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Environmentally induced variation in germination percentage and energy of naked caryopses *of Loxodera ledermannii* (Pilger) W.D. Clayton ex Launert in subhumid Benin (West Africa)

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Kindomihou Missiako Valentin, Romain Lucas Glele Kakai, Achille Ephrem Assogbadjo, Roland Ahouelete Yaovi Holou, Brice Augustin Sinsin: Environmentally induced variation in germination percentage and energy of naked caryopses *of Loxodera ledermannii* (Pilger) W.D. Clayton ex Launert in subhumid Benin (West Africa)

ABSTRACT

This study investigated the conditions for maximizing germination of *Loxodera ledermannii,* an earlier and nutritional tropical fodder grass species. We examined the correlation of percentage germination with seed container, substrate, sowing depth, methods and date of sowing. Naked caryopses of *L. ledermannii* were subjected to various growth conditions. Results showed that percentage germination depended on growth conditions ($P = 0.001$) and energy of germination ($P = 0.0001$). Effects of the seed container, substrate, sowing depth, methods and sowing period were significant ($P < 0.05$). Refining of the substrate improved the percentage germination. Seed container coverage and sowing depth substrates increased the energy of germination ($P = 0.000$); their magnitudes were dependent on substrate types, being average for sterilized soil (56%) and higher with blotting paper (84.9%) and refined soil (121%). Highest germination energy was recorded for covered and deeper seed containers (< 5 days). Tamping increases notably the caryopses germination and the plant density through the growth period. Further studies are needed to well characterize constitutive variation of these traits.

Key words: Germination percentage, energy, caryopses, *Loxodera ledermannii*, growth conditions.

Introduction

Perennial pasture species are essential for the long-term economic and environmental sustainability of Sudanian farming systems in Benin. Because their roots are deeper than those of most annual plant species, their water uptake and use throughout the year is improved [8]. Therefore, such species can provide critical ground cover, reducing erosion while providing high-quality livestock feed. They can also assist in the management of dryland salinity and soil acidity [30].

About 70 native pastures have been identified and described in Benin [19,22,34,32,17,31,14]. Most of the northern pastures are dominated by *Loxodera ledermannii, Andropogon* and *Brachiaria* species [33]. These perennial pasture species are widely used by livestock and mixed farmers to provide high-quality feed on a year-round basis [22]. As the natural availability of forage has become a major constraint to ruminant production [28,6], *L. ledermannii* might offer a useful fodder option, as it grows well in the dry seasons, flowers early and produces good-quality fodder [34,35].

Because this species is strongly grazed by ruminants, it is also favoured by stockbreeders and pastors [34-35]. *L. ledermannii* has spread naturally on the Sudanian and Sudano–Sahelian savanna in the following countries: Nigeria, Uganda, Cameroon, Benin and Niger [15,37,34]. It has also recently been

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identified in the Guinea–Sudanian and Sudano–Guinean zones. The species has been threatened by desertification, overgrazing and climate change [31]. Apart from studies of its spread area, caryopses and morphological characteristics [18], no published information exists on the establishment of the species.

The domestication of this species will certainly be important in order to save a potentially important fodder crop and to increase its availability. To be able to propagate the species through domestication, a better understanding is needed of the optimum conditions for its germination. Previously, suitable temperature and light environments, water inhibition and a cold pre-treatment were cited as species-specific prerequisites to ensure the germination of grasses during reclamation work [3].

Imbibed water quantity, stratification, light and harvest time have also been found to affect seed germination in the dune-building grass *Leymus arenarius (L)* Hocht [13] and *Echinochloa crus-galli* (L) [P.Beauv.] [20]. Appropriate experimental conditions for germination clearly should reflect the environmental conditions of the microhabitat in the field and may act as phenological signals for germination.

Parihar and Pathak's [26] study of the flowering phenology and seed biology of selected tropical perennial grasses revealed that seed germination was very low (depending on percentage seed set), whereas germination in caryopses was much higher (up to 92%).

As constitutive and ecological adaptation of caryopses has been proven with a few temperate grass species such as *Chenopodium* spp. and *Rumex* sp. [27], we hypothesized that this may also be true with *L. ledermannii.* To this end, we investigated the effects of environmental factors on the germination rate and energy of the caryopses of *L. ledermannii*, sward establishment. Our main objectives were: (i) to determine the germination abilities of naked caryopses *of L. ledermannii* and (ii) to determine the optimal sowing depth.

Materials and Methods

Study area:

The experiments were carried out from March to June 2003 at the Laboratory of Applied Ecology and from May to October 2004 at the experimental station of the Faculty of Agronomic Sciences of Abomey Calavi University (Benin Republic). The perimeter is located in the subequatorial zone (latitude 6–7° N, and longitude 2–3^{o} E). The region experiences a climate with a bimodal rainfall. There are two dry seasons – mid-July to mid-September, and mid-November to mid-March. In 2003, the annual rainfall averaged 1300 mm, including 915.8 mm for the long rainy season. Annual temperatures ranged from 25.9°C to 29.1°C. There were 2300 hours of annual sunshine and relative humidity ranged from 30 to 90%; the potential evapotranspiration (ETP) of Penman rate in the period was 1650 mm [1]. The variations in climatic parameters from March to October during the years 1975 to 2004 are presented in Table 1. The soil in the garden of FSA/UAC is ferralitic, relatively acid, with a fragile structure, poor in exchangeable bases, nitrogen and phosphorus, but with appreciable sodium concentration; C/N mass ratio is 10.6. The vegetation of the experimental site environment is the prairie type of *Panicum maximum* C1, and soil ranges from alluvial to sandy of the littoral Table 2, [18].

Plant materials and experimental design:

A collection of caryopses of *L. ledermannii* (Pilger) W.D. Clayton ex Launert, collected from a full Sudanian field of northern Benin (Tanguieta-Natitingou: 10–12°N, 0.16–2°E; Nikki-Kalale: 9.5–10°N, 3–3.58°E), was thrashed in a mortar and pestle, and a dissection kit. These naked caryopses were conserved at 15–25°C and 40–90% relative humidity. The naked caryopses were used at a rate of 30 caryopses per seedling. Temperature in the Laboratory ranged from 9 to 13°C.

Laboratory trials: To study the effects of the seed container on percentage germination, three types of substrate were compared – blotting paper (BP – Weifa Co., Ltd.), fine soil (FS) from Fairfield, Cotonou, and sterilized soil (SS). (The soil was sterilized by heating at 65°C for 30 minutes). The BP substrate was placed in molded containers (V = 3695 cm^3; length = 36.5 cm; width = 22.5 cm; depth = 4.5 cm); the FS was placed in tubular vats (V = 2669 cm^3: diameter = 20 cm; height = 8.5 cm); and the SS was placed in conical pots (V = 175 cm^3: height = 6.3 cm; lower diameter = 5 cm; upper diameter = 6.8 cm). Both uncovered and seed containers covered with glass plates were prepared. All the seed containers were watered (30 ml in the molded containers, 20 ml in the tubular vats and 15 ml in the conical pots) before being planted with 30 naked caryopses of *L. ledermannii*. About 10 ml of water was provided every 3 days until day 45. The whole design was replicated three times and the resulting combinations of three substrates and three types of seed container were arranged in complete randomized blocks in the laboratory. Two sowing depths were tested – at 1 cm and 3 cm; the growing period effect was also observed on days 15 and 30 of the month.

Field trials: In the field, 100 m^2 was divided into subplots sized 15 m^2 (5 m × 3 m) to test the effects of the planting depth and method on percentage germination. The sowing in broadcast was compared to that in-line. In-line sowings were undertaken in furrows opened at the depths of 1 cm, 3 cm and 5

cm; a slight soil tamping was applied and the seeds were spaced out at 50 cm × 30 cm. Controls and treatments were compared. Among the three in-line depths, only 3 cm-depth was considered in the test of seedling planting method and growth period on germination rate.

Parameters measured:

The time (days' number) between the sowing and the appearance of the first shoots, the number of days between sowing and the maximum germination, and the number of caryopses that germinated were recorded. The number of days between sowing and the appearance of the first shoots was used as a proxy for the germination energy of the caryopses. Percentage germination (GR) was calculated as follows:

GR=NFC/USQ × 100%; with NFC = number of fertile caryopses; USQ = number of plants produced per total caryopses used. As used here, the germination energy is the time (number of days) at which 50 percent of the total germination has been attained.

Statistical analysis:

Statistical analyses were performed using SAS (SAS, Institute Inc., Cary, NC, 27513-2414 USA). The effects of the substrates, sowing depths in covered and non-covered treatments, growth periods and sowing methods on the germination energy, the germination rate, were compared in a 3-way analysis of variance (ANOVA) using the general linear mixed models procedure (Proc GLM). Factors were the types of seed containers, substrates and replicates, seed container and sowing depth, sowing depth and growth period, and sowing method and growth period. A one-way ANOVA was used to compare the percentage of germination of the naked caryopses of *L. ledermannii* with the different groups of factors and treatments. Significant differences were identified using Student Newman–Keuls tests as *post-hoc* procedures (at $\alpha = 0.05$).

Results:

Effects of seed container and substrate type on the germination rate:

The germination rate of naked caryopses of *L. ledermannii* ranged from 21.7% to 93.3%, depending on the type of seed container and whether the seeds were covered or uncovered (Figure 1). These caryopses showed highly significant differences for percentage germination (Table 3). The covered seed containers had much higher values (> 41.6%), whereas the non-covered generally had lower values (< 60%).

Apart from the seed container effect, the effect of the substrate was also significant (Table 3). The germination rate of the caryopses generally increased significantly in response to the substrate refinement ($P < 0.0001$). The germination rate increased significantly with the depth of substrate coverage in all cases (Figure 1). The magnitude ranged from 58.2% to 93.3%. The caryopses planted on the blotting paper substrate had much lower germination rates (<41.7%) than those with other substrates. The germination rate for caryopses planted in the sterilized soil was generally higher (≥ 59%) and ranked first in all treatments. In addition, the time for the first appearance of shoots and for achieving maximum germination varied significantly from a substrate to another (Table 4). Generally, the first appearance of shoots after sowing did not exceed 7 days (Tables 3 & 4). The time taken to germinate was significant in respect to the substrate type ($P < 0.001$). Fine sand had the lowest value (≈ 4 days) and the blotting paper had the highest (≈ 6 days).

Effect of seed container and substrate type on the energy of germination:

The germination energy of the caryopses ranged from 5 to 10 days, depending on the seed container and substrate types. There was a significant difference between the germination energies of the caryopses in the different seed containers (Table 3). The uncovered seed containers had much lower values for germination energies (5 days for 59% of the caryopses), whereas the covered seed containers generally had higher values (8 days for 93% of the caryopses). The sterilized soil generally had the best germination energy, ranging from 5 to 8 days (Table 3). Pair-wise, the treatments showed a significant increase in germination energy in only two out of six cases. The increases were 52% and 56% according to the seed container and substrate type, respectively. There was a highly significant seed container × substrate interaction ($P = 0.002$), indicating that germination energy generally increased in response to covering the substrates. This change was significant in all cases. The effect varied strongly depending on substrate type, being average for sterilized soil (56%) and largest for fine sand and blotting paper (121% and 84.9%, respectively).

Effects of seed container and sowing depth on the germination rate:

The germination rate of the naked caryopses ranged from 41.2% to 56.7%, depending on the seed container and the depth of planting. Naked caryopses of *L. ledermannii* showed highly significant differences for their rate of germination (Table 4). The deeper seedlings (≥ 3 cm) had much higher values (> 56.5%), whereas the less deeply planted seedlings (≤ 1 cm) generally had lower values (< 41.5%) (Table 4). In addition, the sowing depth

× covered seed container interaction is highly significant for the germination rate of naked caryopses ($P < 0.0001$). This result indicated that the germination rate generally depended on the sowing depth. For the covered containers, there appeared to be a limit to the sowing depth after which the seeds would no longer germinate. In the uncovered containers, this was not the case. The 37% increase in the germination percentage for seeds in covered containers was significant. There was a 10% decrease in the germination rate for seeds in uncovered containers. Otherwise, the germination energy was the best (< 5 days) for the containers with the more deeply covered seeds (Table 4). The last new shoots of caryopses planted at a shallow depth in covered containers appeared about 4 days before those planted more deeply, indicating that the ability to germinate and the energy of naked caryopses generally decreased with increased sowing depth. However, this finding was not true with the uncovered seed containers.

Effect of sowing methods and period on the germination rates:

The sowing methods had a significant effect on the germination rates of naked caryopses in the field experiments. The density of the plants ranged from 9 to 28 per m^2 (Table 5a and table 5b). Differences were highly significant. Seeds that had been broadcast showed a much lower plant density (< 10 plants/m^2) compared to the other methods of sowing. The in-line sowing at a depth of 3 cm (with a backfilling and tamping of the soil) generally had higher values (> 28 plants/m^2). Apart from the sowing methods, the period in which these methods were used significantly affected the density of the plants. First, the comparison of treatment periods (15 days, 30 days) (Table 5a and table 5b) showed a significant increase in the plants density in all cases – an increase of 31–50%.

Second, the intra-specific comparison of methods of sowing (broadcasting + tamping and in-line sowing at 3 cm + tamping) with their controls (broadcastings and in-line sowing at 3 cm-depth) (Table 5b) showed a significant increase in the plants density – between 44% and 85%. And, finally, the comparison among treatments also showed a significant increase in plant density between broadcasting and in-line sowing at a depth of 3 cm-depth; the increase being in the range 18–44%.

Discussion:

Naked caryopses of *L. ledermannii* were subjected to different growth conditions and the percentage germination and energy of germination were analyzed. In general, growth conditions significantly affected the percentage germination and the energy of germination. Our results showed the influence of seed container type, substrate, sowing depth and method, and sowing period on the germination rate of the naked caryopses. Moreover, the energy of germination was similarly affected by those variables. These results suggest that the germination processes of naked caryopses of *L. ledermannii* significantly depend on growth conditions. The hypothesis that the percentage germination and energy of germination of caryopses are the result of caryopses' adaptation to ecological conditions has rarely been tested. In contrast, El Hassani [11] found that, for most cultivated and invasive species, which spread by means of seeds 7–30 days after germination, the embryos and plants depend completely, except for water, on the nutrient reserves stocked in the endosperm. Moreover, the difference in germination abilities may be related to differences in the energy of germination, physiological maturity, and seed harvest and conservation conditions.

Effect of environmental factors:

The hypothesis that seed germination is environmentally inducible is not well documented in tropical grass species. Our results showed that the germination rate of caryopses strictly varies across environmental factors. Water, temperature and irradiance are known to be crucial factors that affect seed germination. The data we report are noteworthy. In general, the environmental factors that we tested (i.e. recovery rate, humidity, seedling depth, sowing method, growth period) for their effect on percentage germination and the germination energy were significant (Tables 3, 4). The factors that did not have a significant effect on percentage germination and the germination energy were seed container type and the amount of shade (i.e. whether the containers were covered or not). The caryopses responded in similar ways to substrate, degree of shading, growth period, depth and methods of sowing.

Relationship between soil conditions and caryopses' germination rates:

Covering to a depth of 3 cm resulted in the highest percentage germination and the best energy of germination, whereas covering to a depth of 1 cm produced the lowest percentage germination and the longest period for the first shoots to appear. This provides evidence that germination of caryopses of *L. ledermannii* depend on temperature and soil moisture. In addition, those caryopses planted 3 cm deep showed the lowest number of days to the last germination, whereas those planted 1 cm deep showed the highest. These results suggest that seeds that are not superficially sown germinate easily due to the temperature and humidity and also the required distance that they need to cross before emerging from the soil. *L. ledermannii* might join such perennial

grass species as *Stipa tenacissima L.* and *Lygeum spartum L.*, which grow in arid conditions. Like *Ammophila arenaria* caryopses grown in the laboratory [2], the caryopses of these species have an optimal germination in the temperature range 15–25°C. It is known that seeds dried with less than 15% humidity [23,36] and grass seeds thrive by being sown at the start of the rainy seasons [5]. Planting at a depth of 1 cm may prove fatal. It is, therefore, essential that the caryopses are placed on top of freshly prepared and rolled soil so that the seed settles into the soil in close contact with the soil particle following some rain [4]. Our results showed that the long time taken for the caryopses to germinate might be related to the intrinsic performance of the seeds. Generally, with grass seed embryos, the coleoptiles shrivel up in the ground when the seeds are sown deeply and the plant dies because the organ lacks firmness [12] Conversely, we found a higher percentage of germination in response to deeper sowing. Previously, Manske [24] had found that the seed reproductive phase, which is triggered primarily by photoperiod, can also be slightly modified by temperature and rain.

The naked caryopses may show a lower germination rate in particular conditions. Because the caryopses are generally resistant, it may be possible that the embryo of *L. ledermannii* was destroyed, as is the case with *Phleum pratense* L., a well-known temperate grass species [12]. Apart from morphological parameters, which have been previously elucidated [18], no data exist for the parasitism and purity of *L. ledermannii* seeds. These seeds might be immature or harvested very early, or badly conserved or dormant, which can affect about 0–70% of grains [12]. Part of the variability in germination rate might be related to an extra environmental investment. The much higher germination rate in sterilized soils suggests that microbial activity affects the germination of caryopses. Microorganism contamination might either occur in soils that were not sterilized or microorganisms might interact with blotting paper. This is compounded with the effects of aeration – that is, depth, humidity, temperature, pH and exchangeable Ca presence. Furthermore, Chiang and Soudi [9] hypothesised that soil aeration might affect seed germination, as it ranges from 0.7 to 1 kg per square meter and from 10 to 15 cm in the superficial soil layer. However, our experiment was conducted within depths of 1 cm and 3 cm; in hot weather, topsoil temperatures rise and seed desiccation may occur. Changes in shallow soil sowing depths are related to the higher presence of algae in wetland soils and horizons and where sunbeams are available for the oxygen capture of photolithotrophs [9]. In addition, temperatures drop when it is raining and seeds consequently get soaked. Parts of the seed might perish if the conditions are too extreme or when they change too abruptly.

Effects of seed containers on caryopses'germination rates:

Different temperatures and moisture regimes in different seed beds presumably elicit different patterns of physiological response from the developing embryo [10,12,29,7]. In our study, this is highlighted by the fact that embryos from covered seed containers grew more rapidly in larger numbers than embryos from the non-covered seed containers (table 3). Humidity and temperature reserves were mobilized in the scuttelum during the germination process. Consequently, only a small energy reserve remained for sustainable germination processes. Our results showed that 50% of *L. ledermannii* caryopses germinated between 2 and 3 weeks after sowing. In addition, many caryopses that took only a few days for the first appearance of shoots to occur also showed a low percentage of germination. This is consistent with the general trend [12]. Moreover, the extraction of such caryopses by rough manipulation in a pestle and mortar may affect the percentage of germination, improving the energy of germination. On the other hand, differences in germination can be related to differences in germination energy, physiological maturity, and crop conditions and seed conservation. Therefore, lack of seed rising might result from deteriorated embryos caused by mechanical thermal shock [11]. For example, incomplete development of the embryo might relate to the amount of light received. Therefore, manipulating temperatures and scarification in order to remove tegument inhibition could cause caryopses to rise.

Effect of substrate type on caryopses' germination rate:

Temperatures inside a substrate are usually lower, on average, and less variable near the bottom than at the top, and water potential typically increases with depth in the soil [10,6,36]. Our laboratory experiment results showed a low percentage of germination of the naked caryopses, depending on the type of substrate – that is, non-optimal temperatures, a bad water supply and a highly compacted soil. Every pot and plot was consistently watered without compacted soil. The covered treatments showed a significant difference, with low percentage germination occurring mainly in the laboratory. For Boudet [5], the sowing of small seeds was carried out on a sharply crumbled soil and tamped to avoid soil erosion. In studies by Boonman [4], (i) light coastal sands develop high soil temperatures at the end of the dry season and at the beginning of the rainy season (direct sunshine and dry, windy conditions may kill seedlings); (ii) black clays (e.g. vertisols) shrink, leaving cracks in the drought-ridden soil – this causes the young roots to break off and swelling occurs when it starts to rain;

therefore, pasture establishment is very difficult.

The time taken for growth to occur may also be attributed to the environmental conditions. We observed a significant increase in the number of plants per square meter in seedlings from the 15^{th} to the 30^{th} day ($P < 0.001$). These findings suggest that naked caryopses respond to change in soil temperature and moisture, and these factors play a significant part in germination percentage; this has been corroborated by previous studies. Indeed, Boonman [4] and Sounon et al. [36] observed that the rate of germination declined as the rainy season advanced. At the onset of the rainy season, the soil was warmer and showed a better structure. Soil temperatures decline rapidly as the rainy season proceeds.

Effect of sowing methods on caryopses'germination rate:

Variations in plant density depend on variation in germination of naked caryopses in the field which might result from developmental plasticity. Our results show that higher plant density occur when tamping is applied to the soil where seedlings have been planted, suggesting that tamping creates the best ambient conditions for caryopses germination. Consequently, non-tamping caryopses had low plant density (Table 5b). Additionally, 63% and 71% of the variations in germination percentage of naked caryopses were induced by tamping in broadcasting and in-line sowing at a depth of 3 cm (Table 5b). This is not the case of temperate caryopses as the seedling emergence decreased with increasing depth of sowing. Indeed, Maun and Riach [25] observed that the highest germination rate of caryopses and the emergence of seedlings of the temperate grass *Calamovilfa longifolia* (Hook) Scribn. occurred from a depth of 1–2 cm in Canada. They found that the maximum depth of sand from which a seedling can emerge is about 8 cm. But, the tropical grass species have been less documented. Kindomihou et al. [18] have characterized caryopses of *L. ledermannii* (i.e. 4.5 mm long and 1.0 mm wide) enclosed in lemma and palea. In this study, we have tested only three sowing depths have been tested: (i) sowing at 1 cm-depth; (ii) sowing at 3 cm-depth and (iii) sowing at 5 cm-depth (data partly treated). More sowing depths are needed to be tested. Nevertheless, for pasture purposes, broadcasting is customary, resulted in low seed rates, because the risk of the seeds scorching in the soil are small as the tropical grass seeds are very slight, with 1000-seed (spikelet) weight of usually less than 500 mg [4]. The most common method of broadcasting for grass seeds is to bury the seeds deeply in the furrow, ensuring that sufficient overlapping occurs. In addition, seeds establish faster when sown in rows, which enable a more rapid establishment. Boonman [4] observed good contact between seeds and the soil when seeds are sown in freshly drawn furrows.

Specific adaptation:

We observed that caryopses of *L. ledermannii* take 8–12 days to germinate depending on the substrate used (Table 3), and 37–41 days depending on the sowing depth, with the best value (>37.5) being achieved in covered, deeply sown seedlings (Table 4). These results suggest that the establishment of *L. ledermannii* pasture depends on the environment. First, the speed or vigour with which the caryopses germinate depends on the species and might be poorly correlated with seed weight. Indeed, Boonman [4] proved that, under good conditions, Rhodes grass (*Eustachus paspaloides*) seeds usually take 8–10 days to emerge, and grow rapidly some weeks after. Second, dormancy is affected by temperature, so that any method that manipulates temperatures is likely to produce an effect on dormancy. The naked caryopses of *L. ledermannii* have shown different morphological measurements (length, width, thickness, weight) [18], that all might affect the germination process. These results suggest a somatic polymorphism, i.e the seeds that have been used belong to a population of different morphology and behaviour. Similar processes were reported to occur in other perennial herbaceous species, such as *Chenopodium* spp. and *Rumex* spp., the seeds of which have different characteristics in dormancy; these characteristics explain why their seed populations showed the same germination rate at different times of the year [27]. Seeds might also have been produced in unfavourable conditions (i.e. dryness, extreme temperature), as it has been reported that many dormant seeds in agricultural lands provide vapour raised from CO_2 and low temperatures in O_2 in soils [27]. Moreover, seedlings develop favourable moisture and temperature conditions in reduced competition and when resources are easily available to the seedlings for growth [24].

As conclusion, the germination of the caryopses of *L. ledermannii* depends on environmental factors. The percentage and energy of germination might be phenotypic in response to environmental factors. Caryopses might be sown 3 cm deeper and may be subjected to slight tamping. Further studies are needed to appreciate whether these abilities are also constitutive of somatic polymorphism. Moreover, adaptive dormancy and growth speed needed to be characterized for this earlier forage grass species.

Table 1: Climatic data for 1975-2004

	RHMi (%)		RHMa (%)		RHM (%)		Tmi (ºC)		Tma (ºC)		TM (ºC)		P (mm)		IR (hours)	
Months	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
J	62.2	11.3	93.7	2.4	77.5	6.5	24.1	1.3	32.3	3.6	28.3	2.0	12.7	21.5	209.5	27.9
F	63.7	6.1	91.8	2.1	77.8	3.4	25.6	0.8	32.5	0.8	28.8	0.6	41.5	40.4	208.3	21.5
M	67.5	2.5	90.5	1.9	78.6	1.6	26.2	0.6	32.6	0.6	29.6	0.5	76.8	61.4	219.3	22.0
A	68.8	2.7	90.9	2.1	79.6	1.9	26.4	0.8	31.9	0.6	28.9	0.6	132.7	71.0	219.1	19.3
M	70.5	1.9	93.3	1.7	81.7	1.5	25.5	0.5	31.8	0.6	28.4	0.5	205.5	83.9	210.7	20.2
J	74.7	1.8	94.5	1.4	84.1	1.3	24.7	0.4	29.8	0.5	26.9	0.4	308.9	143.9	152.2	27.3
J	76.9	1.5	92.9	1.7	84.7	1.4	23.8	0.5	28.8	0.7	25.9	0.6	123.5	112.7	147.3	31.5
A	75.8	1.7	93.6	1.6	84.3	1.3	23.6	0.5	27.9	0.5	25.8	0.5	55.4	79.5	148.4	28.3
S	75.2	1.9	93.4	1.4	84.8	1.2	23.9	0.4	28.7	0.5	26.4	0.4	114.2	82.3	161.2	23.5
O	72.7	1.8	93.9	1.7	82.8	1.3	24.5	0.5	29.9	0.6	27.2	0.5	130.7	76.7	209.6	15.3
N	67.9	2.4	93.8	1.9	80.7	2.1	24.6	0.6	32.1	0.6	27.9	0.5	37.5	27.8	244.3	15.4
D	63.4	6.1	94.5	1.9	78.5	3.7	24.3	0.8	31.7	0.7	27.6	0.6	19.7	26.2	219.7	25.1

Note. RHMi: relative humidity minima; RHMa: relative humidity maxima; RHM: mean relative humidity; Tmi: temperature minima; Tma: temperature maxima; TM: mean temperature; P: Precipitation; IR: Sunny period. M: mean; SD: standard deviation.

Table 2: Characteristics of the soil of experimentation garden of the Faculty of Agronomic Sciences where the field trials were performed.

Granulometry (%)	0 - 2 mm	10.4
	2 - 20 mm	2.7
	20 - 50 mm	1.9
	50 - 200 mm	19.7
	200 - 2000 mm	65.2
pH (H_2O)		6.4
pH (KCl)		5.4
Ca++ ech.meq/100g		2.58
Mg++ ech.meq/100g		1.32
K+ ech.meq/100g		0.47
Na+ ech.meq/100g		0.10
CEC meq/100g		6.23
P_2O_5 p.p.m		6.89
N (%)		0.077
Organic matter (%)		1.40
C (%)		0.81
C/N		10.60

Source: Kindomihou *et al.* (2009)

Table 3: Effect of seed container and substrate type on the germination percentage (GerP) and respectively on the time (number of days) to the first appearance of shoots (TFAS), the maximum germination (TMAG) or germination energy in the laboratory trials. The adjusted means of the traits and the results of 3-way ANOVA, mixed model to test these effects are reported.

		TFAS (days)		TMAG (days)		GerP (%)	
		m	SE	m	SE	m	SE
Seed container	NCC	5.2a	0.2	5.6b	0.2	40.8b	1.1
	CC	4.7a	0.2	10.3a	0.2	66.1a	1.1
Substrate	BP	5.8a	0.3	9.5a	0.3	31.7c	1.4
	FS	4.3b	0.3	7.5b	0.3	52.5b	1.4
	SS	4.7b	0.3	6.8b	0.3	76.2a	1.4
Seed container (SC)		NS		***		**	
Substrate type (ST)		***		***		***	
Replicats (R)		NS		NS		*	
SC x R		NS		NS		NS	
ST x R		NS		NS		NS	
SC x ST		NS		NS		NS	
Coefficient of variation		12.61		7.81		6.30	

a,b,c are means within a column with different superscripts differ significantly ($P < 0.05$). m: mean; SE: standard error. *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant. For each factor, values with the same letter in the same column are not significantly different. NCC: non covered containers; CC: covered containers. BP: blotting paper; FS: fine soil; SS: sterilized soil

Table 4: Effects of the sowing depth and the seed container on the germination percentage (PGer) and the time (i.e. number of days) from sowing to: (i) the first appearance of shoots (TFAS); (ii) the maximum germination (TMAG)**;** (iii) the latest germination (TLAG) in the laboratory trials. The adjusted means and the results of 3-way ANOVA, mixed model are reported.

		TFAS (days)		TMAG (days)		TLAG (days)		PGer (%)	
		m	SE	m	SE	m	SE	m	SE
Sowing depth	S1	5.8a	0.3	17.2a	1.7	45.7a	0.5	44.4b	0.4
	S3	5.2a	0.3	12.8a	1.7	43.3a	0.5	49.7a	0.4
Treatments	NCC	5.7a	0.3	14.3a	1.7	49.7a	0.5	45.2b	0.4
	CC	5.3a	0.3	15.7a	1.7	39.3b	0.5	48.9a	0.4
Sowing depth (SD)		NS		NS		*		**	
Seed container (SC)		NS		NS		**		*	
Replicate (R)		NS		NS		NS		NS	
SD x R		NS		NS		NS		NS	
SC x R		NS		NS		NS		NS	
SD x SC		NS		NS		NS		**	
CV (%)		13.89		27.15		2.59		1.92	

a and b are means within a column with different superscripts differ significantly ($P < 0.05$). m: mean; SE: standard error. *$P < 0.05$; ** $P < 0.01$; NS = not significant. For each factor, values with the same letter in the same column are not significantly different. NCC: non covered containers; CC: covered containers.

Table 5a: Effects of the sowing method and the period on the plants density (number of plants per m^2) in the field trials. The adjusted means of the plants density and the results of 3-way ANOVA, mixed model to test these effects are reported.

		Plants density (Number plants /m^2)	
		m	SE
Periods	P/1-15	9.7b	0.4
	P/1-30	19.5a	0.4
Sowing method	Broadcasting	9.8d	0.5
	LiSo1cm	12.8c	0.5
	LiSo3cm	15.7b	0.5
	LiSo5cm	19.5a	0.5

Sowing method (SM)		****	
Period (P)		**	
Replicate (R)		NS	
SM x P		NS	
SM x R		NS	
P x R		NS	
R^2		0.99	
Coefficient of variation		8.35	

a, b, c and d are means within a column with different superscripts differ significantly ($P < 0.05$).
*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant. For each factor, values with the same letter in the same column are not significantly different. m: mean; SE: standard error.

Table 5b: Effects of the sowing method and the period on the plants density (number of plants per m^2). The adjusted means of the plants density and the results of 3-way ANOVA, mixed model to test these effects and are reported.

		Number of plants per m^2	
		m	SE
Period	P/1-15	15.3b	0.3
	P/1-30	21.5a	0.3
Sowing method	Broadcasting	11.7d	0.5
	Broadcasting + Tamping	20.3b	0.5
	LiSo3cm	16.5c	0.5
	LiSo3cm+Tamping	25.0a	0.5
Sowing method (SM)		****	
Period (P)		*	
Replicate (R)		NS	
SM x P		NS	
SM x R		NS	
P x R		NS	
Coefficient of variation (%)		6.32	

a, b, c and d are means within a column with different superscripts differ significantly ($P < 0.05$).
*$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant. For each factor, values with the same letter in the same column are not significantly different. m: mean; SE: standard error.

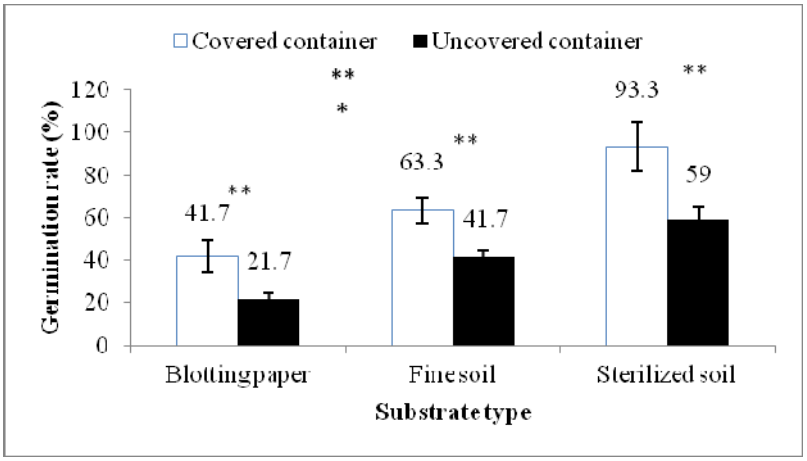

Fig. 1: Change in germination percentage following substrate type and amount of shade.

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