1st**
SM2	22.10 ± 7.57	0.62 ± 0.16	6.02 ± 4.55	1.92 ± 0.91	0.16 ± 0.13	**3rd**
SM3	24.20 ± 5.99	0.64 ± 0.17	6.60 ± 4.18	2.18 ± 0.71	0.17 ± 0.11	**2nd**
P in ANOVA (f)	0.541	0.347	0.110	0.607	0.097	
Sig.	0.585	0.708	0.896	0.548	0.908	

In conclusion, storage temperature and duration had a considerable effect on *P. adolfi-friederici* seed longevity. Similarly, seedlings quality index confirmed that SM1 was the best soil mixture for raising *P. adolfi-friederici* seedlings at Greenhouse. But this result might not be applicable at field establishment of *P. adolfi-friederici* seedlings. Thus, early survival rate and growth performance of seedlings raised with different soil mixtures should be further carried out at field to evaluate the adaptation potential of seedlings.

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