Agricultural Sciences, 2021, 12, 769-782
https://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Variation in Seed Germination and Seedling Growth in Five Populations of *Vitellaria paradoxa* C.F. Gaertn. Subsp. *Nilotica* in Uganda

Juventine B. Odoi[1,2], Thomas L. Odong[2], Clement A. Okia[3,4], John Bosco Lamoris Okullo[2], Moses Okao[5], Harriet Kabasindi[6], Alice Mucugi[3], Samson Gwali[1]

[1]National Forestry Resources Research Institute (NaFORRI), National Agricultural Research Organization (NARO), Kampala, Uganda
[2]Department of Crop Production, College of Agricultural and Environmental Sciences, Makerere University, Kampala, Uganda
[3]World Agroforestry (ICRAF), Nairobi, Kenya
[4]Muni University, Arua, Uganda
[5]Ngetta Zonal Agricultural Research and Development Institute (NettaZARDI), Lira, Uganda
[6]Faculty of Natural Resources and Environmental Sciences, Busitema University, Busia, Uganda
Email: juventineboaz@gmail.com

How to cite this paper: Odoi, J.B., Odong, T.L., Okia, C.A., Okullo, J.B.L., Okao, M., Kabasindi, H., Mucugi, A. and Gwali, S. (2021) Variation in Seed Germination and Seedling Growth in Five Populations of *Vitellaria paradoxa* C.F. Gaertn. Subsp. *Nilotica* in Uganda. *Agricultural Sciences*, **12**, 769-782.
https://doi.org/10.4236/as.2021.127050

Received: October 15, 2020
Accepted: July 24, 2021
Published: July 27, 2021

Abstract

We studied seed germination and seedling growth performance in an economically and socially important fruit tree species *Vitellaria paradoxa* C.F. Gaertn. subsp. *nilotica* (shea tree) in Uganda. The study aimed at determining variations in germination among five shea tree seed provenances and seedling growth performance based on five growth traits (total height, root collar diameter, leaf length, leaf width and leaf petiole length). Five populations were considered from four agroecological zones comprising of 180 candidate "plus" trees. The seed trees consisted of 16 ethnovarieties selected based on their traits for fast growth and high oil yield. A total of 1,204 biological seeds were collected and sown in a tree nursery at Ngetta Zonal Agricultural Research and Development Institute during the month of June 2018 in a randomized incomplete block design with three replications. Significant variation ($\chi = 708$; $p < 0.01$) was observed in seedling phenotypic traits within and between populations. Regression equation for height growth and leaf size index were given as $y = 0.3787 + 12.671x$ and $y = 0.6483 + 15.413x$ respectively. Root collar diameter was more correlated to leaf size index (0.425) than to height growth (0.30). Clustering of shea tree seedlings based on phenotypic growth traits revealed one aggregated cluster indicating that most of the seedlings from the five populations were similar (Jaccard index 0.92, $p < 0.01$). However, clustering based on SNP markers revealed three different populations. We recorded higher growth ($\chi = 708$; $p < 0.001$) in Arua shea population. Red seeded; thin pulped and hairy fruited shea tree ethnovarieties recorded faster growth than the rest. The results reveal useful traits in selection for tree growth and further identified shea tree ethnovarieties that could be selected for fast growth.

Keywords

Ethnovarieties, Plantlets, Root Collar Diameter, Leaf Size Index, Population, Cluster

1. Introduction

The shea tree (*Vitellaria paradoxa*) is a commercially useful indigenous fruit tree species found within Sudano-Sahelian Africa growing within a region of 600 – 1400 mm of annual rainfall [1]. It grows up to 20 m in height and plays economic, ecological roles and supplements food and nutrition among the communities where it grows [2]. The tree's importance is derived from a number of useful products; it provides which include: oil, tasty fruits, cosmetic and medicinal ointments, soaps, fuels as well as various services and income derived from sell of many products [3]. The fruit pulp is consumed by communities in the shea growing areas and is therefore important in subsidizing household food and nutrition during planting periods when other foods are not yet ready. It is mostly consumed before or as a substitute for the main meals. In fact, the species is now considered a traditional African species whereby its nutritious fruit pulp makes it a good source of food improving nutrition and boosting food supply during "annual hunger season" [4]. The fat/oil derived from its seed kernel is highly traded both locally and internationally for cooking, chocolate and cosmetics.

Shea trees grow mainly in northern, eastern and west Nile regions of Uganda where they greatly contribute to communities' livelihood, mostly for women and children. Despite its importance, the tree remains undomesticated probably because of its slow growth or lack of tradition to plant indigenous tree species [5]. Farmers simply protect the trees found growing in their farms, an approach termed "farmer managed natural regeneration" resulting into a parkland system [6]. Furthermore, few studies have been done on shea tree growth parameters due to its slow growth. A recent study [7] highlighted the growth performance of shea tree under cultivation. Although natural regeneration is highly promoted by farmers, the species grows slowly taking over a decade before fruiting. Worse still, because it is an excellent source of fuelwood, many mature trees are converted for charcoal production and this has modified the parkland. These modifications differ in space, intensity and time scale depending on the socio-economic backgrounds of the surrounding communities forming slight variations across the shea belt.

More effort is required by research institutions to establish demonstration plots in form of gene bank collections of shea varieties for local communities to learn its agronomic practices. One example of such collections in Uganda is the shea gene bank jointly established and managed by the world Agroforestry Centre (ICRAF) and National Agricultural Research Organization (NARO) in Northern Uganda which has demonstrated to communities that it is possible to grow and manage shea trees in a planted form.

Shea tree exists in varied morphological forms named differently by local communities where they grow. The varied morphological forms such as fruit/seed shapes, color, taste or texture are used to characterize the trees [8] [9] [10]. Several approaches have been employed by a number of researchers to characterize the species both at sub-species and ethnogamies levels [10] [11].

2. Materials and Methods

2.1. Study Area

Shea fruits were collected from the districts of Katakwi, Otuke, Amuru, Moyo and Arua and seedlings raised in a tree nursery at Ngetta Zonal Agricultural Research and Development Institute (NgettaZARDI) in Lira district (**Figure 1**). The districts are generally described as savannah woodland interspersed with shea butter trees (*Vitellaria paradoxa*). The predominant tree species are the *Combretum* species, *Terminalia* species, *Acacia* species, *Grewiya mole*, *Piliostigma*, and *Hyperemia* species. The mean annual precipitation is ranges between 900 and 1200 mm with bimodal rainfall having high peaks during April-May and August-October. The district mainly comprises of rolling plains rising.

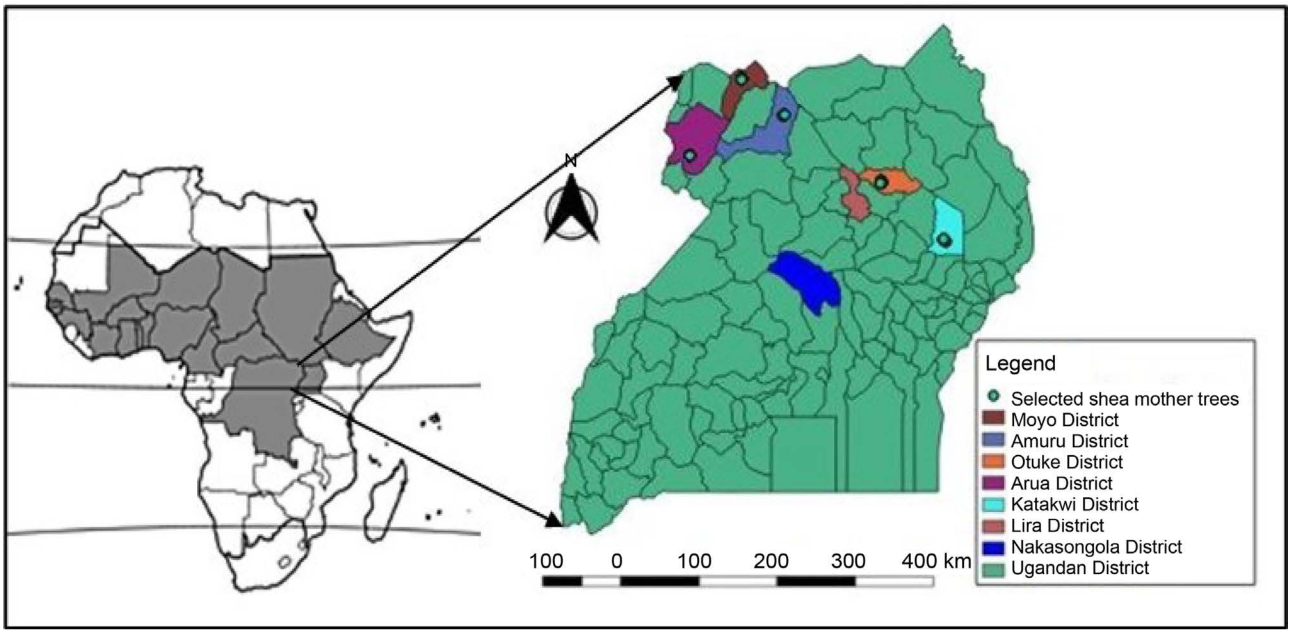

Figure 1. Map of Africa indicating distribution of shea trees within the continent and map of Uganda showing the study sites/districts where shea trees seed were collected.

2.2. Plant Materials and Planting Medium

Only mature and freshly fallen ripe undamaged shea fruits were carefully picked directly from under 180 selected seed trees across the study sites. A total of 1204 fruits were collected during the month of June 2018 and transported to Ngetta-ZARDI for processing before sowing. The fruit pulp was removed by hand and each ethnovariety put in small cloth bags to avoid mixing. These were then placed in thick gunny bags soaked with water and kept under shade for two weeks to allow kennels to crack before sowing directly into polythene pots filled with planting medium. Up to sixteen ethnovarieties were collected from the five populations and sown. The planting medium composed of a mixture of 70% top fertile soil and 30% clay soil. After mixing thoroughly using a spade, the medium was filled into polythene pots 6 inches width by 8 inches length, sealed at the bottom and punctured to let off excess water and avoid seed rot before germination.

2.3. Experimental Design

The seed kernels were sown three days after collection due to its recalcitrant nature. The experiment was laid in a randomized incomplete block design with three replicates to measure the variations among the experimental units after fourteen days when the radicles had cracked the kernels. The kernels were sown directly into the pots filled with the growing medium and watered twice a day with 10 Liters of water per square meter for a period of six months when it was noted that most of the seeds had germinated.

2.4. Data Collection

The seeds were germinated and kept in the nursery at NgettaZARDI for 18 months to attain required height of ≥15 cm measured up to the highest leaf point. The following were recorded from the seedlings for phenotypic analysis: date of first seedling sprout, Root collar diameter, seedling height, number of leaves developed, leaf length, leaf width and petiole length. The root collar diameter (mm) was measured right where the stem leaves the soil, leaf length was taken from the point of attachment to the stem to the leaf apex and the leaf width was taken from the widest point of the leaf lamella. Measurements were rounded to the nearest millimeter and taken monthly for eighteen months. The seedlings were then planted in two different sites as shea tree Breeding Seed Orchard intended for genetic trial of superior phenotypes for mass production of genetically improved materials.

2.5. Data Analysis

Data was subjected to Principal Component Analysis and ANOVA to determine the variation among the five shea populations. Hierarchical clustering was carried out using the web-based platform Glabstat (https://www.glabstat.com/analyze-your-data) that uses R package Shiny to run analyses through R environment and functions.

3. Results

3.1. Seed Germination

Germination of the sown seeds started after three weeks and continued up to the tenth week from the date of sowing. The overall germination percentage of the whole seed lot was 62.8%. Arua population had the highest (61.2%) germination percentage followed by Amuru, Otuke and Moyo population, respectively. The Katakwi population had the lowest germination percentage (43%) (Figure 2).

Arua, Amuru and Otuke seed lots had three weeks' peak germination implying that the seed lots were more viable and therefore had longer germination time which increased their germination percentages (Figure 3). The average shea seed germination rate per month was 8.7%. The seeds gained highest germination rates in the second month (16.1%) after sowing from which it started decreasing till the sixth month (2%) from which no more germination was recorded among all the seed lots. The difference in seed germination among the 5 seed lots was significant ($P \leq 0.001$). All the seed lots gained highest germination vigor in the second and third months. Moyo seed lot had the highest germination vigor where over 50% of the seeds had germinated by the second month. While Katakwi seed lot registered the lowest seed vigor (Figure 3).

3.2. Seedling Growth

Hairy variety, thin pulped, hard pulped and tasteless pulped ethnovarieties vigorously grew and surpassed the other ethnovarieties. These were still the same seedlings which had larger root collar diameters and leaf size. On the other hand, Dwarf; black seeded and red seeded ethnovarieties had much inferior growth traits than the rest (Figure 4).

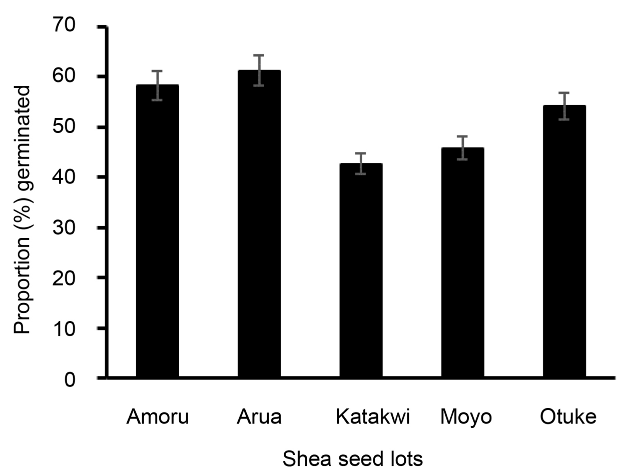

Figure 2. Proportions of germinated shea seeds sown in Ngetta ZARDI tree nursery, northern Uganda.

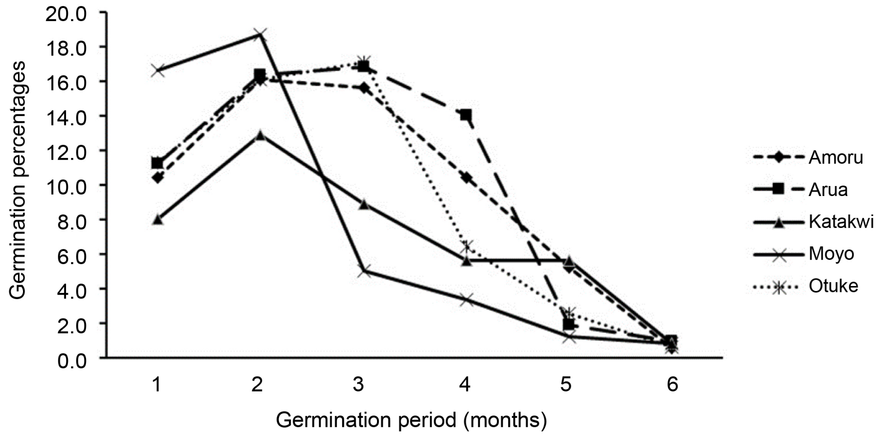

Figure 3. Shea seeds monthly germination percentage for the five seed lots collected from Arua, Amuru, Katakwi, Moyo and Otuke districts, Uganda.

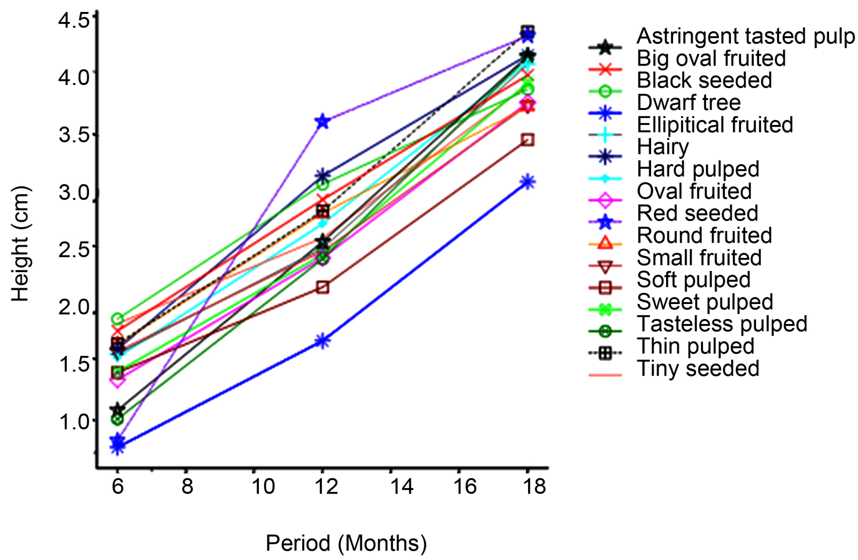

Figure 4. Growth performance of the sixteen-shea ethnovarieties in Uganda. The plantlets were measured thrice at an interval of six months after germination.

Seedling height; root collar diameter; number of leaves; leaf length; leaf width and petiole length were recorded and presented in **Figure 5** below. The tallest plantlets were from Arua population, they also had more leaves per plant. There was a significant variation ($\chi = 708$; $p < 0.01$) in seedling phenotypic traits within and between populations.

Meanwhile Arua and Moyo populations had the largest leaf sizes. Otuke and Katakwi populations generally had lower expression of the studied traits as compared to the other three populations. **Table 1** presents the average performance of the different growth traits. Average growth varied significantly ($\chi = 708$; $p < 0.001$) in Arua shea population (19.69 cm) compared to the rest of the populations. There were some few ethnovarieties which exhibited better phenotypic growth traits as compared to the rest. Hairy (6.02 cm), Thin pulped (5.68 cm)

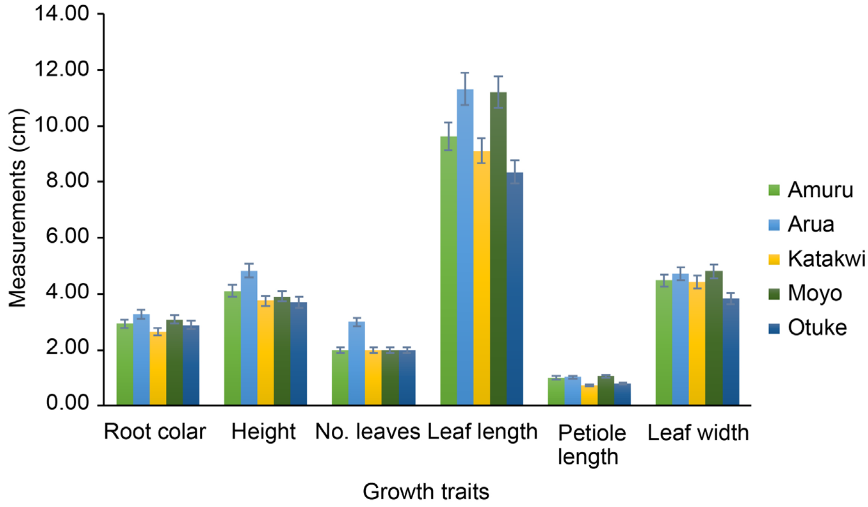

Figure 5. Shea tree phenotypic growth traits for five populations measured at 18 months.

Table 1. Comparison of growth traits among shea ethnovarieties measured at 18 months after sowing.

Conventional descriptor	Traditional (Luo) nomenclature	Height (cm)	Root collar diameter (mm)	No. of leaves	Leaf length (cm)	Leaf diameter (cm)	Petiole length (cm)
Sweet pulped	Malim	5.49	4.18	4	13.55	6.16	1.17
Soft pulped	Apocopoco	4.51	3.61	3	12.76	5.19	1.08
Hard pulped	Acogo	5.62	4.24	4	12.17	5.35	1.25
Small seeded	Lagili	4.99	3.71	3	11	5.05	0.94
Oval fruited	Acula	5.17	3.56	4	11.54	5.35	1.02
Astringent tasted	Yao cot	5.06	4.12	3	12.34	5.7	1.57
Round fruited	Alulung	4.8	3.45	3	12.26	5.44	1.21
Thin pulped	Lo'koroc*	5.68	4.18	3	14.17	6.16	1.51
Hairy	Jayer	6.02	4.15	3	17.07	6.67	1.4
Elliptical fruited	Maboco	5.37	3.91	3	14.20	7.9	1.3
Tasteless pulped	Yao mabot	5.63	3.92	3	13.44	5.58	1.38
Big oval fruited	Yao Madongo	5.39	3.63	4	12.33	5.52	1.23
Tiny seeded	Lalet	5.16	3.86	3	12.81	5.5	1.11
Black seeded	Macol/Molilo	3.97	2.93	2	10.37	4.3	0.86
Red seeded	Aremo	4.49	2.77	2	7.92	3.58	0.82
Dwarf	Yao wigweng	3.17	2.7	3	7.69	3.71	1.31

*Ateso.

Tasteless fruited (5.63 cm) and Hard pulped (5.62 cm) ethnovarieties were taller than the rest. These were the same ethnovarieties that had the largest root collar diameters and leaf size indices. These ethnovarieties could also be selected for when selecting traits for shea tree breeding programme in Uganda.

3.3. Relationship among the Different Growth Traits

The regression lines in Figure 6 show a strong positive relationship between root collar diameter and shea tree height and leaf size index. It reveals that 85.9% of the variation in shea tree leaf size and 93% of the variation in shea tree height respectively were explained by the root collar diameter. The linear regression equation for shea tree plantlet height is therefore presented as y = 0.3787 + 12.671x and for leaf size index as y = 0.6483 + 15.413x.

It is evident that shea tree height and leaf size index exponentially increased with increasing root collar diameter. Leaf size index increased at a slightly higher rate than the tree height.

Correlation of the five traits showed that root collar diameter is more correlated to leaf length (0.4) than shear tree height (0.3) (Figure 7). There was a weak correlation between tree height and leaf width. Number of leaves per plant had a weak negative correlation with leaf width and petiole length (−0.04 and −0.02 respectively) but weak positive (0.09).

There was however a relatively higher correlation (0.45) between the leaf length and petiole length. Root collar diameter and number of leaves on the different plantlets varied making it possible to do directional selection for various purposes. Besides tree height and leaf length, the rest of the traits were negatively skewed (skewed to the left). All the plantlets had more less the same leaf width except the Hairy (6.02 cm), Thin pulped (5.68 cm) Tasteless fruited (5.63 cm) and Hard pulped (5.62 cm) ethnovarieties which was an outlier. Analysis of PC1 explained 86.3% of the variance while PC2 explained 4.8% totaling to 91.1% of the whole phenotypic variation in the population (Figure 8). It is also important

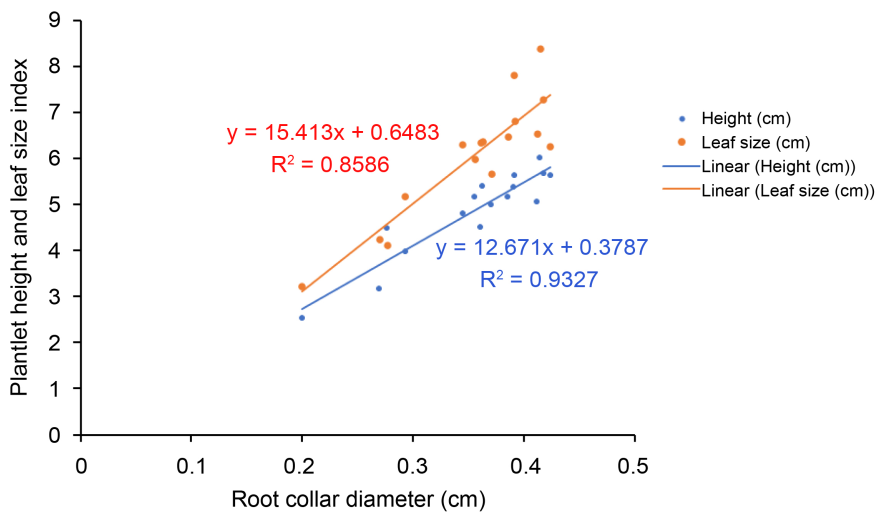

Figure 6. Regression of shea tree the root collar diameter against shea tree height and leaf size index in five populations plantlets. The dots/points in the figure represents each shea tree family, they represent either average shea tree root collar diameter on the X-axis or the average shea tree height and leaf size index of the plantlets on the Y-axis. The leaf indices were derived by getting the averages of each leaf length and width to get a single parameter referred to here as the leaf index.

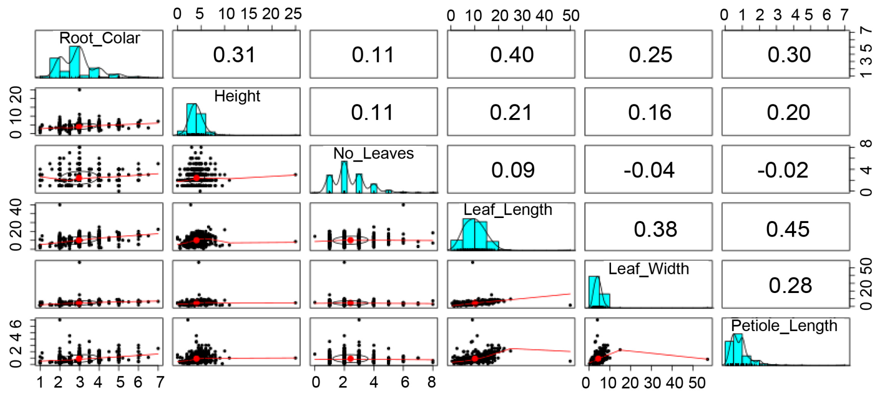

Figure 7. Plot of pairwise Pearson correlations and regressions among traits and their scatter plot matrices. The phenotypic data for each trait was used for the analysis.

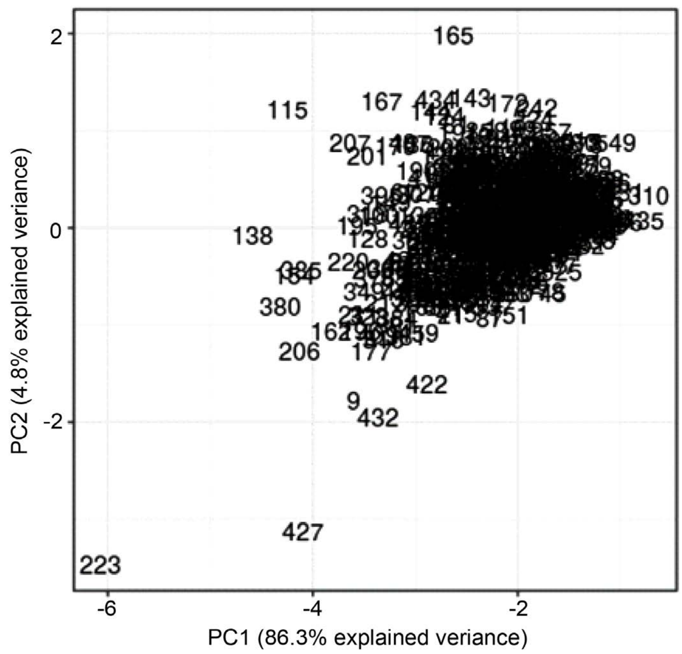

Figure 8. Hierarchical clustering of shea tree lines (numbers onset) based on their phenotypic values for the 5 traits.

to note that a majority of the population were clustered into only one aggregated cluster with some few outside the cluster.

Almost all lines shown in **Figure 5** were grouped together in one side of the graph indicating little differentiation among them based on the five phenotypic traits. In fact, their Jaccard index for the growth traits was 0.92 indicating that the plantlets were 92% similar given their phenotypic growth traits. This proved how similar they were in phenotypic composition that resulted in the majority forming one cluster with very few phenotypes (8%) dissimilar. This is contrary to the hierarchical clustering based on the SNP data in **Figure 9** where individuals clustered themselves within a population according to their genetic similarities and nearest relatives.

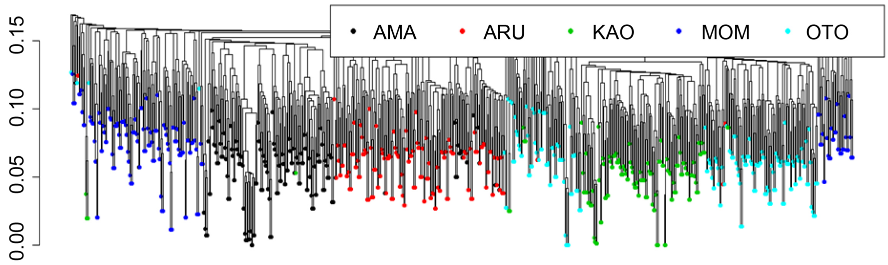

Figure 9. Hierarchical clustering performed on the Duplicate removed SNPs data using the R package SNP Relate. It shows how the different populations (colors) clustered based on their genetic similarities and dissimilarities.

The clusters were divided into groups of similar colors according to areas of origin although some few members with different colors identified with different clusters. Three major clusters were formed meaning there are three populations according to SNP data. Amuru, Moyo, Arua, some few Katakwi and Otuke members formed the first major population; Otuke, Moyo and few Katakwi members formed the second population and Katakwi population the third. In **Figure 9**, the different colors show different populations.

4. Discussion

4.1. Seed Germination and Seedling Growth

Arua population generally exhibited high germination and growth rates than the rest of the populations. The seed lots varied significantly in germination vigor and growth performance although they did not significantly differ in germination percentage. Seed germination vigor is a good predictor of seed lot quality and health. It is an indication that the seed lot quality has not deteriorated and therefore still retains good physiological potential for vigorous growth. It also implies that the species or seed lot is well adapted to the environment [12]. This study however, considered seedlings raised under a similar environment thus negating claims that the variation in ethnovarieties is due to environment. The results of this study suggest that the variation in growth performance could be genetic rather than environmental. Where such variations exist given similar treatments such as variation in height and root collar diameter of seedlings grown under similar conditions it was observed that the variations could be due to additive genetic factors [13]. The genetic effects on the seedlings could have been inherited due to the narrow sense heredity which is important for shea tree breeding (improvement). Related studies [14] recommended selection of *Populus tremuloides* genotypes with taller heights and larger diameter growth for the species improvement. Meanwhile it can also be noted that environment can greatly affect growth performance in species, however this is not additive and therefore can change with changing environment. Growth medium like compost amended medium may yield better results than other medium [15]. In fact, similar tree species from different sources grown in one environment and conditions can still show some variation in growth traits indicating that there are other factors in control than the environment. In a bid to rule out this claim, *Cordia africana* and *Azadirachta indica* seedlings collected from 19 provenances and grown in one location registered different growth rates beyond simply phenotypic or environmental characteristics [16]. This is true with the expression that any phenotype is as a result of genetic and environmental factors; (P = G + E). Growing different populations within a similar environment eliminates the claims on environmental effects leaving additive genetic effects at play.

4.2. Seedling's Growth Traits

The relationship between collar diameter, height growth and leaf size index showed a strong positive correlation at juvenile stage indicating that increase in diameter, height and leaf development uniformly took place. The diameter increased with height and leaf growth confirming existence of larger root collar diameter growth which was population based. Populations from different regions can differ in their trait performance even if they are the same species. This is in line with [17] who reiterated that variation in root collar diameter of *Azadirachta indica* from western, central and eastern Sudan was due to better adaptive and hereditary traits within the different populations. Other related studies [7] [18] associated faster tree growth to bigger crown size/crown biomass which is partly contributed by the available leaf quantity. In fact, a one report [7] indicated that broad lived shea trees from Palabek in northern Uganda were growing faster than the rest of the accessions. Despite all the above, tree diameter has been registered as the best tree growth estimator compared to any other tree growth parameters [19].

4.3. Relationship and Correlations among the Growth Traits

Hierarchical clustering (**Figure 8**) revealed three populations performed from SNP data of five populations (Katakwi, Otuke, Amuru, Moyo and Arua). Katakwi population formed a standalone population although few of its members were more similar with members in the second cluster. The population is located in the eastern lowlands agroecological zone separated from the second population/cluster (Otuke) by a wide swamp that feeds into lake Kyoga which could have disrupted genetic flow between the two populations. Although pollination in shea is mainly carried out by bats and birds that can fly over such swamps. Land use changes that have claimed a large number of shea tree stands through clearance for agricultural expansion has further widened the gap between the two populations that pollen cannot be transported from one population to another. This could be more difficult with shea fruits which are heavier to be carried by avian species over longer distances. This could have caused isolated population clusters that encouraged inbreeding among close relatives resulting in strong similarity within the population but dissimilarity with other populations. This is in line with the report by [20] who reported substantial genetic differentiation between smaller populations and larger populations of *Salvia pratensis* and *Scabiosa columbaria* The second cluster comprised majorly of Otuke population with a mixture of some Katakwi and Arua population. Otuke population however, had some combination from Katakwi and Arua suggesting genetic similarity with those genotypes. These could have been due to gene flow that caused the transfer of genetic materials form other populations into Otuke population. Since shea tree is a widely out crossing species, recombination during cross pollination could have had much more effect that caused the similarity of such genotypes to those in Katakwi and Arua populations [3]. Likewise, the allele frequencies of such genotypes must have been similar due to the recombination effects working within the population. This could be true given the geographical distance from Arua district (West Nile region) to Otuke district (Lango region) separated by a rift valley and river Nile.

The third cluster however, comprised of aggregation of populations from Moyo, Arua and Amuru districts. Since Arua and Moyo are within the same agroecological and political region where communities easily interact, there is a high possibility that genetic materials could have been moved from one location to the other facilitating mixing, although cross pollination could have also played a big role. On the other hand, Moyo district is kind of land locked district with exit and entry routes through Arua to the south and Amuru to the East. It is therefore most likely that genetic materials moved from these populations as communities traveled and got mixed within the original local populations across the districts. Following the history of the Luo migration from Bar-El-Gazzel (Equatorial province in Sudan) through Amuru point, some shea tree genetic materials could have been dispersed to Amuru which later on found their way to West Nile (Moyo and Arua) since the Alur tribes in West Nile was as a result of intermarriages between the original West Nile people and the Luo.

There was considerable variation in germination and growth performance among the five shea tree populations under this study. The variations within and between populations indicate existence of some few genotypes with both quite good growth traits that could be selected for faster growth and thus early maturity important for shea tree improvement in Uganda. The significant difference in the germination and growth performance among the populations suggest that the seedlings exhibited different physiological and genetic characteristics that made them to perform differently.

5. Conclusion

We therefore concluded that the seedlings from different ethnovarieties grow at different rates at juvenile stage. Diameter and leaf index are the major determinants of the seedling's growth rates. We recommend that directional selection could be targeted to conserve both the good growth performers and poor performers which may possess other good traits of interest like high oil yield and tasty fruit/nutritious fruit pulps for conservation.

Acknowledgements

The authors would like to thank the staff and administration of Ngetta Zonal Agricultural Research and Development Institute (NettaZARDI), Ms. Gloria Mutoni, the Agroforestry Technician, for her tireless contribution towards management of the experiments and data collection used in this paper.

This work was supported through a collaborative project between the World Agroforestry (ICRAF) and National Forestry Resources Research Institute (NaFORRI) under Gene bank Platform (GCDT-1213).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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