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Research Article

Accelerated Ageing Tests of *Nauclea diderrichii* Seeds: Evaluating Seed Vigour from Different Forest Provenances to Aid Plus-trees Selection in Ghana

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Abstract

Background and Objectives: The selection of plus-trees with high seed vigour is an important step towards efficient *ex situ* conservation of forest genetic resources and plantation development. The study classified seeds collected from plus-trees of *Nauclea diderrichii* from 4 different provenances within the high forest zones of Ghana, namely: Abofour (KABO), Amantia (KAMA), Benso (KBEN) and Gambia (KGAM). **Materials and Methods:** A total of104 individual trees of *N. diderrichii* were identified across the natural distribution range of the species. These were marked as candidate plus-trees based on a set of standardized phenotypic scores during field surveys. Fruits were collected at maturity from 32 plus-trees and seeds extracted for storage and subsequent accelerated ageing tests. The time and temperature regime for the AA tests were 72 h and 41°C, respectively. Seed samples were placed in hermetically sealed bottles and stored at 4-5°C for 12 months. Germination of seeds before and after AA test was undertaken under ambient temperature by sowing 2.0 g of seeds in 4 replicates. Sterilized river sand was used as germination media for each accession. **Results:** Significant differences ($p<0.05$) in germination before and after AA test across all 4 provenances were found. Provenance KAMA produced the highest mean germination of 400 seedlings/2.0 g of seeds compared with KGAM (248), KABO (276) and KBEN (285) during germination after extraction. However, seed lots from provenances KABO and KAMA showed more vigour than the rest after 12 months of storage followed by AA tests. **Conclusion:** Although provenance KGAM recorded the highest number of plus-trees, seed lots from provenance KABO, KAMA and KBEN will be more suitable for long-term *ex situ* conservation as they gave higher vigour seeds after 12 months of storage.

Key words: Plus-trees, provenances, accelerated ageing, seed vigour, *Nauclea diderrichii*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

To achieve the desired outcomes of sustainable forestry, forest genetic resource conservation must be of global priority. Conserving forest genetic resources is of the essence because, despite the immense contribution of forest to the welfare of humankind, the rate of global deforestation continues to rise FAO[1]. Much of the world's biodiversity is being irreversibly lost through extinction caused by the destruction of natural habitats IUCN[2]. The FAO[3] estimates that the annual rate of deforestation in Ghana is 2.1%/year which corresponds with an average forest loss of 115,000 ha since the turn of the century.

There have been efforts in the past by the state to undertake large scale reforestation programmes in the country. For instance, the National Forest Plantation Development Programme (NFPDP) in 2001, aimed at planting 20,000 ha annually throughout the country Cobbinah *et al.*[4] Also, the Ghana Forest Plantation Strategy (2015-2040) seeks to achieve about 60% increases in forest estates of the country by the year 2040 FC[5].

Seeds play a vital role in the conservation and propagation of most plants species. Tree seeds form part of the primary input for productive forestry and reforestation programmes. As a result, the requirement for tree seeds in most tropical countries has multiplied manifold. Certainly, a plentiful supply of high quality seeds (of high viability and vigour) in good time is a prerequisite for any successful reforestation programme Schmidt[6].

Any successful breeding or reforestation programme for the improvement of timber quality and stock requires the identification and proper management of plus-tress Clark and Wilson[7]. Plus-trees often form the basis for reliable and efficient seed collection expeditions. These trees are mostly of good form relative to neighbouring individuals of the same species. In the past, the best trees have been felled, leaving poorer individuals as seed trees or founders of the next generation. However, the selection of plus-trees for propagation and conservation purposes is a critical step towards successful plantation forestry.

The use of accelerated ageing (AA) test as a complement to the conventional phenotypic selection of plus-trees is an innovative approach towards successful tree seed production and utilization Filho[8]. Identifying plus-trees using their phenotypes together with standardized seed testing techniques will no doubt ensure high seedling performance and subsequent timber quality. The accelerated ageing (AA) test is one of the most widely used techniques to evaluate seed vigour and predict storage potential of a seed lots Tekrony[9], Leeks[10], Miloševic *et al.*[11]. The procedure was developed by Delouche and Baskin[12] to measure seed storability and evaluate vigour. Over the years, AA technique has been employed by tree seed researchers as a means for evaluating the efficiency of *ex situ* gene conservation methods. The AA is the recommended technique for evaluating the physiological potential of a seed lots after a specified storage period Panobianco *et al.*[13]. This technique involves the exposure of seeds to adverse levels of temperature (40-45°C) and relative humidity (about 100%) for varying length of time followed by normal germination test. The seeds absorb moisture from the high humid-high temperature environment causing rapid ageing. The AA increases the seed deterioration process. As a result, several cellular and metabolic chemical alterations occur which impair DNA and mRNA, slows down protein synthesis and results in the loss of membrane integrity Kibinza *et al.*[14].

The basis for this test is that higher vigour seeds tolerate the high temperature-high humidity treatment and thus retain their capability to produce normal seedlings in the germination test[15]. The differences in germination before and after ageing provide a relative measure of seed vigour. Higher vigour seed lots result in higher normal seedling germination percentage than seed lots of low vigour. Wang *et al.*[16] proposed the use of an "Index of Ageing" (AI), since simple percentage differences lack the precision required for a real quantitative test. AI is the ratio of the difference in germination (initial germination and germination after AA), to the initial germination of the seed lot expressed in percentage.

Nauclea diderrichii is a tropical African hardwood species belonging to the family Rubiaceae. The species is widely distributed across tropical Africa, from Liberia eastward through the Congo Basin to Uganda and Angola, Hawthorne and Gyakari[17]. It is semi heavy and of medium hardness. Because of its good mechanical properties and natural durability, *N. diderrichii* can be enhanced by preservative treatment. It is sought after as a timber for outdoor uses (harbour works, railway sleepers), buildings (carpentry, floors, indoor and outdoor woodwork) and for cabinet making. The wood is also suitable for fence posts and bridges, as it is moderately resistant to termites and fungi wood infestations. In Ghana, its most popular use is for mortars, but it is also used to make telegraph poles, pit props, mine-shaft guides, furniture and drums Orwa *et al.*[18]. The species is heavily exploited in West Africa for timber and it is considered vulnerable IUCN[19].

Nauclea diderrichii, locally known as Kusia in Ghana has been selected as one of the priority indigenous species to be planted and conserved. However, illegal logging and uncontrolled exploitation of the species make it difficult to obtain seeds from the desired seed sources in natural stands. Hence a large-scale collection of germplasm from selected plus trees of *N. diderrichii* are imperative for its conservation and utilization in the long term. The fact that *N. diderrichii* has a wide range of ecological adaptations, suggests that there is a considerable amount of genetic variability. This can be exploited for potential domestication in other parts of the country. Comprehensive work on collection of tree germplasm, seedling performance evaluation, morphology, seed characteristics and yield traits are still in its infancy stage for most tropical trees species Rao *et al.*[20].

Again, the use of the AA seed testing technique to aid the phenotypic selection of plus-trees in Ghana has barely been explored. Specific objectives for this study were to: (1) Identify plus-trees of *N. diderrichii* within the selected provenances based on phenotypic superiority scores, (2) Examine the feasibility of using the AA tests to determine the seed vigour categories in the species and (3) Determine the provenance that houses plus-trees with the highest vigour and storage potential.

MATERIALS AND METHODS

Selection of plus-trees, seed collection and seed extraction: Candidate plus-trees of *N. diderrichii* were selected from 4 different provenances across 4 forest ecological zones in Ghana from January to June, 2016. These were Abofour (KABO), Amantia (KAMA), Benso (KBEN) and Gambia (KGAM) (Fig. 1). A twelve-point phenotypic superiority score established by Clark and Wilson[7] was adopted and modified for identifying candidate plus-trees during field surveys. Phenotypic attributes were assigned scores between 0-10 after a careful examination by 2 independent field assessors. These were denoted as (1) For stem straightness, (2) For timber height, (3) For above 45 cm diameter at breast height, (4) Less forking, (5) Branch angle, (6) Crown dimensions, (7) Epicormic susceptibility and (8) Absence of pest and diseases. Each of these criteria was scored and a total superiority score of above 70%, indicated that the individual tree qualified as a candidate plus-tree.

After monitoring the flowering and fruiting cycle, fruits were collected from 32 plus-trees in August and September, 2016. Seeds were extracted and assigned unique accession numbers. Seed lots were shade dried for 3 days to achieve uniform moisture content. Moisture content (MC) of

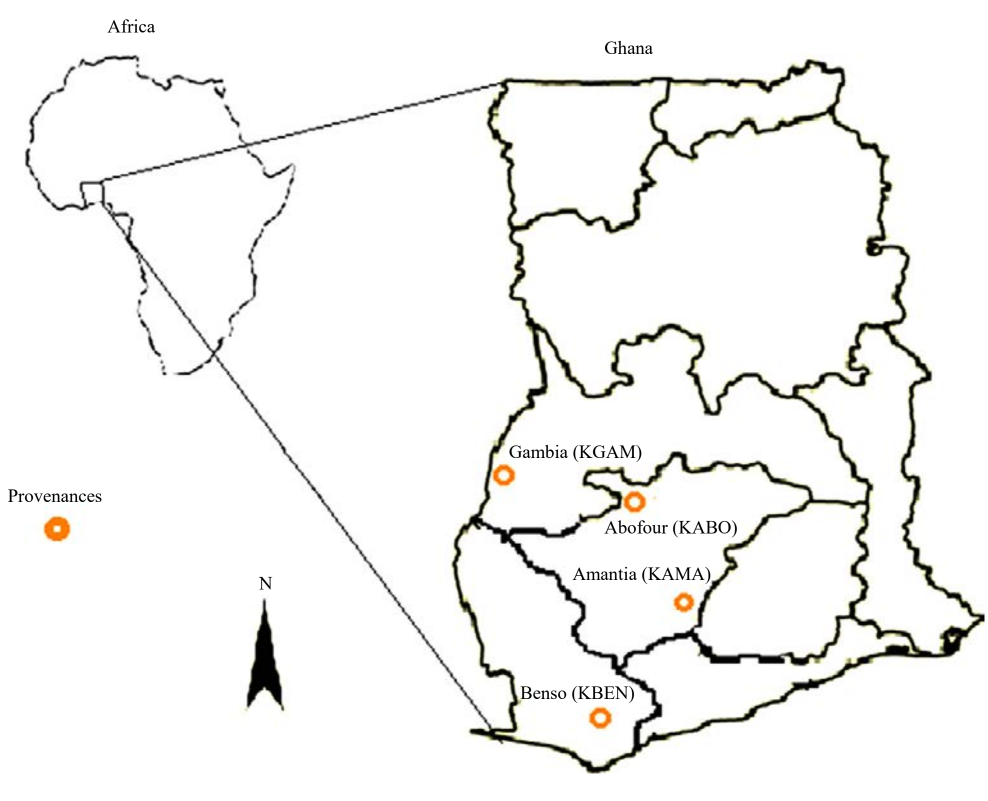

Fig. 1: Map of Ghana indicating the locations of the 4 provenances where plus-tress were selected for seed collection

the working samples on fresh weight basis was determined by the oven-dry method ISTA[21] with 2.0 g of seed samples. The MC was calculated by the equation:

$$MC\ (\%) = \frac{FW - DW}{FW} \times 100$$

Where:
FW = Weight before drying
DW = Weight of sample after drying

Seed storage and germination test: Seed samples were placed in hermetically sealed bottles and stored at 4-5°C for 12 months. The weighed replicate method was used for sampling seed lots for germination before and after AA tests. This method is recommended for species with very tiny seeds where counting of specific numbers of seeds is arduous FAO[22]. 2.0 g of seeds from each accession was weighed and sown in 4 replicates in a medium of sterilized river sand. The germination media was placed in round plastic germination bowls with holes at the bases to ensure good drainage. Tests were conducted at a plant house with ambient temperature of 27-33°C. Mean germination days and number of seedlings obtained after germination were carefully recorded.

Accelerated ageing test: The inner chamber (tray) set-up was adopted to conduct the accelerated ageing tests.Tekrony[23,24]. About 2.0 g of each seed lot from all accessions was placed (spread) uniformly on a plastic mesh tray suspended over 50 mL of water in the inner chamber (plastic sample box of dimension 22×17×9 cm with lid). The inner chambers were placed on trays in an outer ageing chamber (a laboratory oven) for an ageing period of 72 h at a temperature of 41°C (±0.5°C). The "aged" seeds were then subjected to standard germination test procedures. Period for germination was approximately 30 days and the results were expressed as mean number of normal seedlings recorded for each lot.

Statistical analysis: A completely randomized design was used for the germination experiment. A one-way ANOVA with Tukey's (HSD) *post hoc* mean comparison was conducted at the 5% level of significance using R statistical software.

RESULTS

There were a total of 104 individual trees sampled as candidate plus-trees during reconnaissance field surveys across the 4 ecological zones. However, only 32 plus-trees were subsequently selected for seed collection and the AA tests. These trees were georeferenced and assigned a specific plus-tree number based on their provenances. Provenance Gambia (KGAM) recorded 13 plus-trees followed by 9, 7 and 3 plus-trees from Benso (KBEN), Amantia (KAMA) and Abofour (KABO) respectively. Summary of *N. diderrichii* plus-trees identified across the 4 ecological zone is presented in Table 1.

Seed germination tests: There were significant difference ($p<0.05$) in mean seed germination data across all 4 provenances. Also, mean germination after extraction and before AA tests was higher (400 seedlings/2.0 g of seeds) for provenance KAMA relative to mean germination range of 248-285 seedlings/2.0 g of seeds from KBEN, KABO and KGAM (Fig. 2a). Markedly, provenance KAMA recorded the highest mean germination of 285 seedlings/2.0 g of seed after AA prior to seed storage compared with the rest as shown in Fig. 2b.

Germination of seeds after 12 months of storage indicated that provenance KAMA, followed closely by provenance KABO, produced the highest number of seedlings/2.0 g of seeds before the AA test (Fig. 3a). However, provenance KABO, followed by provenance KAMA recorded the highest mean germination of 180 and 155 seedlings/2.0 g of seeds compared with the rest after AA of stored seeds (Fig. 3b). A look at the performances of the various plus-trees in terms of germination following storage and AA,

Table 1: Summary of plus-trees identified across the 4 forest ecological zones and their locations

Forest types	Provenances	Accession	Latitude (N°)	Longitude (°)	Number of tree surveyed	Number of plus-trees
MSD NW	Gambia	KGAM	06.23390	00.05560 E	39	13
MWE	Benso	KBEN	06.82670	00.05030 E	28	9
MSD	Amantia	KAMA	06.29670	00.03150 W	18	7
DSD	Abofour	KABO	06.19430	00.05090 E	19	3

MSD: Moist semi-deciduous North-West KGAM means Kusia from Gambia, MWE: Moist wet evergreen KBEN means Kusia from Benso, MSD: Moist semi-deciduous KAMA means Kusia from Amantia, DSD: Dry semi-deciduous KABO means Kusia from Abofour, Kusia is the local name of *N. diderrichii* in Ghana

Table 2: Individual plus-trees and their mean seedlings produced/2.0 g seeds after accelerated ageing test following 12 months of storage

Accession codes	No of seedlings/2.0 g seed	Accession codes	No of seedlings/2.0 g seed	Accession codes	No of seedlings/2.0 g seed
KGAM 2	89[d]	KGAM 16	121[c]	KBEN 9	130[c]
KGAM 3	116[c]	KGAM 17	87[d]	KAMA 10	142[b]
KGAM 4	125[c]	KGAM 18	74[d]	KAMA 1	153[a]
KGAM 6	118c	KGAM 19	68[d]	KAMA 3	148[a]
KGAM 7	74[d]	KBEN 1	148[a]	KAMA 4	172[a]
KGAM 9	88[d]	KBEN 2	122[c]	KAMA 5	142[b]
KGAM 9	113[c]	KBEN 3	142[b]	KAMA 8	132[c]
KGAM 11	107[d]	KBEN 4	119[c]	KABO 1	173[a]
KGAM 12	115[c]	KBEN 5	126[c]	KABO 2	145[b]
KGAM 13	94[d]	KBEN 8	139[c]	KABO 3	150[a]
C.V	15.94		24.51		9.73
SED	5.24		8.87		4.58

Means with the same letters in the table were not significantly different at 5% level of significance

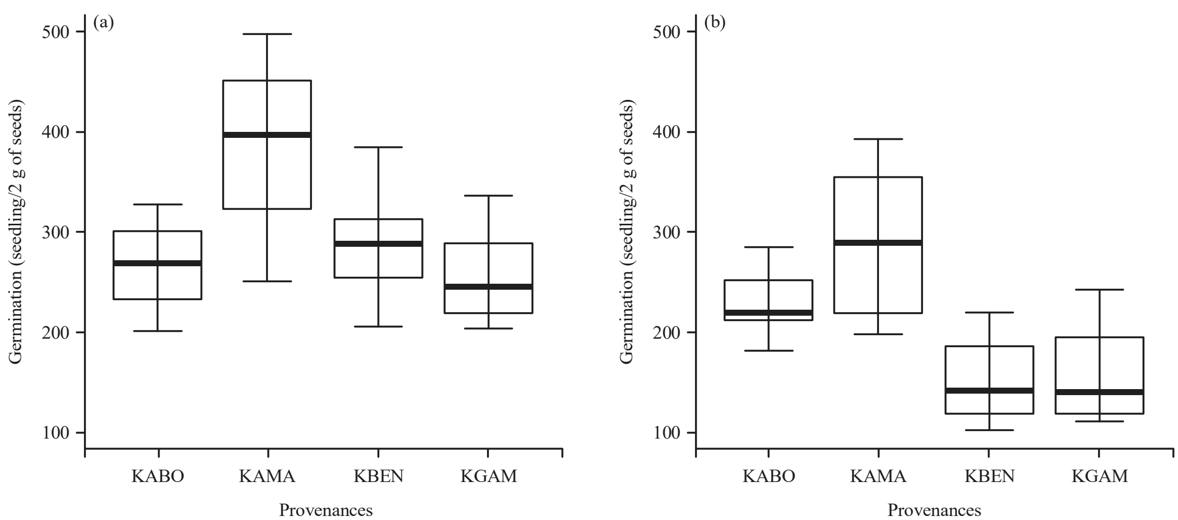

Fig. 2: A box-plot of mean germination from all 4 accessions (a) Before and (b) After AA test

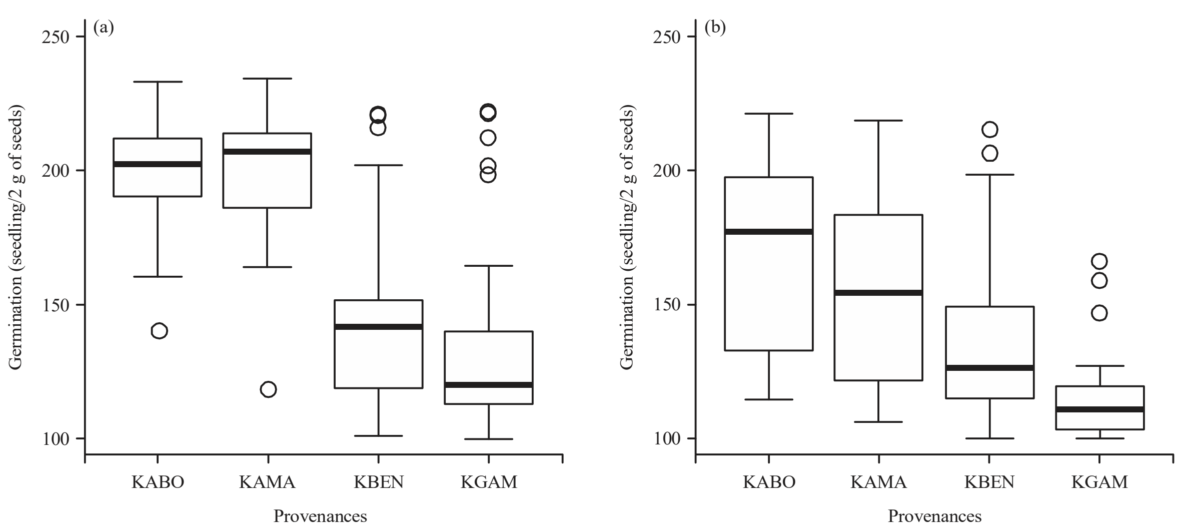

Fig. 3: A box-plot of mean germination from all provenances (a) Before and (b) After AA 12 months after storage

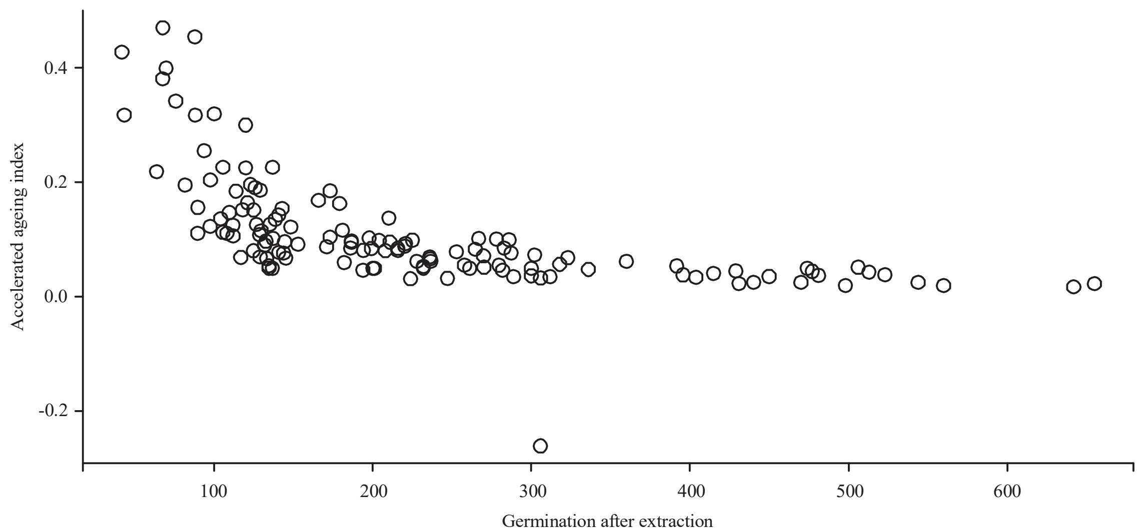

Fig. 4: Accelerated ageing index (AI) for Initial germination after extraction across all provenances

indicated that individually KAMA 1, KAMA 3, KAMA 4, KABO 1, KBEN 1 and KABO 3 plus-trees performed better compared to the rest (Table 2). This observation reflected in the performances of seeds at the provenance level. Generally, seeds with higher vigour indicated decreasing ageing indexes (AI) as shown in Fig. 4.

DISCUSSION

Unequal number of trees surveyed for evaluation as candidate plus-trees was due to their availability, access routes within the forest and initial phenotypic scores. Provenance Gambia (KGAM) recorded the highest number of plus-trees (13 plus-trees)because of the species wider spatial distribution within the low-lying areas across the high forest zone of Ghana[17].

Although provenance KGAM produced the highest number of plus-trees for *N. diderrichii*, mean germination of seedlots from KGAM before and after AA test remain relatively low. Markedly, lots from provenance KAMA recorded the highest mean germination (400 seedlings/2.0 g seed) but declined to <200 seedlings during AA test after 12 months of storage. Differential response of seedlots from plus-trees have been reported, including Kraus and Skier[25] in *Pinus palustris*. Germination and vigour are good indicators of seed physiological potential under various field conditions[8]. Vigour testing has become a more sensitive measure of seed physiological quality. Vigour testing, however, requires more rigorous control of test variables and data interpretation to obtain consistent results. Reliable information of seed vigour and germination from plus-trees will provide a solid basis for anticipating improved stand establishment in the field.

Low germination after AA has been attributed to cell membrane disorganization and significant death of tissues in different seed parts particularly in meristematic tissues McDonald[26]. Additionally, several authors have shown evidence of serious degenerative changes in seed metabolic activities during the AA test procedure Ganguli and Sen-Mandi[27], Basavarajappa *et al.*[28] and Das and Sen-Mandi[29].

These changes cause observed differences in performance of seedlots of the same species from different provenances. However, a vigorous seedlot is the one that is potentially able to endure conditions sub-optimal to the species during the AA test; resulting in higher seed germination ISTA[30]. This trend was clearly observed in seed lots from provenances KABO, KAMA and KBEN compared to KGAM. Consequently, provenances KABO, KAMA and KBEN were found to be of superior vigour compared with KGAM. Similar trend of varying seed vigour of the same species from different provenance was reported by Bhering *et al.*[31] and Torres *et al.*[32] when evaluating seed lots of cucumber (*Cucumis sativus*) and coriander (*Corriandrum sativum*), respectively.

CONCLUSION

The use of AA tests as a complement benchmark for classifying plus-trees is an efficient means of increasing tree seed productivity. Provenance KGAM hosts the highest number of *N. diderrichii* plus-trees and therefore, should serve as major source of collection for *N. diderrichii* seeds for general plantation programmes. However, seeds from provenances KAMA and KABO will be more suitable for long term *ex situ* conservation due to their observed superior vigour after 12 months of storage. Seeds from these provenances are therefore, recommended for long term *ex situ* conservation for the species.

SIGNIFICANCE STATEMENT

The study discovered that there are significant vigour differences among seeds of *Nauclea diderrichii* collected from trees of the same provenance as well as seeds from trees of different provenances. Thus the choice of seed sources or provenance of *N. diderrichii* can be decisive for the success or failure of future plantings as well as *ex situ* seed conservation programme of the species. The study has also discovered that the accelerated ageing technique is very effective for the classification of seeds of *N. diderrichii* into various vigour levels.

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