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Effects of Sowing Depth and Seed Orientation on the Germination and Seedling Growth in Shea Tree (*Vitellaria paradoxa* C.F. Gaertn.) for Rootstock Production in Nursery

YAO Saraka Didier Martial*, DIARRASSOUBA Nafan, DIALLO Rokia, KOFFI Eric-Blanchard Zadjehi, DAGO Dougba Noël, FOFANA Inza Jesus

Department of Biochemistry and Genetics, Pedagogical and Research Unit (UPR) of Genetics, University of Peleforo GON COULIBALY (UPGC), BP 1328 Korhogo, Côte d'Ivoire
*Corresponding author: didiery@yahoo.fr

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Abstract The study aims to determine the effects of sowing depth and seed orientation on the germination and seedling morphological variability in nursery. The seeds used were collected from an elite shea tree coded P14 located at Boundiali. The two factors that are sowing depth (5 cm, 10 cm and 15 cm) and seed orientation (VH, VB, HH, HB and HL) were tested in a Fisher block design with three replications. Germination parameters and vegetative growing traits of the juvenile shea plants were evaluated in nursery. The results showed that the seeding depth of 5 cm got better final germination rate (84.6 %), a shorter latency time (51.3 days) and a lower median germination time (110 days). Vegetative vigour of shea seedlings was better for the horizontal seed orientation with hilum sidewards (HL). After 12 months in the nursery, the proportions of plants suitable for grafting varied from 20 % (vertical seed orientation with the apical end upwards) to 32.50 % (horizontal seed orientation with hilum sidewards). It appears that sowing the seeds at a depth of 5 cm in the horizontal orientation with hilum sidewards leads to good germination and better vegetative development of shea seedlings in the nursery.

Keywords: *shea seed, sowing depth, seed orientation, germination, seedling morphological variability, nursery*

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1. Introduction

Shea tree (*Vitellaria paradoxa* C.F. Gaertn.) is a Sapotaceae endemic to Africa that grows wild in the Sudanian savannahs of the Southern Sahara in Africa [1]. The natural distribution area of shea tree in Côte d'Ivoire extends from North to Centre between 7°30' and 10°15' North latitude [2]. There are two main subspecies of shea tree that are the paradoxa subspecies which grows in West Africa, also present in Côte d'Ivoire, and the nilotica subspecies which grows specially in Northern Uganda and Southern Sudan [3]. Shea butter is one of the oldest edible oils in Africa. The species are highly valued by local communities for their food, financial and environmental benefits. The economic exploitation of the African shea butter tree has become the subject of a dynamic industry, mainly due to the initiative and courage of African women in rural area [4].

Despite the increasing of socio-economic importance of the shea butter, shea tree populations in agroforestry parks are lost and only established through natural regeneration from seeds [5]. During fruiting period, shea seeds germinate immediately when seeds fall onto tilled soils. Farmers only keep freely saplings that have achieved a minimum size and need time to grow without being disturbed [5]. Previous works on shea seed has revealed relatively low germination rates. This is a major constraint to the development of shea cultivation [6]. The germination of shea seeds on different substrates revealed that the success of seed germination depends strongly on the seed quality (physical, physiological and sanitary qualities), environmental factors (temperature, humidity, *etc.*) in interaction with the richness of the sowing substrates in organic and mineral substances [6]. Studies conducted on the oilseed of the cucurbit [*Lagenaria siceraria* (Molina)] showed that the main factors such as sowing depth and seed orientation during sowing can influence germination, emergence and seedling development [7]. The present study was conducted based on the hypothesis that the control of factors that influence germination would allow to increase the yield of rootstocks produce in nursery for shea grafting in Côte d'Ivoire. The present study aims to know the effects of the sowing depth and the seed orientation on seed germination and morphological variability of juvenile shea plants in the nursery.

2. Material and Methods

2.1. Study Area

The study area includes the site where shea seeds were collected (Boundiali) and the site where germination test was realized (Korhogo). The localities of Boundiali and Korhogo are located in the Savannah District (Figure 1). The climate of the Savannah District is Sudanian with two main seasons: a rainy season (June to September) and a dry season (October to May). Annual average temperature recorded is 30°C. Average rainfall varies from 900 to 1500 mm per year [8,9]. The vegetation is characterized by trees and shrubs, 8 to 12 m high, scattered with a canopy density varying from 25 to 35 %. The relief is monotonous, with altitudes varying from 300 and 400 m. The soils are Ferrisols [10].

2.2. Pant Material

The plant material consisted of the seeds and seedlings of shea tree. The shea seeds were extracted from mature fruits collected under the crown of an elite shea tree coded P14 in Boundiali. This elite shea tree is located at 9°28 North latitude and 6° 27 South longitude. The seeds used for the experiment have a mass that varies from 8 to 20 g, dark, brown or pale in color and ellipsoid in shape.

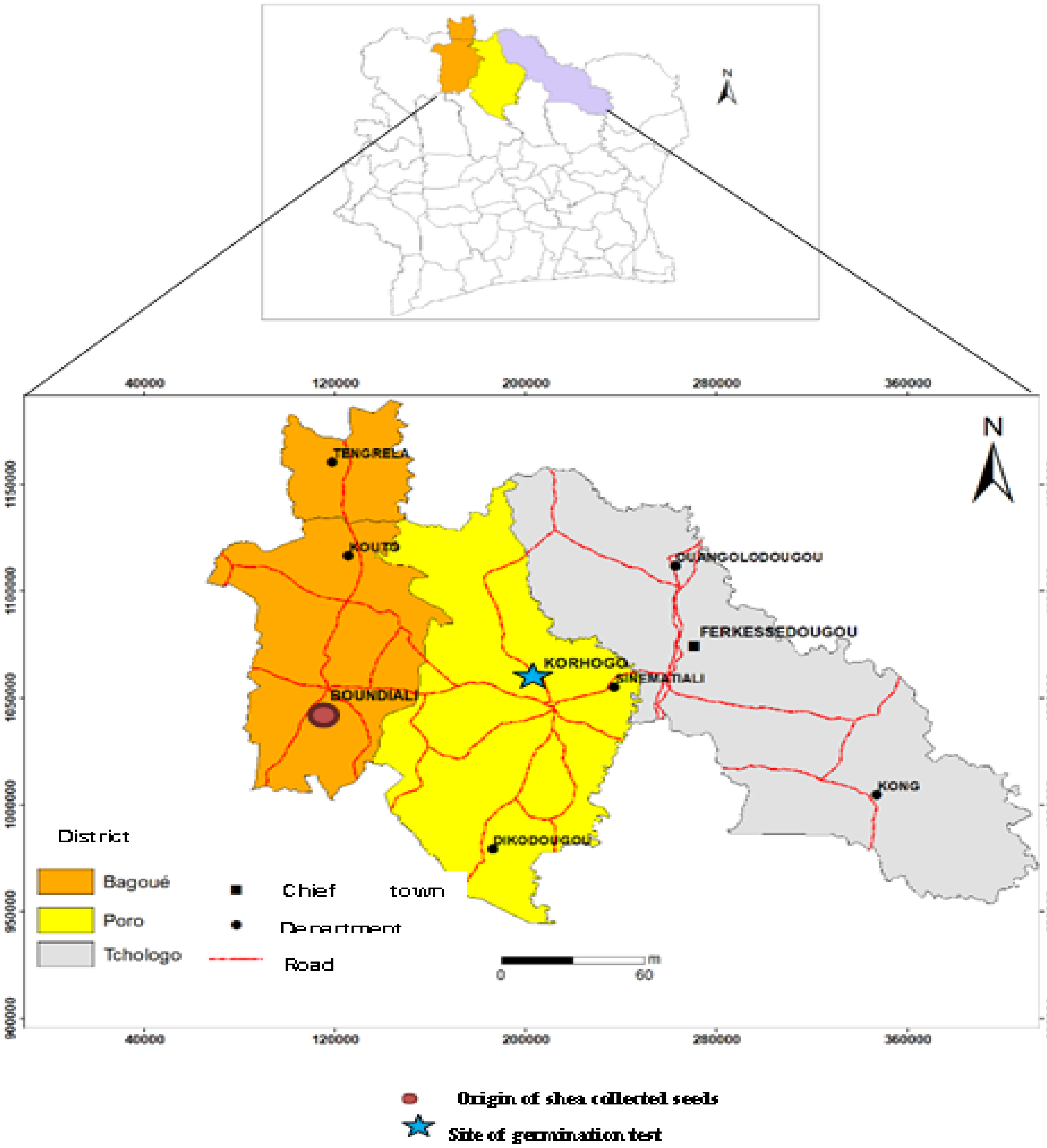

Figure 1. Geographical location of the study sites

2.3. Sowing Substrate

The sowing substrate in the nursery was composed of a homogeneous mixture of equal quantities of river sand, manure and humus soil. The mixture has been treated with two types of pesticides to sterilize the substrate. These are a fungicide and nematicide with Manebe and Carbofuran as active substances respectively.

2.4. Experimental Design

To test the effects of sowing depth and seed orientation on germination and vigour of juvenile plants in the nursery, a Fisher block design with three replications was used (Figure 2). The trial was conducted on individuals in nursery bags that were arranged under shade where no heterogeneity gradient is suspected. The seeds were sown at three different depths (5 cm, 10 cm and 15 cm depths) and arranged according to the orientation concerned (Figure 2). Five seed orientations that are vertical seed orientation with the apical end upwards (VH), vertical seed orientation with the apical end downwards (VB), horizontal seed orientation with hilum upwards (HH), horizontal seed orientation with hilum downwards (HB) and horizontal seed orientation with hilum sidewards (HL) were tested (Figure 3). Within a same block the treatment combining a given sowing depth with a specific seed orientation was repeated five times. Each of the three blocks contained 75 nursery bags. For all three blocks 225 seeds were sowed in nursery bags.

Block 1

VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15

Block 2

VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15

Block 3

VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15
VH5	VH10	VH15	VB5	VB10	VB15	HH5	HH10	HH15	HB5	HB10	HB15	HL5	HL10	HL15

Figure 2. Experimental design used in nursery (VH: Vertical seed orientation with the apical end upwards, VB : Vertical seed orientation with the apical end downwards, HH : Horizontal seed orientation with hilum upwards, HB : Horizontal seed orientation with hilum downwards, HL : Horizontal seed orientation with hilum sidewards, 5: sowing depth of 5 cm, 10: sowing depth of 10 cm, 15: sowing depth of 15 cm)

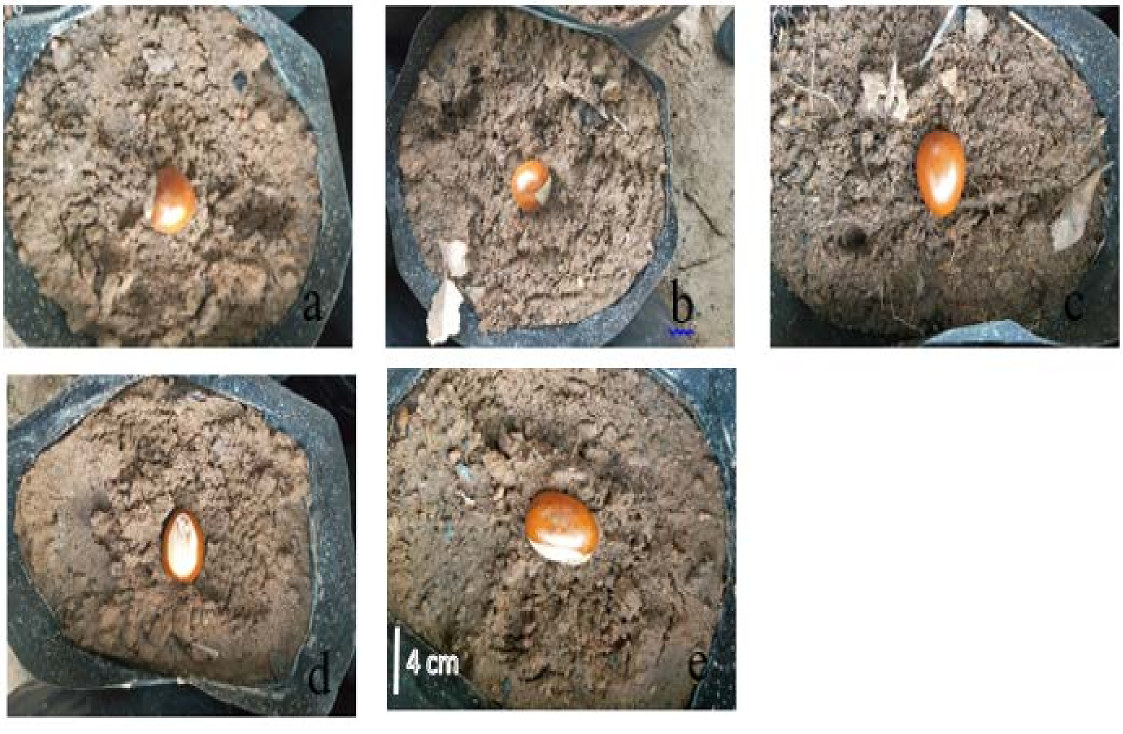

Figure 3. Seed orientations tested during shea seed sowing (a. Vertical seed orientation with the apical end downwards; b. Vertical seed orientation with the apical end up; c. Horizontal seed orientation with hilum downwards; d. Horizontal seed orientation with hilum upwards; e. Horizontal seed orientation with hilum sidewards)

2.5. Germination Parameters Assessment

The process of seed germination includes the emission and growth of the radicule (root) followed by the ones of the plumule (first bud of a plant from which the stem and leaves will grow during seed germination). The shea seed having cryptogeal germination mechanism, a seed was considered as germinated when the plumule emerges on the surface of the sowing substrate. This to approximate field conditions where nursery gardeners or farmers consider germinated seed only when the plumule emerges from the substrate and is visible. Six germination parameters were calculated. These are latency time, final germination rate, germination time, germination speed, germination uniformity and germination synchronism [6,11,12].

2.5.1. Latency Time (TL)

The latency time (TL) refers to the time elapsed from the sowing date to the first germinations.

2.5.2. Final Germination Rate (TGF)

Final germination rate refers to the percentage of seeds that have germinated during the germination process. The mathematical expression of the final germination rate is as follows:

$$TGF\left(\%\right)=\frac{ni}{N}*100 \qquad (1)$$

With ni the cumulative number of germinated seeds at each observation i, and N the total number of germinated seeds. TGF varies from 0 (no seeds germinated) to 100% (all seeds were germinated).

2.5.3. Germination Time (TMG)

The germination time is measured with the median time that corresponds to 50% of the seed germination. This measure allows knowing the germination behaviour of all the seeds in a sample. The median time (TMG) is expressed as follows:

$$\mathbf{TMG}\left(\mathbf{days}\right)=\mathbf{T}1+\frac{0,5-G1}{\left(G2-G1\right)*\left(T2-T1\right)} \qquad (2)$$

G1 = cumulative percentage of germinated seeds at time T1 whose value is closest to 50 % by lower value. G2 = cumulative percentage of germinated seeds at time T2 whose value is closest to 50 % by higher value.

2.5.4. Speed of Germination (GVC)

The germination speed or germination velocity coefficient (GVC) corresponds to the reciprocal of the average germination time.

$$CVG\left(\%\right)=100\left(\frac{n1+n2+\ldots+nx}{n1t1+n2t2+\ldots+nxtx}\right) \qquad (3)$$

With nx: the number of germinated seeds for observation x, tx: the day corresponding to the seed germination.

2.5.5. Uniformity of Germination (CVt)

The variation coefficient of the germination time (CVt) is assessed to evaluate the uniformity of germination. This parameter corresponds to a measure of relative dispersion allowing to quantify the variation of the germination time between each germinated seed:

$$\mathbf{CV}_t\left(\%\right)=\left(St/TMG\right)x100 \qquad (4)$$

With St: the standard deviation of the average time of germination, TMG: the time of germination

2.5.6. Synchronism of the Germination

In general, the germination is asynchronous and it is possible to quantify this characteristic thanks to the index of synchronization noted Ē who expressed as follows:

$$\bar{\mathbf{E}}\left(\mathbf{bit}\right)=-\sum_{\mathbf{i}=1}^{\mathbf{k}}\mathbf{fi}\log 2\mathbf{fi},\mathbf{avec\ fi}=\mathbf{ni}/\sum_{\mathbf{i}=1}^{\mathbf{k}}\mathbf{ni} \qquad (5)$$

With fi the frequency of germination; ni: the number of germinated seeds on day i and k: the last day of observation. The germination is all the more synchronous as the values of Ē are close to 0.

2.6. Growing Trait Measurements in Nursery

Seven vegetative traits were measured on the seedlings in the nursery at 1, 5 and 9 months after seed germination. They are root collar diameter, plant height, leaf number, leaf width, leaf length and petiole length (Figure 4) and vegetative vigour. The vegetative vigour was calculated from the formula proposed by Alexandre [13]:

$$\mathbf{HP/DCP}\left(\mathbf{cm/mm}\right)=\frac{\mathbf{Plant\ height}}{\mathbf{Collar\ diameter}} \qquad (6)$$

Similarly, the parameter reflecting the increase rate of the vegetative traits evaluated was calculated according to the formula proposed by Lekadou et al. [14] and applied in shea tree by Yao et al. [15]:

$$\mathbf{VA}\left(\mathbf{cm/month}\right)=\frac{\mathbf{Xtn}+1-\mathbf{Xtn}}{\mathbf{tn}+1-\mathbf{tn}} \qquad (7)$$

$X_{\_(tn+1)}$-$X_{\_tn}$: the difference between the lengths observed at t_(n+1) and t_n for a given trait and t_(n+1)-t_n corresponds to the duration in months between two successive observations.

Twelve months after seed sowing, the number of plants suitable for grafting was recorded following sowing depth and seed orientation. The selection of shea juvenile plants suitable for grafting was done following the recommendations of Yao et al. [16] stating that the seedlings must have a diameter values between 5-10 cm on the stem at least 0.8 cm. The proportions of plants suitable for grafting (PPG) were calculated according to the formula:

$$PPG\left(\%\right)=\frac{\sum PGi*100}{Total\ plants} \qquad (8)$$

With $\sum\mathbf{PGi}$ the sum of plants suitable for grafting.

2.7. Statistical Analyses

An analysis of variance or ANOVA (factor with more than two levels) or a Student's t test (factor with two levels) was performed to differences between treatment means of each studied trait at a 5 % threshold. When the ANOVA test was significant (p <0.05), post ANOVA test such as Student Newman Keul (SNK) was performed to rank the statistical studied units. All these analyses were performed with SPSS version 20 software (IBM Corp., USA) and Statistica version 7.1 software (StatSoft., USA).

Figure 4. Some vegetative traits measurement on shea seedlings in the nursery. a. Diameter at the neck; b. Length of the leaves; c. Width of the leaves

3. Results

3.1. Effect of Sowing Depth and Seed Orientation on Shea Seed Germination in Nursery

The effect of interaction between the factors sowing depth and seed orientation did not significantly influence the germination parameters (p > 0.05). On the six tested germination parameters, only latency time (F = 5.13; p = 0.01), final germination rate (F = 4.81; p = 0.01) and median germination time (F = 9.06; p = 0.001) parameters significantly differentiated the three levels of seeding depth tested (Table 1). The 5 cm sowing depth had the best latency time (51 days) (Table 1). The 5 cm seeding depth had the highest final germination rate (84.60 %) (Table 1). The lowest median germination time (110 days) was recorded at the 5 cm depth, corresponding to the best median germination time (Table 1).

Only the variation coefficient of the germination time significantly differentiated (F = 3.18; p = 0.024) the five levels of seed orientation during seeding (Table 1). Horizontal seed orientation with hilum downwards (HB) and horizontal seed orientation with hilum sidewards (HL) got the highest variation coefficient of the germination time (43.2 % - 49.8 %). The lowest variation coefficient of the germination time (26.1 % - 27.6 %) was obtained with seed orientation with hilum upwards (HH) and vertical seed orientation with the apical end upwards (VB).

3.2. Effects of Sowing Depth and Seed Orientation on Vegetative Vigour of Shea Seedling in Nursery

The analysis of variance (ANOVA) showed no significant interaction effect between the factors sowing depth and seed orientation for each vegetative trait evaluated in the nursery. The result of the effect of sowing depth on vegetative vigour of shea seedlings is recorded in Table 2. The analysis of variance (ANOVA) did not show a significant difference in each vegetative trait.

According to seed orientation 1, 5 and 9 months after germination the analysis of variance (ANOVA) showed a significant differences (p < 0.05) in vegetative traits such as seedling root collar diameter (F = 5.11; p = 0.002), seedling height (F = 4.53; p = 0.005) and seedling leaf number (F = 3.03; p = 0.03) (Table 3). One month after germination, HL orientation recorded the highest root collar diameter (0.25 cm). The highest value of plant height was obtained by orientation HL (6.23 cm). Regarding the number of leaves of the seedlings, the HL orientation recorded the best average number of leaves (5.75). Five months after germination, HL orientation recorded the highest root collar diameter (0.49 cm). Nine months after germination the HL orientation showed the highest value of root collar diameter (0.49).

The analysis of variance (ANOVA) did not show significant differences between the increase rate of growth of the vegetative traits assessed on shea seedlings in nursery at different sowing depths and seed orientation (Table 4).

Table 1. Effect of sowing depth and seed orientation on germination parameters of shea seeds in nursery

Source of variation	Modalité	TL (days)	TGF (%)	TMG (days)	CVG (%)	CVt (%)	Ē (bit)
Sowing depth	5	51.3 ± 10 b	84.6 ± 19.2 a	110 ± 54 b	0.93 ± 0.54 a	36.8 ± 22.3 a	0.4 ± 0.10 a
	10	83 ± 40 ab	78 ± 18.2 ab	186 ± 33.2 ab	0.54 ± 0.11 a	37.4 ± 22.1 a	0.38 ± 0.09 a
	15	140 ± 115 a	64 ± 10 b	196 ± 63.6 a	0.82 ± 0.55 a	36 ± 27.1 a	0.35 ± 0.08 a
ANOVA statistic test	F	5.13	4.81	9.06	2.26	0.16	0.76
	p	0.01	0.01	0.001	0.112	0.84	0.47
Seed orientation	VH	131 ± 101 a	82.2 ± 26.6 a	176 ± 85 a	0.58 ± 0.19 a	27.6 ± 11.4 b	0.41 ± 0.09 a
	VB	54 ± 9.8 a	81.1 ± 17.6 a	137 ± 59 a	1.01 ± 0.63 a	36.9 ± 20.4 ab	0.38 ± 0.09 a
	HH	131 ± 115 a	76.6 ± 20 a	176 ± 90 a	0.5 ± 0.19 a	26.1 ± 16.6 b	0.4 ± 0.11 a
	HB	90.5 ± 76 a	72.2 ± 22.3 a	180 ± 11 a	0.72 ± 0.47 a	49.8 ± 21.7 a	0.38 ± 0.09 a
	HL	57 ± 9.5 a	65.5 ± 23.3 a	147 ± 25 a	0.95 ± 0.51 a	43.2 ± 24.6 a	0.32 ± 0.06 a
ANOVA statistic test	F	1.76	1.04	0.64	1.63	3.18	0.94
	p	0.16	0.39	0.63	0.19	0.024	0.45

TL: latency time; TGF: final germination rate; TMG: mean germination time; CVG: germination speed; CVt: germination uniformity; E: germination synchrony; F: ANOVA test statistic; p: Probability value associated with the test; VH : Vertical seed orientation with the apical end upwards, VB : Vertical seed orientation with the apical end downwards, HH : Horizontal seed orientation with hilum upwards, HB : Horizontal seed orientation with hilum downwards, HL : Horizontal seed orientation with hilum sidewards, 5: sowing depth of 5 cm, 10: sowing depth of 10 cm, 15: sowing depth of 15 cm. Means assigned the same letter in the same column for each trait are not statistically significant at the 5% probability level.

Table 2. Effect of sowing depth on vegetative vigour of shea seedlings at 1, 5 and 9 months after seed germination in nursery

	Sowing depth (mean ± standard deviation)					
Months after seed germination	Growing trait	5 cm	10 cm	15 cm	F	p
1 month	DCP (mm)	2.20 ± 0.3 a	2.20 ± 0.3 a	2 ± 0.8 a	1.01	0.37
	HP (cm)	5.54 ± 1.21 a	5.40 ± 1.28 a	5.51 ± 2.3 a	0.41	0.66
	HP/DCP	2.51 ± 0.41 a	2.47 ± 0.55 a	2.46 ± 0.39 a	0.03	0.96
	NF	4.93 ± 1.03 a	5.13 ± 1.5 a	5.23 ± 2.06 a	0.22	0.80
	LP (cm)	0.74 ± 0.42 a	0.69 ± 0.32 a	0.62 ± 0.39 a	0.31	0.73
	LF (cm)	5.86 ± 1.78 a	5.26 ± 1.33 a	6.06 ± 1.45 a	0.94	0.39
	LGF (cm)	2.53 ± 0.37 a	2.44 ± 0.73 a	2.78 ± 0.60 a	1.15	0.32
5 months	DCP (cm)	2.70 ± 0.4 a	2.70 ± 0.3 a	2.80 ± 0.4 a	0.19	0.82
	HP (cm)	6.91 ± 1.99 a	7.25 ± 2.31 a	6.90 ± 1.57 a	0.13	0.87
	HP/DCP	2.53 ± 0.67 a	2.60 ± 0.80 a	2.44 ± 0.42 a	0.19	0.82
	NF	6.4 ± 2.06 a	6.64 ± 1.86 a	6.33 ± 2.03 a	0.72	0.49
	LP (cm)	0.74 ± 42 a	0.74 ± 0.31 a	0.65 ± 0.39 a	0.21	0.80
	LF (cm)	5.99 ± 1.78 a	5.67 ± 1.15 a	6.06 ± 1.45 a	0.21	0.81
	LGF (cm)	2.53 ± 0.37 a	2.55 ± 0.74 a	2.85 ± 0.57 a	1.21	0.30
9 months	DCP (cm)	4.30 ± 0.9 a	4.40 ± 1.10 a	4.60 ± 1 a	0.27	0.76
	HP (cm)	13.57 ± 3.95 a	12.61 ± 2.51 a	16.01 ± 7.66 a	1.32	0.28
	HP/DCP	3.15 ± 0.67 a	2.87 ± 0.45 a	3.33 ± 0.93 a	1.15	0.32
	NF	8.5 ± 2.92 a	9.17 ± 4.98 a	9.73 ± 2.32 a	0.36	0.69
	LP (cm)	0.79 ± 0.43 a	0.69 ± 32 a	0.80 ± 0.43 a	0.30	0.73
	LF (cm)	6.39 ± 1.58a	5.65 ± 1.19 a	6.06 ± 1.45 a	0.76	0.47
	LGF (cm)	2.60 ± 0.34 a	2.53 ± 0.75 a	2.96 ± 0.49 a	1.77	0.18

DCP: diameter at the collar of the plant; NF: Number of leaves of the plant; HP: Plant height; HP/DCP: Plant vigour; LP: Petiole length; LF: Leaf length; LGF: Leaf width; p: Value of the probability associated with ANOVA test; F: ANOVA test statistic; 5: sowing depth of 5 cm, 10: sowing depth of 10 cm, 15: sowing depth of 15 cm; Means assigned the same letter in the same column for each trait are not statistically significant at the 5% probability level.

Table 3. Effect of seed orientation on the vigour of vegetative traits of shea seedlings at 1, 5 and 9 months after seed germination in nursery

		Seed orientation (Mean ± standard deviation)						
Months after seed germination	Growing traits	VH	VB	HH	HB	HL	F	p
1 month	DCP (mm)	1.9 ± 0.20 b	2.30 ± 0.3 a	2.20 ± 0.2 a	2.20 ± 0.2 a	2.5 ± 0.1 a	5.11	0.002
	HP (cm)	4.52 ± 1.28 b	5.87 ± 1.06 b	4.88 ± 1.07 b	6.18 ± 0.99 a	6.23 ± 0.73 a	4.53	0.005
	HP/DCP	2.34 ± 0.45 a	2.42 ± 0.44 a	2.09 ± 0.65 a	2.84 ± 0.39 a	2.46 ± 0.23 a	2.65	0.051
	NF	4.13 ± 0.83 a	5.11 ± 1.05 a	5 ± 0.88 a	5.13 ± 0.99 a	5.75 ± 0.88 a	3.03	0.03
	LP (cm)	0.65 ± 0.32a	0.68 ±0.32a	0.66 ± 0.45 a	0.72 ± 0.39 a	0.73 ± 0.45 a	0.69	0.99
	LF (cm)	4.58 ± 0.77 a	5.66 ±1.41 a	5.71 ± 1.27 a	6.52 ± 1.48 a	6.10 ± 2.14a	1.90	0.13
	LGF (cm)	2.19 ± 0.31 a	2.64 ±0.97 a	2.58 ± 0.38 a	2.66 ± 0.22 a	2.80 ± 0.60 a	1.22	0.31
5 months	DCP (mm)	2.30 ± 0.4 a	2.80 ± 0.2 a	2.70 ± 0.1 a	2.90 ± 0.2 a	3.10 ± 0.5 a	4.87	0.004
	HP (cm)	5.34 ± 0.94 a	7.61 ± 2.62 a	7.04 ± 2.03 a	6.92 ± 1.49 a	8.10 ± 1.71 a	2.27	0.085
	HP/DCP	2.29 ± 0.49 a	2.32 ± 0.43 a	2.24 ± 0.52 a	2.41 ± 0.47 a	2.52 ± 0.46 a	0.37	0.82
	NF	6.71 ± 2.62 a	5.5 ± 1.04 a	7.43 ± 2.29 a	6.14 ± 1.06 a	7.43 ± 2.76 a	1.00	0.42
	LP (cm)	0.65 ± 0.34 a	0.84 ± 0.25 a	0.76 ± 0.40 a	0.71 ± 0.47 a	0.80 ± 0.45 a	0.23	0.91
	LF (cm)	4.65 ± 0.81 a	6.35 ± 1.16 a	5.94 ± 1.18 a	6.79 ± 1.38 a	6.54 ± 1.88 a	2.57	0.055
	LGF (cm)	2.26 ± 0.25 a	3.06 ± 0.92 a	2.63 ± 0.38 a	2.69 ± 0.22 a	2.92 ± 0.52 a	1.9	0.15
9 months	DCP (mm)	3.60 ± 0.7 b	4.40 ± 0.9 b	4.70 ± 0.7 a	3.70 ± 0.6 b	4.90 ± 0.6 a	4.35	0.024
	HP (cm)	10.42 ± 2.41 a	15.13 ± 3.93 a	12.06 ± 3.38 a	11.96 ± 2.92 a	14.58 ± 3.71 a	1.84	0.15
	HP/DCP	2.75 ± 0.67 a	3.41 ± 0.49 a	2.74 ± 0.60 a	3.60 ± 0.87 a	2.90 ± 0.54 a	2.65	0.051
	NF	9.20 ± 1.30 a	7.67 ± 3.01 a	9.33 ± 3.05 a	8.67 ± 3.01 a	10.60 ± 1.81 a	0.95	0.45
	LP (cm)	0.66 ± 0.39 a	0.82 ± 0.29 a	0.92 ± 0.64 a	0.75 ± 0.50 a	0.96 ± 0.44 a	0.34	0.84
	LF (cm)	4.93 ± 0.74 a	6.11 ± 1.56 a	6.63 ± 1.54 a	6.57 ± 1.74 a	7.52 ± 1 a	2.31	0.09
	LGF (cm)	2.37 ± 0.21 a	2.96 ± 1.06 a	2.88 ± 0.30 a	2.71 ± 0.24 a	3.14 ± 0.46 a	1.19	0.34

DCP: diameter at the collar of the plant; NF: Number of leaves of the plant; HP: Plant height; HP/DCP: Plant vigour; LP: Petiole length; LF: Leaf length; LGF: Leaf width; p: Value of the probability associated with ANOVA test. F: ANOVA test statistic; VH: Vertical seed orientation with the apical end upwards, VB: Vertical seed orientation with the apical end downwards, HH: Horizontal seed orientation with hilum upwards, HB: Horizontal seed orientation with hilum downwards, HL: Horizontal seed orientation with hilum sidewards.

Table 4. Effects of sowing depth and seed orientation on growth rate of vegetative traits assessed on juvenile shea plants during the first year of breeding in nursery

<table>
<tr><th>Source of variation</th><th>Modalities</th><th>DCP (mm.month-1)</th><th>HP (cm.month-1)</th><th>HP/DCP</th><th>NF (unity.month-1)</th><th>LP (cm.month-1)</th><th>LF (cm.month-1)</th><th>LGF (cm.month-1)</th></tr>
<tr><td rowspan="3">Sowing depth</td><td>5</td><td>2.62 ± 1.94 a</td><td>1.003 ± 0.93 a</td><td>0.08 ± 0.10 a</td><td>0.44 ± 0.11 a</td><td>0.012 ± 0.0007a</td><td>0.066 ± 0.047 a</td><td>0.017 ± 0.0007 a</td></tr>
<tr><td>10</td><td>2.75 ± 2.12 a</td><td>0.90 ±0.62 a</td><td>0.05 ± 0.024 a</td><td>0.50 ± 0.18 a</td><td>0.012 ± 0.0001 a</td><td>0.053 ± 0.068</td><td>0.016 ± 0.015 a</td></tr>
<tr><td>15</td><td>3.25 ± 1.76 a</td><td>1.31 ± 1.36 a</td><td>0.11 ± 0.15 a</td><td>0.56 ± 0.40 a</td><td>0.022 ± 0.021 a</td><td>0.0002 ± 0.0002 a</td><td>0.022 ± 0.007 a</td></tr>
<tr><td rowspan="2">ANOVA test statistic</td><td>F</td><td>0.058</td><td>0.088</td><td>0.172</td><td>0.096</td><td>0.447</td><td>1.049</td><td>0.218</td></tr>
<tr><td>p</td><td>0.94</td><td>0.91</td><td>0.85</td><td>0.91</td><td>0.67</td><td>0.45</td><td>0.81</td></tr>
<tr><td rowspan="5">Seed orientation</td><td>VH</td><td>0.21 ± 0.15 a</td><td>0.73 ± 0.75 a</td><td>0.063 ± 0.072 a</td><td>0.63 ± 0.015 a</td><td>0.0024 ± 0.001 a</td><td>0.043 ± 0.037</td><td>0.022 ± 0.007 a</td></tr>
<tr><td>VB</td><td>0.26 ± 0.19 a</td><td>1.15 ± 1.02 a</td><td>0.14 ± 0.17 a</td><td>0.32 ± 0.31 a</td><td>0.022 ± 0.024 a</td><td>0.11 ± 0.079 a</td><td>0.065 ± 0.022 a</td></tr>
<tr><td>HH</td><td>0.31 ± 0.26 a</td><td>0.89 ± 0.50 a</td><td>0.081 ± 0.061 a</td><td>0.54 ± 0.093 a</td><td>0.032 ± 0.01 a</td><td>0.11 ± 0.08 a</td><td>0.037 ± 0.035 a</td></tr>
<tr><td>HB</td><td>0.18 ± 0.01 a</td><td>0.72 ± 0.76 a</td><td>0.20 ± 0.13 a</td><td>0.44 ± 0.26 a</td><td>0.006 ± 0.005 a</td><td>0.061 ± 0.008 a</td><td>0.006 ± 0.0017 a</td></tr>
<tr><td>HL</td><td>0.30 ± 0.21</td><td>1.04 ± 0.81 a</td><td>1.04 ± 0.81 a</td><td>0.60 ± 0.26 a</td><td>0.028 ± 0.015 a</td><td>0.17 ± 0.095 a</td><td>0.042 ± 0.017 a</td></tr>
<tr><td rowspan="2">ANOVA statistic test</td><td>F</td><td>0.164</td><td>0.116</td><td>0.658</td><td>0.663</td><td>1.801</td><td>1.183</td><td>1.01</td></tr>
<tr><td>p</td><td>0.94</td><td>0.97</td><td>0.54</td><td>0.64</td><td>0.26</td><td>0.41</td><td>0.48</td></tr>
</table>

DCP: diameter at the collar of the plant; NF: Number of leaves of the plant; HP: Plant height; HP/DCP: Plant vigour; LP: Petiole length; LF: Leaf length; LGF: Leaf width; p: Value of the probability associated with ANOVA test. F: ANOVA test statistic; VH: Vertical seed orientation with the apical end upwards, VB: Vertical seed orientation with the apical end downwards, HH: Horizontal seed orientation with hilum upwards, HB: Horizontal seed orientation with hilum downwards, HL: Horizontal seed orientation with hilum sidewards; 5: sowing depth of 5 cm, 10: sowing depth of 10 cm, 15: sowing depth of 15 cm.

Table 5. Correlation between growing traits measured in the nursery on 12-month-old shea seedlings (r values below the diagonal and associated probability values above the diagonal)

Growing traits	DCP	NF	HP	LP	LF	LGF	HP/DCP
DCP	1	<0.001	<0.001	0.019	<0.001	<0.001	0.287
NF	0.591	1	<0.001	0.677	0.59	0.302	0.238
HP	0.776	0.537	1	0.415	0.067	0.115	<0.001
LP	0.255	0.046	0.09	1	<0.001	<0.001	0.199
LF	0.4	0.059	0.199	0.76	1	<0.001	0.158
LGF	0.37	0.199	0.172	0.517	0.814	1	0.246
HP/DCP	0.106	0.117	0.492	0.141	0.155	0.127	1

DCP: diameter at the collar of the plant; NF: Number of leaves of the plant; HP: Plant height; HP/DCP: Plant vigour; LP: Petiole length; LF: Leaf length; LGF: Leaf width; r: Pearson coefficient value; p: Probability value associated with Pearson test.

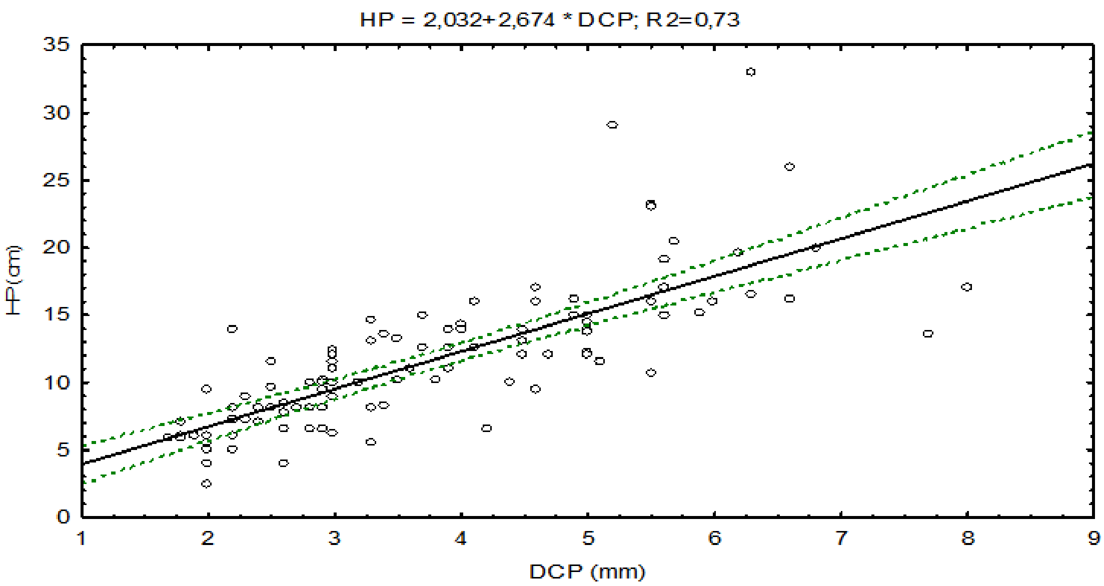

Figure 5. Linear regression between plants height (HP) and root collar diameter (DCP) of shea juvenile plants in nursery

3.3. Correlation between Growing Traits Measured on Juvenile Shea Plants in Nursery

Shea seedlings with higher root collar diameter produced a higher number of leaves (r = 0.591; p <0.001), recorded higher height growth (r = 0.776; p <0.001) with high leaf size (Table 5). Multiple regression analysis showed that only root collar diameter explained the variability in height growth observed in the nursery 73% of the time (Linearity test; F = 18.74; p <0.001) across all traits tested (Figure 5).

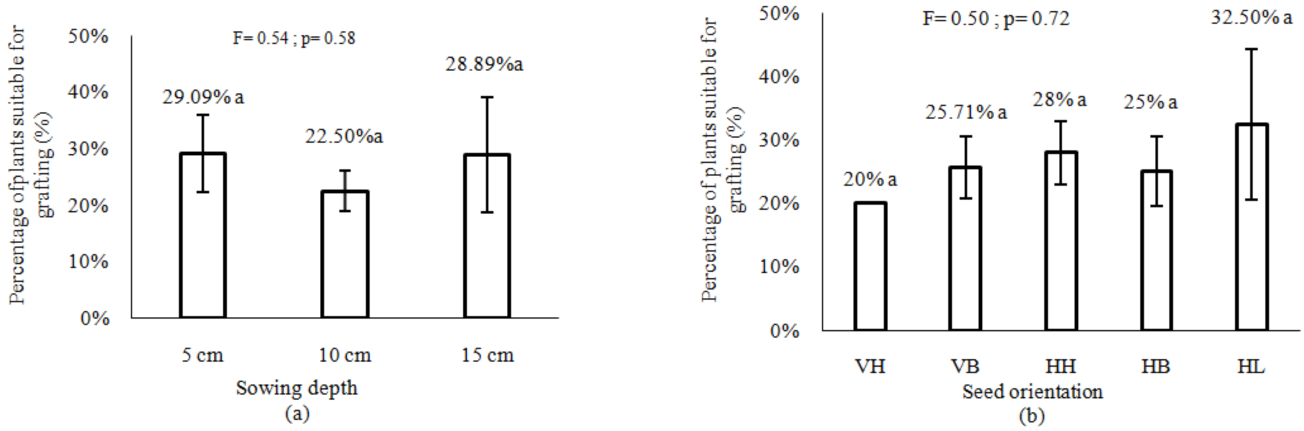

Figure 6. Proportion of shea rootstocks suitable for grafting 12 months after sowing in nursery. **(a)** Percentage of shea plants suitable for grafting 12 months after sowing following sowing depth; (b) Percentage of shea plants suitable for grafting 12 months after sowing following seed orientation (F: ANOVA test statistic; p: Probability value associated with the test; VH: Vertical seed orientation with the apical end upwards, VB: Vertical seed orientation with the apical end downwards, HH: Horizontal seed orientation with hilum upwards, HB: Horizontal seed orientation with hilum downwards, HL: Horizontal seed orientation with hilum sidewards, 5: sowing depth of 5 cm, 10: sowing depth of 10 cm, 15: sowing depth of 15 cm. Means assigned the same letter are not statistically significant at the 5% probability level)

Figure 7. Vegetative aspects of shea rootstocks 12 months after sowing in the nursery (a. Rootstock suitable for grafting; b. Rootstock not suitable for grafting)

3.4. Proportion and Vegetative Characteristics of Shea Rootstocks Suitable for Grafting 12 Months after Sowing

Sowing depth (F = 0.54; p = 0.58) and seed orientation (F = 0.50; p = 0.72) were not affected significantly the rate of shea rootstocks suitable for grafting 12 months after sowing in nursery. Depending on the sowing depth, the percentage of shea rootstocks suitable for grafting varied from 22.50 % to 29.09 % (Figure 6 a). Regarding the seed orientation, the percentage of shea rootstocks suitable for grafting varied from 20 % to 32.50 % (Figure 6 b). Shea rootstocks suitable for grafting recorded the best expressions of vegetative traits than non-grafting rootstocks (Table 6). Twelve months after sowing in nursery the collar diameter of shea rootstocks suitable for grafting (5.37 mm) was higher than the value recorded on non-grafting rootstocks (2.77 mm). Shea rootstocks suitable for grafting had more leaves (11 leaves), higher plant height (16.1 cm), higher values of petiole length (0.92 cm), leaf length (6.9 cm) and leaf width (2.9 cm) (Figure 7).

Table 6. Characteristics of rootstocks suitable and not suitable for grafting 12 months after sowing in nursery

Roostocks status	Vegetative traits (means ± standart deviation)						
	DCP	NF	HP	LP	LF	LGF	HP/DCP
Suitable for grafting	5.37 ± 2.9 a	11.1 ± 3.90 a	16.1 ± 5.1 a	0.92 ± 0.84 a	6.9 ± 3.4 a	2.9 ± 1.25 a	3 ± 0.84 a
Not suitable for grafting	2.77 ± 1.6 b	7.45 ± 3.15 b	8.9 ± 3.01 b	0.55 ± 0.28 b	5 ± 1.63 b	2.3 ± 0.75 b	3.2 ± 0.8 a
Associated statistics test	t = 283.8	t = 26.99	t = 78.51	t = 7.85	t = 10.7	t = 7.28	t = 1.52
	p <0.001	p <0.001	p <0.001	p = 0.006	p = 0.002	p = 0.008	p = 0.22

DCP: diameter at the collar of the plant; NF: Number of leaves of the plant; HP: Plant height; F: Student's t-test statistic; HP/DCP: Plant vigour; LP: Petiole length; LF: Leaf length; LGF: Leaf width;; p: Value of the probability associated with Student test; t: Student statistic test.

4. Discussion

Plant production and the establishment of good agricultural crops are highly dependent on seed germination which is a crucial step in the life cycle of perennial plants. Knowledge of the crop itinerary on aspects such as sowing depth and seed orientation during the sowing of crops are important factors that would optimize shea rootstocks production in the nursery. Thus, this study was undertaken to increase the yields of improved shea plants in nursery for plantation establishment in Côte d'Ivoire. It appears that the sowing depth influences the final germination rate. Thus the depth of 5 cm appears to be the depth giving a short latency time, the best germination rate and the lowest median germination time. This result could be explained by reducing the elongation effort that the seedling must undergo to reach the surface of the sowing substrate. Similar results were reported by Embaye *et al.* [17] who showed that bamboo seeds (Oxy*tenanthera abyssinica* (A.Rich) Munro), sown at a depth of 2.5 mm, also achieved a faster emergence with a higher germination percentage than those sown at depths of 5 mm and 10 mm. Similarly, in cotton seeds (*Gossipium hirsutum* L.), results showed that emergence of sheas decreased with increasing sowing depth and there was no emergence beyond 9.2 cm depth [18]. This result could also be explained by the fact that at a shallow seeding depth, shea seeds would have a good availability of oxygen necessary for their metabolic activities. Indeed, according to Power and Fonteyn [19], the ability of seeds to give better germination and emergence rates at shallow sowing depths is linked to their greater need for oxygen, the availability of which is greater in the shallow soil horizons.

Horizontal seed orientation with hilum sidewards (HL) positively influenced the vegetative vigour of shea seedlings after germination. This result would be due to the fact that the type of germination of shea seed is cryptogeal. Horizontal seed orientation with hilum sidewards would allow a faster and easier rooting. Thus this seed orientation would promote good mineral and water nutrition of the seedling from the roots. Similar results were obtained in beans [20] and bamboo [17] whose seedlings emerge better only when the seeds are sown in a horizontal position.

All germinated seedlings recorded a robustness ratio (plant height/root collar diameter) inferior to 7. This is in accordance with the quality standard of seedlings before planting defined by Lamhamedi *et al.* [21] in agroforestry. According to Lamhamedi *et al.* [21] the quality of the forestry seedlings is estimated by the robustness ratio defined according to the ratio between the plant height and the collar diameter which must be lower than 7. These results corroborate those of Sarir and Benmahioul [22] et Odoi et al. [23] who found similar results on three oak and shea tree species respectively in the nursery. These results attest to the quality of care and good monitoring of shea seedlings in the nursery. Similarly, the technical itinerary for monitoring seedlings adopted by the technicians of shea breeding program at the University Peleforo GON COULIBALY (UPGC) of Côte d'Ivoire seems appropriate.

Significant correlations were recorded between the root collar diameter and other vegetative traits such as the plant height, the leaf number, the petiole length, the leaf length and the leaf width. This result suggests that plant collar diameter alone can estimate vegetative vigour of the juvenile shea plants in the nursery. This result allows reducing the number of agromorphological descriptors to be assessed in the nursery in subsequent studies. Such recommendations from correlations recorded between studied agromorphological traits, have been reported in coconut [24] to reduce the number of descriptors to be considered in subsequent morphological diversity studies.

The shea plant breeding at nursery revealed that at only 12 months, the percentage of juvenile shea plants suitable for grafting when seeds are sowing at 5 cm of depth and horizontal seed orientation with hilum sidewards (HL) was 32.50 %. However, studies conducted by Yao *et al.* [16] showed that shea rootstocks suitable for grafting should remain in the nursery for up to 24 months. This result can be explained by the fact that both 5 cm of sowing depth and horizontal seed orientation with hilum sidewards would promote better development of juvenile shea plants which would have reduced the time to use them as rootstocks in nursery.

5. Conclusion

The objective of this work was to know the effects of sowing depth and the seed orientation on the germination and growth of juvenile shea plants in nursery. It was found that 5 cm of sowing depth gave a better final germination rate of 84.6 %, a shorter germ emergence time (51 days) and the lowest median germination time (110 days). Horizontal seed orientation with hilum sidewards (HL) during seed sowing gave more vigorous juveniles of shea plants in the nursery. During juvenile shea plant breeding in nursery, the seed sowing at depth of 5 cm and the horizontal seed orientation with hilum sidewards allow to dispose of 30 % of the seedlings as rootstocks suitable for grafting in 12 months.

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