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Full Length Research Paper

Seed germination and initial growth in *Moringa oleifera* Lam. 1785 (Moringaceae) in Sudano-sahelian zone

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ABSTRACT

***Moringa oleifera* is commonly used for feeding purposes in the African and Asian communities. Almost all parts of the plant have food interests and socio -economic importance in the agro-ecological region of far north Cameroon. The domestication of this Moringaceae is essential to its good management in this region. In order to select the best seeds, this study assessed the germination and initial growth depending on the position of the seeds in the pod. The seeds were obtained from 12 localities representative of the agro- ecological region. The experimental design was a randomized complete block with three replications. Treatments correspond to the eight positions in the pod and the experimental unit consisted of 30 seeds. The results show that the weight of the seed depends on its position in the pod (0.0000<0.001). The seeds of the middle part are heavier (263. 21±22.52mg) than those of the distal (214.29±0.86mg) and proximal (250.05±17.86mg) positions. Seed germination also depends on the position of the seed in the pod ($p < 0.05$). Position 2 seeds, from the proximal extremity, have the best germination rate (79.58 ± 4.2%).**

Keywords: *Moringa oleifera*, seed position, germination, initial growth, domestication.

INTRODUCTION

Moringa oleifera (Moringaceae) is a species native of the Middle East including India, Pakistan, Bangladesh and finally Afghanistan (Fahey, 2005). It was introduced in East Africa in the early 20th century (Foidl et *al*., 2001). Today production has grown in the tropics. It is a traditional leafy vegetable adapted to agro-ecological conditions in the Sudano-Sahelian region, easy to produce and which requires little inputs. *Moringa oleifera* is cultivated in the Sudano sahelian zone in different farmers' operating systems (Madi et *al.,* 2012). It grows well on marginal land and does not require much maintenance, with high resistance to drought. The species is especially a food source in the tropics (Bosch et *al*., 2011). Well operated and maintained, it could fight the scourges food and raise the income level of farmers (Saint Sauveur, 2001).

This fast-growing shrub has recently been identified by the World Vegetable Centre as a vegetable with the highest nutritional value among 120 food species (Ray-Yu et *al*., 2006). The fresh leaves of M. *oleifera* are seven times richer in vitamin C than oranges. Similarly, they are four times richer in vitamin A than carrots, have as much protein as eggs and are four times richer in calcium than milk. They triple bananas in potassium (Palada and Chang, 2003; Nancy, 2004). Flowers and pods are also nutritious. The discovery of its many nutritional functions has prompted researchers and NGOs to promote the cultivation of *M. oleifera* as a source of income (Saint Saveur, 2010). Despite of its nutritional value, resistance to natural disasters and socio -economic importance, cultivation of *M.oleifera* has remained the traditional state area.

In Cameroon, any scientific work is still interested in the plant except those Tedonkeng Pamo et *al*. (2004), Madi (2008) who discussed some aspects of the germination of the plant but none addressed the influence

of the position of the seed in the pod on the weight and quality of the seed. The hypothesis of this paper is that the weight and quality of the seed in a long fruit are influenced by its position.

It is therefore necessary to find ways to fast spread of the plant and identification of good seed sources in the area that allow farmers to increase their production capacity. The main objective of this study is to contribute to the domestication of the plant so that the seeds of quality within the reach of farmers. Specifically, it is firstly to examine the characteristics and capabilities of seed germination M.*oleifera* according to the rank they occupy in the pod, and secondly to assess the influence of the position seed in the fruit on the initial growth of the plant.

MATERIALS AND METHOD

Study Site

The region of far north Cameroon is located between 10° and 13° north latitude and 12° and 15° east longitude. The climate is Sudano-Sahelian with two seasons, dry season from October to June and rainy season from July to September. The average annual temperature is 28º C and the average annual rainfall varies between 400 and 900mm. The soil is composed of sandy soils and vertisols. The vegetation is a woody and /or shrub savannah (Harmand *et al*., 1997). In this region, 50 indigenous ethnic groups are encountered (Iyebi – Mandjek and Seignobos, 2000) and the most important are formed by the Fulbe, Kotoko, Choa Arabs, the Guiziga, the Moudang, the Massa, Toupouri, the Bornoua, the Mafa, the Kapsiki, the Mofou, Mandara and the nations of the Massif de Mora (Padlewski, 1966).

Sampling

The seed pods of *Moringa oleifera* were collected from 12 different localities (Djouta bébal, Guirvidik, Pétté, Guidiguis, Papata, Yagoua, Boula, Gazawa, Maroua, Mandaka, Kosséhon and Mokolo) representative of the study area. After harvest, the pods are packed in polyethylene bags labeled and transported to the laboratory where the number of seeds per pod is determined. After this preliminary work, in the majority of fruit, the average number of seeds was found equal to 8. For this reason, the pods containing 8 seeds were chosen, and numbered P1, P2, P3…P8 from the distal to the proximal position, for this study. A total of 90 pods were used and 720 seeds have allowed us to perform the test.

Assessment of seed weight

8 seed pods used are gutted and seeds are collected in different bags in accordance with the position of seeds in the pods. Each bag bears a label indicating the position of the seed in the pod. The Seed weight was measured using a sensitive (0.01 mg) electronic balance (Teldo brand). Weight gain has made seed by seed. 90 seeds were weighed for each position.

Germination

The germination test was conducted in the experimental field of the Institute of Agricultural Research for Development (IRAD) of Maroua in April 2012. The land was plowed (one month before sowing) and irrigation of the plot began two weeks before the start of planting. The field was watered every night because of the high temperature. The experimental field was protected from animals and weeds were manually removed. No fertilization has been made. The experimental design is a completely randomized 3 replications and 8 treatments block; treatments correspond to eight positions stored in the terminal. The experimental unit consists of 30 seeds. Each repetition was performed on an area of 3m x 4m. The spacing of pockets was between 30cm and 20cm between column lines. According to Tedonkeng Pamo *et al.* (2004), evaluation of germination is stopped 30 days after sowing. The number of germinated seeds was recorded after every four days. A seed was considered germinated when the stalk appears above the ground (Mapongmetsem *et al*., 1999ab).

Growth and seedling development

Seedlings from the previous test are monitored and maintained until the 60^{th} day. After the 30^{th} day, 10 seedlings arising from each previously numbered seeds were randomly labeled. The height and diameter of seedlings were measured using a tape measure and calipers. In addition to the number of axes/plant, numbers of lateral branches at each airline axis and the number of leaves were determined. This evaluation was done every 10 days.

Data Analysis

Data analysis focused on the variance. Significant means are separated by the Duncan Multiple Range Test (DMRT). The software is put to use Statgraphics plus 5.0.

RESULTS

Assessment of seed weight

Seed weight in the pod varies from 214.29 mg for first seed, in the proximal position, to 292.87 mg for seeds from the sixth position, to the distal pole of the pod. These results show that the position of the seed has an

Table 1. Seed weight

Rank seed in pod	Seed weight (mg)
P1	214.29± 0.86 a
P8	250.05± 17.86 a
P2	253.42±21.03 a
P3	263.94±8.43 a
P7	266.97± 15.06a
P5	272.40±9.12 ab
P4	291.75±13. 14b
P6	292.87±0.79b
Average	**263. 21±22.52**

Means followed by the same letter are statistically identical
P1, P2, P8 ...Corresponds to the rank occupied by the seed in the pod

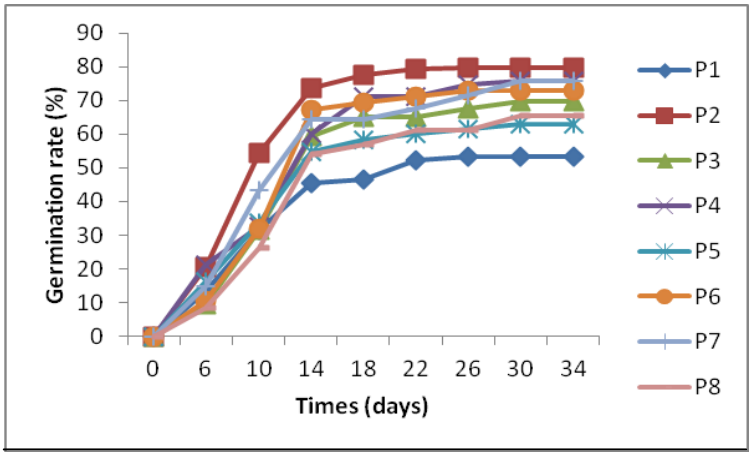

Figure 1. Daily variation of germination rate M.*oleifera* field.
P1, P2, P8 ... Corresponds to the rank occupied by the seed in the pod.

influence on its weight and the maximum is reached in the middle zone (4-6) (Table 1). This variability in seed weight following the seed position is evidenced by a significant difference (0.0000<0.001). The average weight of 8 seeds' pods was 263.21 ± 22.5 mg. A Duncan Multiple ranks test showed two groups of seeds (P< 0.05).

The first group consists of seeds with low weight. These are the seeds from positions 1, 8, 2, 3 and 7. The second group is constituted of higher weight seeds. These are the seeds from the 5, 4 and 6 positions.

Seed germination

The first seeds began to germinate 6 days after sowing. The germination rate is a function of the position of the seed in the pod ($p < 0.05$). Regarding the evolution of germination, 6 days after sowing positions 4 (21.18 ± 1.3) and 2 (20.48 ± 1) exhibit the highest rate (Figure 1). The lowest rates are observed in the positions 8 (8.33 ± 3.2) and 3 (9.33 ± 2.6). At 10th day after sowing position 2 seeds double their germination rate while position 7 seeds triple their germination rate. Seeds from positions 3, 6 and 8 have also tripled their germination at this time. Two weeks after sowing all positions' seeds have nearly doubled their rate of germination. After the 18th day, the evolution of germination slowed considerably. At day 22, all the seeds of the position P2 completed their germination. Seed from positions 1, 6 ceased to germinate after the 26th day while those from positions 3, 4, 5, 7 and 8 reached the 30th day.

The evolution of the germination time showed a significant difference ($p \leq 0.00$ 1). The germination rate is similar between the 18th and 22th day, in one hand, and between the 26th and the 30th day, in the other hand. The 30th day after sowing , it ranged from 54.10 ± 14.31% in the first position of the pod proximal portion to 79.58 ± 7.28% in the second position of the proximal pole of pod. The average germination percentage was 69.66 ± 12.12% (Table 2).

Table 2. Percentage of germination

Rank seed in pod	Germination (%)
P1	54.10±14.31a
P5	64.85±15.34ab
P8	65.27±8.67ab
P3	69.74±5.42ab
P6	72.94±10.04ab
P7	75.57±15.51b
P4	75.92±7.32b
P2	79.58±7.28b
Average	69.66±12,12

Means followed by the same letter are not statistically different.
P1, P2, P8 ...Corresponds to the rank occupied by the seed in the pod

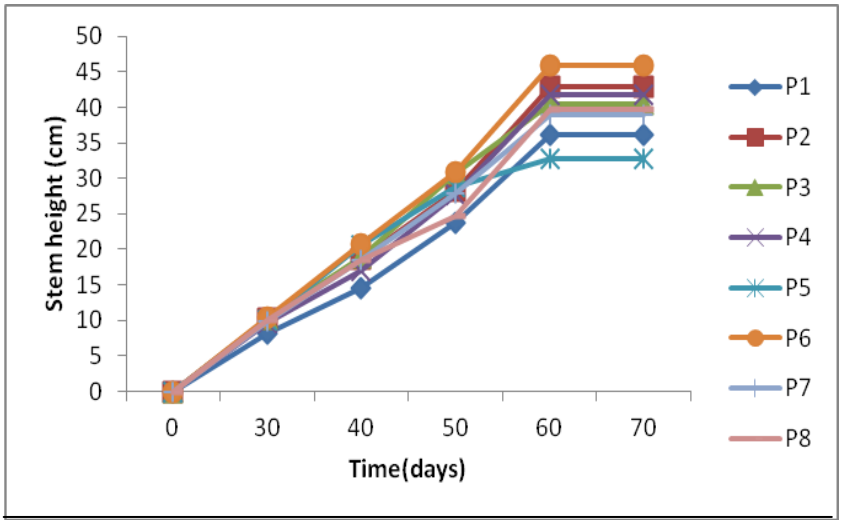

Figure 2. Daily variation of vertical seedlings growth M.*oleifera* in field.
P1, P2, P8 ....Corresponds to the rank occupied by the seed in the pod.

Initial seedling growth

Seedling growth in height is 39.79 ± 3.81 cm on average, 60 days after germination. The average height varied from 32.57 ± 5.46 cm for seedlings derived from the germination of seeds of the middle position (P5) to 45.92 ± 4.35 cm for seedlings derived from the germination of seeds of the sixth position from the distal pod end (Figure 2). Seedlings from seeds located on the 2nd, 3rd and 4th positions follow had average heights of 42.83 cm, 41.82 cm and 40.44 cm respectively. The P8 and P7 positions (distal pod end) and P1 (proximal end) showed low seedling height values: 39.68 cm, 38.91 cm and 36.1 cm, respectively. This difference is not significant (P> 0.05).

Regarding the evolution of growth, after 30 days the average elongation of seedlings was 9.86 ± 0.68 cm and 40 days after sowing it reached 18.42 ± 1.92 cm. After 50 days, the seedling height of 28.43 ± 2.53 cm was obtained and at 60th day, it was 39.79 ± 3.81 cm. The evolution of this growth over time showed a significant difference (0.0000<0.001).

Concerning the radial growth, the average diameter of the seedlings stems was 0.85 ± 0.15 cm at the 60th day after germination. It ranged from 0.78 ± 0.21 cm for the median position 5 to 0.92 ± 0.16 cm for the second position, at the distal end (Figure 3). Seedlings stems of positions P6, P8, P3, P7 , P1 and P4 respectively followed with averages of 0.2 ± 0.05 cm, 0.89 ± 0.21 cm, 0.86 ± 0.05 cm, 0.85 ± 0.09 cm, 0.4 cm ± 0.14 and 0.82 ± 0.24 cm. The curve shows that the position 2 had the better radial growth. This difference between positions was not significant (P>0 .05).

The diameter of the seedlings is independent of the positions of the seeds in the pod after two months of growth. The temporal evolution of the radial growth showed a significant difference (p < 0.0000) between the periods of growth.

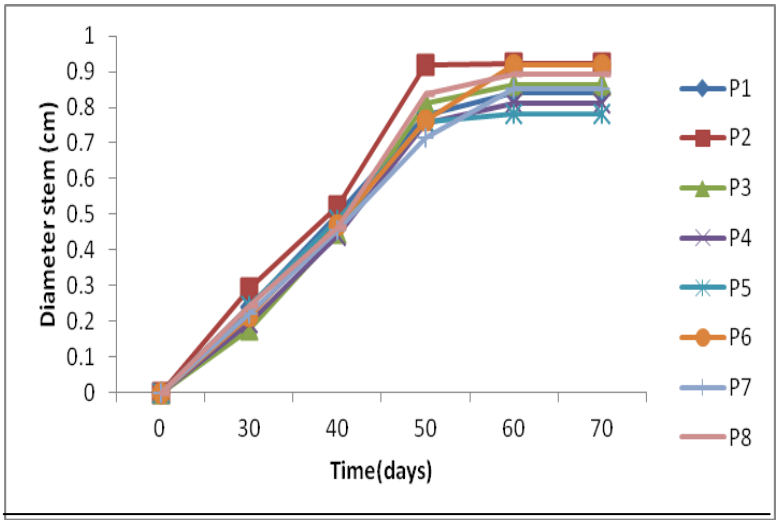

Figure 3. Daily variation of radial growth of seedlings M.*oleifera* in field. P1, P2, P8 ....Corresponds to the rank occupied by the seed in the pod.

Table 3. Number of leaves and branches 60 days after sowing

Rank seedling	Number of leaves	Number of axe air	Number of ramifications
P7	11.7±2.1	1.5	3,66±1.66
P1	12.56±2.68	1.5	6±5.88
P8	12.88±3.88	1	10±8.6
P4	12.89±1.39	3	8±6.16
P3	12.91±2.57	1.5	5.66±3.29
P6	13.10±3.49	1.5	6.33±3.86
P2	13.69±3.02	1	8±6.16
P5	18.44±6.25	2	5.33±3.29
Average	**13.52±3.52**	**1.65±0.64**	**6.62±1.92**

Number of leaves and branches

At day 60, the average number of leaves per seedling was 13.52 ± 3.52. Extreme values belong to the seedlings of the following positions: 7 (11.7 ± 2.1), second near the proximal end and position P5 (18.44 ± 6.25) (Table 3). Plants derived from seeds of positions 1, 8, 4 and 3 had respectively 12.56 ± 2.68, 12.88 ± 3.88, 12.89 ± 1.39 and 12 ± 2.57 91 leaves while those from seeds of positions 6 and 2 had 13.10 ± 3.49 and 13.69 ± 3.02 leaves, respectively. The analysis of variance did not show a significant difference between the numbers of leaves among the position of seeds in the pod (P> 0.05). There was a significant increase in number of leaves for position 3 at 40th day, but this trend was reversed after 10 days. Analysis of variance showed that the number of leaves varied significantly over time ($p < 0.0000$).

Concerning the number of ports, plants from positions 2 and 8 are single-stemmed. The plants from positions 1, 3, 5 and 7 showed two air lines (Table 3). Position P5 plants had twice two air lines, and position 4 is the only one to get plants with three axes (one time). The analysis of variance did not show significant difference (P> 0.05).

In terms of branching, the lowest number of branching is observed for position P7 (3.66 ± 1.66) and the largest number belongs to the proximal position (P8), 10 ± 8, 64. Positions 2 and 4 had higher number of branching (8 ± 6.16) than the overall average (6, 62 ± 1, 92). Positions 1, 3, 5 and 6 had lower numbers of branches than the average, they were respectively 6 ± 5.88, 5.66 ± 3.29, 5.33 ± 3.29 and 6, 33 ± 3. The difference observed is not significantly dependent on the position of the seed in the pod (P> 0.05).

DISCUSSION

About the seed weight, the fact that the heaviest seeds are localized in the central region of the pod can be explained by the trophic and structural assumptions made by other authors (Nkongmeneck et *al*, 1996; Mapongmetsem et *al*, 2004):

- according to the trophic hypothesis, there would be a competition between seeds in the pod for receiving nutrients provided during fruit set, seeds of the middle zone are the most appropriate;

- concerning the structural hypothesis, the configuration of the pod is such that the seeds of the middle zone have more space to grow than those of distal and proximal poles. It emerges from this analysis that the seeds situated in the middle part of the pod are heavier than those at the ends. Similar results were obtained on *Parkia biglobosa* (Mapongmetsem et *al*., 2004).

In terms of germination, work on annual Medicago and those of Taylor and Palmer on *Trifolium subterraneum* (IbnTattou 1981 cit. Nkongmeneck et *al*., 1996) showed sequential germination of seeds of a pod. The results of this work have shown that the seeds of the distal end germinate less than the others. The germination rate of 69.66 ± 12.12% is contrary to the results obtained by Ofoh *et al.* (2010) in south-eastern Nigeria, where 50 % of the seeds had germinated at fourth day.

Considering the average percentages of germination of the same plant in India, West Africa and Cameroon (Palada and Chang, 2003; Tedonkeng Pamo et al. 2004; Saint Sauveur, 2010), they were all above those of this work. This difference would be due to the different pretreatments (soaking, scarification) applied to seeds or to differences in agro -ecological zones. The seeds of this investigation have not undergone any pretreatment and the ground has not been mulched. However, the average germination rate of 69.66 ± 12.12% is higher than that obtained by Jahn (2003) in Sudan, in similar weather conditions. The germination rate obtained by this author was 40% for *Moringa oleifera* and 45% for *Moringa stenopetala*. Seeds at position 2 in the pod have a high germination rate (79.51 ± 4.2), approximately double of that obtained in Sudan (40%). The germination rate of fresh seeds is around 80% and may fall to about 50% after 12 months of storage (Bosch *et al*., 2011).

The germination rate of seeds of *Tetrapleura tetraptera* and *Parkia biglobosa* is also influenced by the position of the seed in the pod (Nkongmeneck *et al*., 1996 and Mapongmetsem et al., 2004).

The vertical growth of seedlings is low compared to those of Ofoh *et al*. (2010) in south-eastern Nigeria 7 weeks after sowing, as seedling height varied between 46 cm and 69 cm. The position of the seed in the pod of *Moringa oleifera* does not affect significantly seedling growth 60 days after sowing. The spacing between seedlings gives the opposite results. Indeed, Amaglo *et al*. (2006) showed that the smaller spacing (5 x 5cm) generates the largest increase in the size of plants in Ghana. At the 60th day, daily average elongation of the stalk was 0.66 cm. This growth is much higher than that of ecotypes of Togo where this growth was 0.29 cm per day at 22nd day (Kokou, 2001). Plants reached the size of 15-25 cm in 2 months (Bosch *et al.,* 2011). The decrease in the number of leaves during growth is not a new phenomenon in *Moringa oleifera*. Similar results were obtained in Ghana by Amaglo *et al*. (2006). The lower number of leaves is attributed to the competition between the seedlings.

CONCLUSION

The seeds of the middle part of *M. oleifera* pod were heavier than those of the extreme positions. The germination of seed was influenced by its position in the pod. The average germination rate was 69.66 ± 12.12%. The vertical and radial growth were not affected by the position of the seed in the pod during 60 days. The number of leaves, branches and ports were also influenced by the position of the seed in the fruit. The evolution of the initial seedling growth over time showed significant differences.

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