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Comparative study of oil palm seedlings morphological development of from three stages of germinated seed differentiation

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Abstract

The oil palm pre-nursery corresponds to the cultivation of young plants during the four (4) months following seed germination. Before transplanting, the pre-germinated seeds were subjected to quality control. The control made it possible to eliminate anomalous or undifferentiated sprouted seeds by using well-defined sorting criteria. Nevertheless, these criteria are sometimes poorly known to the sorting operator and provide oil palm producers with seeds with insufficient development to allow transplantation, leading to a low emergence percentage in the pre-nursery.This study evaluated the morphological development of oil palm (Elaeis guineensis Jacq. 1760) obtained from the three (3) stages of differentiation of sprouted seed differentiation. These were long differentiated seeds (DL), medium differentiated seeds (MD), and undifferentiated seeds (ND). Of this study, it appears that undifferentiated seeds (ND) and medium differentiated seeds (MD) recorded the highest number of dead seedlings with 111.00 ± 10.13 and 102.75 ± 38.93 dead seedlings, respectively. The long-differentiated seeds (DL) have given Seedlings longer and higher fresh weight with 43.55 ± 9.34 cm and 25.05 ± 11.18 g, respectively. The moderately differentiated (MD) seeds produced seedlings with a crown diameter of 0.75 ± 0.32 cm, larger and a root system 26.17 ± 7.75 cm more developed than the undifferentiated and long differentiated seeds. However, no difference was observed between the number of leaves emitted from the seedlings and the root system with regard to the three (3) stages of differentiation of the sprouted seeds.

Keywords: Oil Palm; Sprouted Seeds; Nursery; Differentiation; Seedling

1. Introduction

Oil palm *(Elaeis guineensis* Jacq.) is a strict cross-pollinated perennial plant grown for its oleaginous fruits in approximately 20 countries and represents the world's leading source of vegetable oil, with 39 % vegetable oil production [1]. The surface area of oil palm on the Ivory Coast covers approximately 250,000 ha divided between the industrial sector (29%) and the village sector (71%). The annual production is estimated to be more than 550,000 tons of crude palm oil [2]. The Ivoiry Coast is a leading exporter of palm oil in Africa. The oil palm sector provides a living for approximately 2 million people with 200,000 regular jobs in Ivory Coast and generates an average of nearly 500 billion turnovers [3]. Improvement in the productivity of oil palm, an allogamous perennial plant, has been made possible by adapting since 1957, the reciprocal recurrent selection scheme (SRR) [4] applied to maize by [5]. In this diagram, the two (2) groups of populations with complementary characteristics are improved relative to each other. These are the populations of group A, consisting of palms with a small number of big bunches, mainly originating from Southeast Asia, and the populations of group B, characterized by palms with a large number of small bunches, mainly palms of African origin. Overall, the evaluation of the agro-morphological and technological characteristics of the progenies of the two groups of parents led to the selection of a few elite palm trees that were used for the production of hybrid seeds of the Tenera variety [6]. This important place occupied by the oil palm is due not only to the extensions of the areas planted with oil palm, but also mainly because of the remarkable gains in productivity of the improved plant material put in

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place by agronomic research and distributed since 1960 to planters in the form of germinated seeds [6]. The germinated seeds before delivery for transplanting underwent rigorous sorting to eliminate those presenting anomalies that are not yet differentiated. This elimination is performed by a sorting operator with well-defined criteria [7]. However, these criteria are sometimes poorly known to the sorting operator and make available to oil palm producer seeds with ungerm insufficiently developed to allow their transplanting, leading to a low success rate of seedlings in the pre-nursery despite the different substrates provided to enrich the potting soil [8]. The objective of this study was to evaluate the morphological development of oil palm seedlings (*Elaeis guineensis* Jacq.) due to the three stages of differentiation of the germinated seeds.

To achieve this general objective, two (2) specific objectives were assigned to this study:

- Determine the number of dead seedlings in the pre-nursery;
- Determine the morphological parameters of seedlings in the pre-nursery.

2. Material and methods

2.1. Study site

The study was conducted at the National Agronomic Research Center (CNRA) La Mé Station. This station is located in the northeast of the city of Abidjan, 27 km east of Abidjan, in the region of La Mé on the Abidjan-Alépé axis. Its geographical coordinates are 5°26' North latitude and 3°50' West longitude.

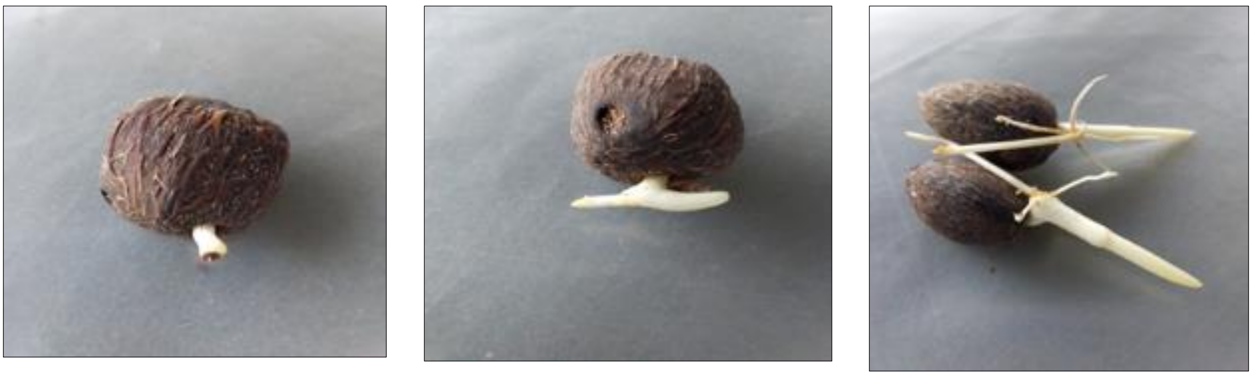
2.2. Plant material

The plant material used in this study consisted of oil palm seedlings from seeds of category C1001 F, whose parental origin was the parent DA115 D AF. The choice of these hybrids is based on their tolerance to Fusarium wilt, and is intended for replanting. The evaluation focused on the percentage of seed success and development in the pre-nursery of 6000 germinated seeds from three (3) different stages of differentiation. Two thousand (2000) non-differentiated seeds (ND), 2000 medium differentiated seeds (MD), and 2000 long differentiated seeds (DL) were used (Figure 1). Measurements were made on seedlings aged 3 months in the pre-nursery.

2.3. Technical equipment

The materials used to determine the seedling development parameters consisted of the following elements:

- A graduated tape and a graduated ruler were used to measure the length of the seedlings and the longest root.
- Electronic scalefor weighing the seedlings
- A caliper was used to measure the diameter of the collar.



a. Non differentiated seeds **b.** Medium differentiated seeds **c.** Long differentiated seeds

Figure 1 Different differentiation stages of germinated oil palm seeds

3. Methods

3.1. Experimental Device

The device used was a completely randomized Fisher block with three (3) treatments (differentiation stages) and four (4) repetitions (**Table 1**). Each treatment was subdivided into 4 blocks of 500 germinated seeds from the C1001F category. A total of 6 000 germinated seeds were randomly distributed in each replicate. The substrate was 100% potted soil. All seeds were transplanted on the same date into pre-nursery bins at the CNRA research station in La Me.

Table 1 Totally randomized Fisher block experimental design

Replicate 1	C1	C2	C3
Replicate 2	C3	C1	C2
Replicate 3	C2	C3	C1
Replicate 4	C3	C1	C2

3.1.1. Evaluation of morphological parameters

Observations were made on the variables related to the development and growth characteristics of six thousand (6000) oil palm seedlings from the three (3) stages of differentiation of germinated seed differentiation: undifferentiated germinated seeds, medium differentiated seeds, and long differentiated seeds.

3.1.2. Number of dead plantlets

In each block and in each subblock, three (3) months after sowing the germinated seeds, a count of dead seedlings was made every two weeks in order to record the number of dead seedlings. The number of dead seedlings was grouped by seed differentiation stage to compare the emergence rate of the three (3) germinated seed differentiation stages. The number of dead seedlings made it possible to evaluate the mortality rate as follows:

Mortality rate (%) = (Sum of dead plantlets) / (Total number of seedlings)

3.1.3. Seedling length (cm)

In each block, three (3) months after emergence, thirty (30) seedlings were randomly chosen for measurement of the longest leaf. Seedling length was measured from the collar to the tip of the longest leaf using a graduated tape and a graduated ruler (**Figure 2).** The average seedling length expressed in centimeters (cm) was obtained using the following relationship:

Seedling length (cm) = (Sum of seedlings measurements) / (Total number of seedlings).

3.1.4. Number of leaves

The number of leaves emitted from the thirty (30) plantlets was counted. The average number of leaves was calculated using the following formula:

Number of leaves = (Total number of leaves of the seedlings) / (Total number of seedlings)

3.1.5. Fresh seedling weight

The thirty (30) plantlets were uprooted, washed (**Figure 3**), and weighed individually using an electronic balance (**Figure 4**). The average weight of the seedlings expressed in grams (g) was determined using the following formula:

Average seedling weight (g) = (Total seedling weight) / (Total number of seedlings)

3.1.6. Root system of seedlings

The longest root was measured using a graduated ruler (**Figure 5**). Measurements of the average root system expressed in centimeters (cm) were obtained using the following relationship:

Root system (cm) = (Sum of measurements carried out on seedlings) / (Total number of seedlings)

3.1.7. Circumference at collar

The diameter at the collar was measured at the collar of the seedlings using a caliper (**Figure 6**). The average collard diameter expressed in centimeters (cm) was obtained using the following formula:

Circumference at collar (cm) = (Sum of measurements taken on the seedlings) / (Total number of seedlings)

Figure 2 Length Measuring Method of the plantlet

Figure 3 Root rinse

Figure 4 Method of weighing the plantlet

Figure 5 Longest root measurement method

Figure 6 Method of measuring the diameter at collar

3.2. Statistical data analyzes

Descriptive analyses (mean, standard deviation, and coefficients of variation) [9] were performed to describe the various variables evaluated. Levene's test was used to verify the assumptions of normality and homogeneity of variances necessary for the use of parametric tests. An analysis of variance (ANOVA) [10] followed by the Newman-Keuls multiple comparison test of means at a risk of 5% [11] was applied to compare the means of the measured variables of the three (3) stages of development of germinated oil palm seeds. A correspondence factor analysis (CFA) was carried out to establish similarities between seed differentiation stages and to group variables according to differentiation stage. All analyses were carried out using STATISTICA 7.1 and XLSTAT 2016.02 software.

4. Results

4.1. Seedling mortality according to the stage of differentiation of germinated seeds

Undifferentiated (ND) and medium differentiated (MD) seeds recorded on average the highest numbers of dead seedlings with 111.00 ± 10.13 and 102.75 ± 38.93 dead seedlings, respectively. However, the coefficient of variation of non-differentiated seeds (ND) was low (9.13%), indicating low variability within them, unlike medium differentiated sprouted seeds (MD), where the CV was large (37.89%). A low number of dead seedlings was observed at the level of long differentiated (DL) germinated seeds (61.50 ± 14.84 dead seedlings) for a respective coefficient of variation of 24.13%. Significant differences were observed between the number of dead seedlings recorded with regard to the three (3) stages of differentiation of germinated seed differentiation (P = 0.04 < 0.05) (**Table 2**).

Table 2 Comparison of the mortality of seedlings from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	**Number of seeds transplanted**	**Dead plantlets**	**CV (%)**
Long differentiated seeds (DL)	2000	61.50 ± 14.84 [a]	24.13
Medium differentiated seeds (MD)	2000	102.75 ± 38.93 [b]	37.89
Non differentiated seeds (ND)	2000	111.00 ± 10.13 [b]	09.13

CV = Coefficient of variation; Means with the same letter in the same column are not significantly different

4.2. Effect of differentiation stage of germinated seeds on seedling length

Long differentiated (LD) seeds produced significantly greater plantlets with an average length of 43.55 ± 9.34 cm for a coefficient of variation of 21.45%. The length of the seedlings from medium differentiated (MD) seeds was intermediate (41.12 ± 9.71 cm and CV (23.61%). Undifferentiated (ND) seeds produced seedlings with a smaller average length of 33.35 ± 7.85 cm for a coefficient of variation 23.54%. Significant differences were reported between the lengths of seedlings from the three (3) stages of differentiation of germinated seeds (P = 0.001 < 0.05) (**Table 3**).

Table 3 Comparison of the length of seedlings from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	**Number of plantlets**	**Seedling length (cm)**	**CV (%)**
Non differentiated seeds (ND)	120	33.35 ± 7.85 [a]	23.54
Medium differentiated seeds (MD)	120	41.12 ± 9.71[b]	23.61
Long differentiated seeds (DL)	120	43.55 ± 9.34 [c]	21.45

CV = Coefficient of variation; Means with the same letter in the same column are not significantly different

4.3. Effect of differentiation stage of germinated seeds on the number of leaves emitted from seedlings

Medium differentiated (MD) seeds recorded on average the highest number of leaves with 7.00 ± 1.22 leaves for a coefficient of variation equal to 17.43%. They are followed by undifferentiated seeds which gave 6.84 ± 1.30 leaves with a coefficient of variation of 19.01%. Long differentiated (DL) seeds provided on average the lowest number of leaves with 6.55 ± 1.04 leaves for a coefficient of variation equal to 15.88%. The coefficient of variation was low for the three (3) differentiation stages, indicating reduced variation within them (**Table 4**). However, no significant difference was observed between the leaves emitted from the three (3) stages of differentiation of germinated seeds (P = 0.3 > 0.05).

Table 4 Comparison of the number of leaves emitted from seedlings from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	**Number of plantlets**	**Number of sheets output**	**CV (%)**
Long differentiated seeds (DL)	120	6.55 ± 1.04 [a]	15.88
Non differentiated seeds (ND)	120	6.84 ± 1.30 [a]	19.01
Medium differentiated seeds (MD)	120	7.00 ± 1.22 [a]	17.43

CV = Coefficient of variation; Means with the same letter in the same column are not significantly different

4.4. Effect of differentiation stage of germinated seeds on fresh weight of seedlings

The differentiated mean (MD) seeds recorded on average the highest fresh weight seedlings with 25.05 ± 11.18 g, for a coefficient of variation equal to 44.63%, followed by the long differentiated (DL) seeds with 23.06 ± 10.25 g for a coefficient of variation of 44.45%. Undifferentiated seeds (ND) gave seedlings of smaller fresh weight with 18.98 ± 9.21 g for a coefficient of variation equal to 48.52%. The coefficients of variation were high, indicating large variation between the three (3) stages of differentiation of germinated seeds for this trait (**Table 5**). Significant differences were observed between the fresh weights of the seedlings obtained from the three (3) stages of differentiation of the germinated seeds (P = 0.01<0.05).

Table 5 Comparison of fresh weight of seedlings from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	Number of plantlets	Fresh weight of plantlets (g)	CV (%)
Non differentiated seeds (ND)	120	18.98 ± 9.21 [a]	48.52
Long differentiated seeds (DL)	120	23.06 ± 10,25 [b]	44.45
Medium differentiated (MD)	120	25.05 ± 11.18 [b]	44.63

CV = Coefficient of variation, g = gram; Means with the same letter in the same column are not significantly different

4.5. Effect of the stage of differentiation of germinated seeds on the development of the root system of seedlings

The medium differentiated seeds (MD) recorded on average seedlings with a most developed root system with 26.17 ± 7.75 cm, for a coefficient of variation equal to 29.61%. The non-differentiated seeds (ND) recorded an intermediate root system with 24.02 ± 8.70 cm with a coefficient of variation of 36.22%. Long differentiated seeds (DL) gave on average a weak root system equal to 22.59 ± 8.60 cm for a coefficient of variation of 38.07%. Statistical analyses revealed that no significant difference existed between the development of the root systems of seedlings from the three (3) different stages of differentiation of germinated seeds (P = 0.06 > 0.05). The coefficient of variation was high for the three (3) stages of differentiation, which explains the strong variation within them (**Table 6**).

Table 6 Comparison of the development of the root system of seedlings resulting from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	Number of plantlets	Root system (cm)	CV (%)
Long differentiated seeds (DL)	120	22.59 ± 8.60 [a]	38.07
Non differentiated seeds (ND)	120	24.02 ± 8.70 [a]	36.22
Medium Differentiated (MD)	120	26.17 ± 7.75 [a]	29.61

CV = Coefficient of variation; Means with the same letter in the same column are not significantly different

4.6. Effect of the differentiation stage of germinated seeds on the diameter at the collar of the seedlings

The moderately differentiated (MD) seeds produced on average seedlings had a larger collar diameter (0.75 ± 0.32 cm, with a coefficient of variation of 42.67%. At the level of undifferentiated seeds (ND), the collar diameter of the seedlings produced was average with 0.61 ± 0.39 cm and a CV of 63.93%. The long differentiated (DL) seeds, for their part, made it possible to produce seedlings with a small diameter at the collar (0.49 ± 0.27 cm) and an average CV of 55.10%. Significant differences were reported between the diameter at the collar of seedlings from the three (3) different stages of germinated seed differentiation (P = 0.001<0.05). The variabilities obtained for collar diameter were very strong within the seedlings from the three (3) stages of seed differentiation (**Table 7**).

Table 7 Comparison of the diameter at the collar of seedlings from the three (3) stages of differentiation of germinated seeds

Stage of differentiation	Number of plantlets	Diameter at the collar of the seedlings (cm)	CV (%)
Long differentiated seeds (DL)	120	0.49 ± 0.27 [a]	55.10
Non differentiated seeds (ND)	120	0.61 ± 0.39 [b]	63.93
Medium differentiated seeds (MD)	120	0.75 ± 0.32 [c]	42.67

CV Coefficient of variation, cm = centimeter;Means with the same letter in the same column are not significantly different

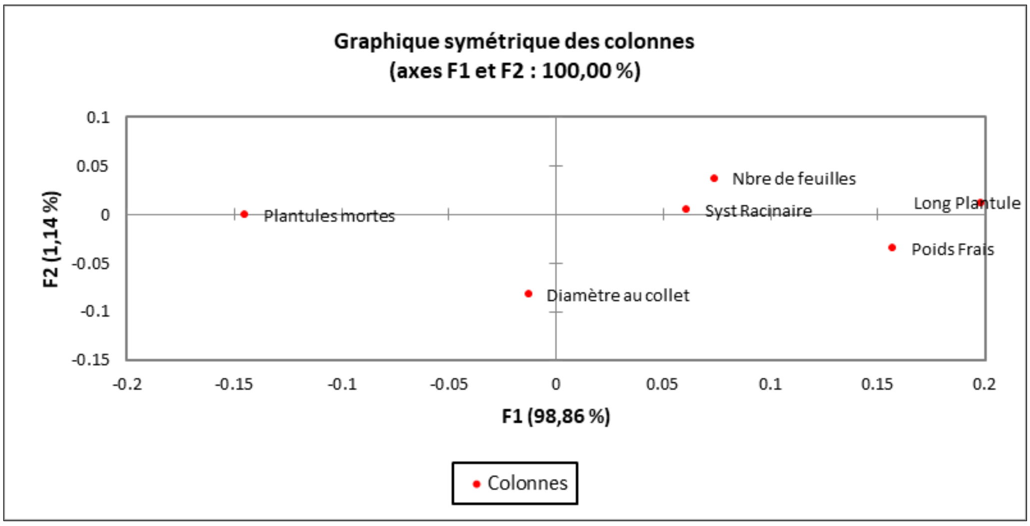
4.7. Link between the vegetative parameters of seedlings and the three (3) stages of differentiation of germinated oil palm seeds

The results of the factorial correspondence analysis (AFC) revealed two main axes expressing 100% of the total variability according to the eigenvalues (Table 8; Figure 7). The correlation circle made it possible to differentiate between the two groups of variables based on the vegetative development of seedlings. The 1st group containing the length of the seedlings, the number of leaves, the fresh weight, the root system, and the dead seedlings, strongly correlated with the F1 axis (98.86% of the total variability) with respective contributions of 0.373 0.009, 0.133, 0.022, and 0.464 and respective squared cosines tending towards 1 (0.996, 0.797, 0.952, 0.994, and 1). The 2nd group was strongly linked to the F2 axis (1.14% of total variability). It includes the diameter at the collar, which contributes 0.088 to the formation of this axis F2, with a square cosine of 0.977. Regarding the observations, the AFC shows that the non-differentiated seeds (ND), medium differentiated seeds (MD), and long differentiated seeds (DL) are strongly correlated to the F1 axis because they have high respective contributions of 0.376, 0.008, and 0.616 to the total inertia and the respective squared cosines of 0.992, 0.537, and 0.998, respectively.

The projection of the observations in the factorial plan (F1, F2) made it possible to categorize the three (3) stages of differentiation of germinated seeds based on the vegetative growth of the seedlings. Indeed, long-differentiated seeds (ND) are characterized by a high rate of dead seedlings. Medium-differentiated seeds (MD) produced seedlings with larger collar diameters. Long differentiated seeds (DL) were distinguished by seedlings of larger size and fresh weight. In contrast, the number of leaves emitted from the seedlings and root system was not influenced by the stages of differentiation of the germinated seeds. This explains why there was no significant difference between the number of leaves emitted and between the root systems with regard to the different stages of GG differentiation (Figure 8).

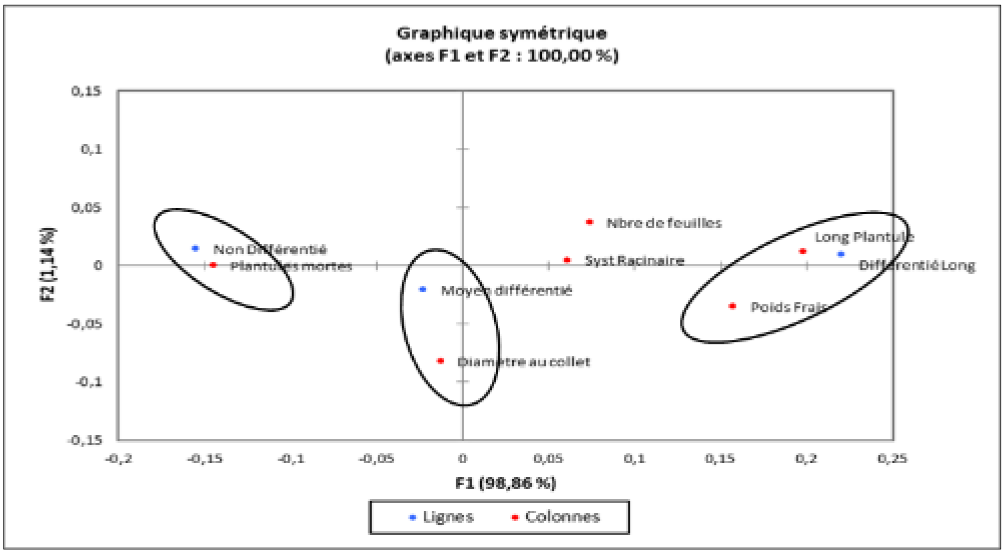
Table 8 Formation of axes according to variables and observations

	F1	F2
Eigenvalue	0.022	0.00
Inertness (%)	98.62	1.13
% cumulative	98.86	100.00



Long plantule: Plantlet length; Nbre: Number; Syst: System

Figure 7 Projection of seedling vegetative development variables onto the correlation circle in the plane (1; **2)**



Long. Plantule : Plantlet length; Nbre : Number; Syst : System

Figure 8 Projection of the three (3) stages of differentiation of germinated seeds in the factorial plan (1; 2).

5. Discussion

Seedlings produced in oil palm pre-nurseries were obtained from the selected seeds. To obtain good quality seedlings, the germinated seeds must undergo quality control by eliminating those presenting anomalies before being transplanted into bags, where they remain for four (4) months before being transferred to the nursery. This operation is performed by sorting operators according to well-defined selection criteria. The results obtained on seed survival showed that undifferentiated seeds (ND) had a significantly higher mortality rate in the pre-nursery than medium differentiated seeds (MD) and long differentiated seeds (DL). The low success rate of undifferentiated seeds could be due to the poor transplanting of these seeds not having perfectly differentiated germs that can distinguish the stem from the radicle [7]. Indeed, the selection of a normal germinated seed is performed by observing long differentiated seeds (DL) and medium differentiated seeds (DM). The selected germinated seeds must necessarily have a well-differentiated stem and radicle, whose total length must be between 8 and 15 mm, a stem and a radicle opposite, and very straight. Unfortunately, undifferentiated seeds (ND) are also chosen for transplantation, but their development is insufficient to allow transplantation. Thus, when transplanting these undifferentiated seeds (ND), the future stem and the future radicle can be confused and once the stem is placed downwards, this could lead to rotting of the seed and generate a low success rate of seedlings in oil palm nurseries [12]. Regarding the length, growth, and fresh weight of the seedlings, our results revealed that long differentiated seeds (DL), the so-called normal seeds, gave on average seedlings of significantly larger size and fresh weight than seedlings obtained from medium differentiated seeds (MD) and non-differentiated seeds (ND). The good growth of seedlings from long-differentiated seeds (DL) could be explained by the fact that the seeds already having a well-differentiated stem and radicle had no difficulty in continuing their growth in the pre-nursery. Indeed, [13], and [14]. Seed germination capacity, speed of seedling emergence, and ability to establish vigorous plants are related to seed quality. Therefore, a good seed is essential to reduce the rate of loss in the pre-nursery and ensure good vigor of seedlings. Although the success rate in the pre-nursery was lower with non-differentiated seeds (ND) than with medium differentiated seeds (MD) and long differentiated seeds (DL), which recorded a higher success rate of seedlings, no difference was observed between the number of leaves emitted by the seedlings from the three (3) stages of differentiation of the germinated seeds. This result shows that apart from the enormous losses recorded in the pre-nursery with non-differentiated germinated seeds (ND), the seedlings obtained from this type of germinated seeds also showed better vegetative development than seedlings from long differentiated seeds (DL) and medium differentiated seeds (MD). Significant dispersion was also observed within the seedlings from each stage of differentiation of germinated seeds for all characters evaluated. This high variability could be explained by the existence of genetically different individuals within the same category, as shown by [15] and [16]. Indeed, the oil palm is a plant with sexual reproduction, where different genotypes coexist within the same offspring according to the homozygous or heterozygous state of the genes involved in a character, as well as their number and the relationships that link them. These results confirmed those of [17] on the determination of the sex ratio at different ages in the Dura, Pisifera, and Tenera varieties of oil palm. The authors showed that the variations in the sex ratio were very high between different lines, but also between palms of the same line.

6. Conclusion

The results obtained showed that long differentiated seeds (LD) gave a high rate of dead seedlings. Medium differentiated seeds (MD) are characterized by seedlings with a larger collar diameter. Long differentiated seeds (DL) were distinguished by seedlings of larger size and fresh weight. On the other hand, the number of leaves emitted from the seedlings and the root system were not influenced by the stages of differentiation of the germinated seeds. This really justifies why there was no significant difference between the number of leaves emitted and between the root system with regard to the different stages of differentiation of the germinated seeds. This result reveals that after seed emergence, the three (3) types of germinated seeds evaluated develop normally and at the same rate in the pre-nursery.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contributions

Concept, supervision and writing, TANO Ekra Kouamé. and KONAN Jean Noël; statistical analysis and correction of the manuscript, N'GUESSAN Assiénin Hauverset; editing and translation, NIAMKETCHI Gilles Léonce.

All authors have read, accepted and approved the published version of the manuscript.

Data availability statement

All tables and figures in the text contain the data used in the current survey.

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