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Effect of different substrates on the domestication of *Saba comorensis* (Bojer) Pichon (Apocynaceae), a spontaneous plant used in agroforestry system

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ABSTRACT

Saba comorensis is a multipurpose woody liana which is threatened in Western Africa due to deforestation. To protect it from future extinction, the present study was carried out and aimed to evaluate the effect of different substrates on seed germination and seedlings growth in nursery. Fresh unpulled seeds were sown in polyethylene bags arranged in sub-blocks and containing the following substrates: cocoa pod + topsoil (¼; ¾), sawdust + topsoil (¼; ¾), poultry dung + topsoil (¼; ¾), rice bran + topsoil (¼; ¾) and only topsoil serving as control. The germination data obtained after regular watering for 35 days and growth data during 5 months were analyzed throughout R software for a one-way analysis of variance using the appropriate tests to establish significant differences. Results showed that the highest germination rate was obtained with the sawdust + topsoil mixture and the control (58.33% each). On the other hand, the latency time was better with the sawdust + topsoil mixture (16 days) than all the others. There was a significant difference ($p<0.05$) in height between the plants obtained from the sawdust + topsoil mixture (28.45 ± 1 cm) and those from the other substrates, except for the plants bred in poultry dung + topsoil mixture (25.70 ± 1.25 cm). Thus, in view of these results, the mixture of sawdust + topsoil (¼; ¾) is suitable for ensuring the domestication and conservation of *Saba comorensis*.

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INTRODUCTION

There is generally a current use of spontaneous plant species by the populations from rural areas in Africa (Abalo *et al.,* 2012; Effoe *et al.,* 2020) and particularly in Côte d'Ivoire to assure livelihood (Aké, 2015; N'Guessan *et al*., 2015; Ouattara *et al*., 2016). These plant species morphologically shaped either as tree, shrub, herb or liana provide many services (food, drug, timber, shade, agricultural tools, building material or economical products) to local people.

For many years, researches through ethnobotanical surveys have been conducted in the African continent for establishing their potentials and their abundance (Ambé *et al*., 2001; Djaha and Gnahoua, 2014; Aké *et al*., 2015; Aké *et al*., 2019). Such studies still continue in several ecological areas. They are guided by field observations that show a decrease or a scarcity of some local plants. Indeed, over 83% of the forest area have been lost since 1960 in Côte d'Ivoire due to urbanization, timber exploitation, intensive agriculture, unstable program of forests management (Aké-Assi, 2001). The political and military crisis of 2010 has also contributed to this decline, particularly in forest relics (Bamba *et al.,* 2018). Thus, many local plant species are threatened, some like *Ricinodendron heudelotii* are actually mentioned on the International Union for the Conservation of Nature (IUCN) red list.

Saba comorensis is a plant resource found in western Africa either in the savannah, either in the forest (Abalo *et al.,* 2012; Vanié-Bi *et al*., 2021). This plant is also concerned with threat as mentioned Lawin *et al.* (2016) in Benin. These authors declared that there was no strategy being developed for its preservation. However, some strategies should be performed for the conservation of *S. comorensis* since it is established to be a multipurpose liana. Its food, medical and economic potential are significant for the population's welfare (Aké *et al*., 2006). Unfortunately, reports on its domestication by seeds germination are lacking. The regeneration by cutting is sometimes seen at a small scale in Northern Côte d'Ivoire as some farmers use it to build fences around their crops to protect them from trampling by livestock.

The domestication of tree species throughout seed germination is an approach that has already been explored in many works before (Hien *et al*., 2023). The success of this practice focuses on the environment condition, the seed viability or the quality of the substrate. On this last topic, there are the works of Ouattara *et al.* (2005), Djaha and Gnahoua (2014) and Nguema *et al.* (2014) that helped to the understanding of the impact of the substrate on the germination of seeds.

This present study conducted in this scheme highlighted to the domestication of *S. comorensis*. Its aim was to determine the effect of different substrates on the germination of unpulled seeds and the growth of seedlings in the nursery, with a view to identifying the best substrate.

MATERIALS AND METHODS

Study area

The study was carried out at the Jean Lorougnon Guédé University (JLoGU) of Daloa. Daloa is located in the Western Centre of Côte d'Ivoire, at the geographical zone 29N especially at the 783869 E and 759500 N of the UTM coordinates system (Fig. 1).

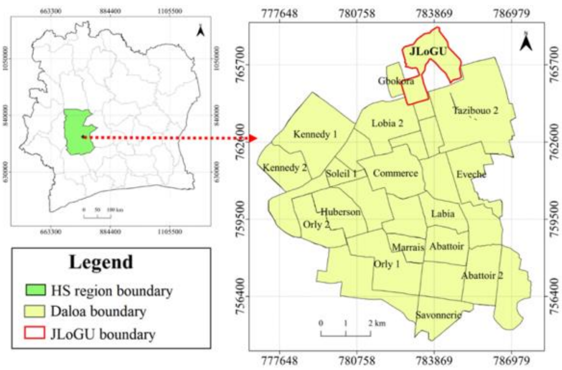

Fig. 1. Localization of the study area, according to UTM coordinates

The town of Daloa belongs to the Haut-Sassandra region and is surrounded by the towns of Vavoua and Zuénoula in Northen part, Issia and Sinfra in the South, Bouaflé at the East, Zoukougbeu, Bangolo and Duékoué at the West. The total area of Daloa is 5 305 Km^2. The Haut-Sassandra region is characterized by two seasons, a dried season from November to February and a rainy season from March to October. The temperatures vary from 24.25 °C to 26.95 °C (Kouman, 2018). The soils are essentially of the reworked ferralitic type (Perraud *et al.,* 1970). The vegetation is dominated by the dense humid and semi-deciduous forests (Guillaumet and Adjanohoun, 1971) which is unfortunately being growing to forest relics due to anthropical actions.

a: Ripped fruits; b: Fresh unpulled seeds

Fig. 2. Pictures of *Saba comorensis*

CP: cocoa pod + topsoil (¼; ¾); SDT: sawdust + topsoil (¼; ¾); PD: poultry dung + topsoil (¼; ¾); RB: rice bran + topsoil (¼; ¾); CTRL: control (topsoil)

Fig. 3. Experimental dispositive

Collect of the plant material, proceeding and sowing

The fresh fruits of *S. comorensis* were supplied from a local market in May 2024 (Fig. 2). An experiment dispositive made of polyethylene bags arranged in five sub-blocks and kept under shade was established (Fig. 3). Each sub-block contained a specific substrate. Though, five substrates were used for the experiments, and they were: cocoa pod + topsoil (¼; ¾), sawdust + topsoil (¼; ¾), poultry dung + topsoil (¼; ¾), rice bran + topsoil (¼; ¾) and topsoil serving as control. After setting the dispositive, the fresh fruits were broken to take out the seeds. The unpulled seeds were sown without treatment one per polyethylene bag at a 2 cm depth and watered twice a day.

Germination and growth parameters

Three germination parameters were assessed during 35 days after the sowing of seeds. They were the germination rate, the latency time and the duration of germination. Each parameter is described in Table 1. Then the number of leaves, the number of nodes visible on the stem, the height and the collar diameter of the seedlings were considered for the evaluation of the growth. These measurements were performed every week from the second month, and lasted 13 weeks.

Table 1. Description of the germination parameters considered in this study

Parameter	Definition, calculation and expression
Germination rate	The capacity of seeds to germinate $\% GR = \frac{NSG}{NS} \times 100$ %GR = Germination rate; NSG = Number of seeds germinated; NS = Number of seeds sown (Logbo *et al.*, 2022)
Latency time	It is the time in day required for first germination, from the sowing day to the day the first seed germinates (Adji *et al.*, 2021)
Duration of the germination	This is the time between the day the first seed germinates and the day the last seed in the same batch germinates, expressed in day (Adji *et al.*, 2021)

Statistical analysis of data

A one-way ANOVA test was performed to analyzed the data with R software version 4.4.3. Before that, the tests of Shapiro and Bartlett were realized to check respectively and the egality of variances. The Newman Keuls test completed the analysis to establish groups. Significance was considered for $p<0.05$.

RESULTS AND DISCUSSION

Germination parameters

The germination rate along 35 days varied from 42% to 58%. The highest germination rate was obtained with the sawdust-based substrate and with the control, reaching about 58% each (Fig. 4). These observations prove that the pulp of *S. comorensis* fruit was not an obstacle to germination comparatively to the pulp of *S. senegalensis* fruit. According to Diawara *et al.* (2020) the unpulled seeds of *S. senegalensis* didn't germinate. That occurrence suggests a potential role of the pulp of *S. comorensis* fruit to preserve the seeds viability and their capacity to germinate. Understanding of the fact that the unpulled seeds have germinated in this work is probably due to the acidity content of the pulp of *S. comorensis* seed (Aké *et al.,* 2006). The acidity probably keeps the seeds safe. That corroborate with the results of Tokpa *et al.* (2024), who showed that acidity was suitable for plants' nutrition. Consequently, this step of de-pulping the seeds before sowing may not be necessary**.** It has also been established that acidity weakens the seed coat, making easier their germination (Diallo *et al.*, 2023).

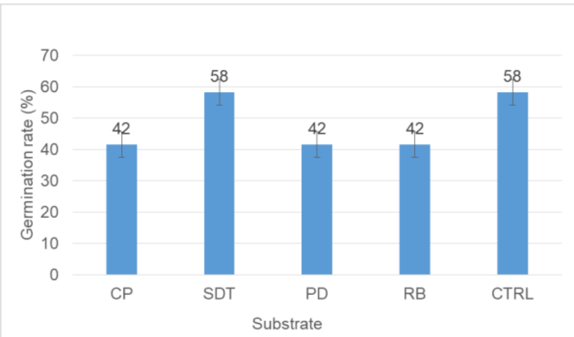

Fig. 4. Germination of *Saba comorensis*

Additionally, the sawdust probably removes the pulp from the seeds more quickly than topsoil or the other substrates and facilitate the seed germination. In natural conditions, fresh seeds of ripe fruits which escape from human or animal consumption germinate properly. In this case, it is difficult to determine which mother tree had grown up from an unpulled seed or simple seed (the one sucked then thrown away by animals or humans). The germination of an unpulled seed is probably a way this wild plant species regenerates naturally in the ecological area.

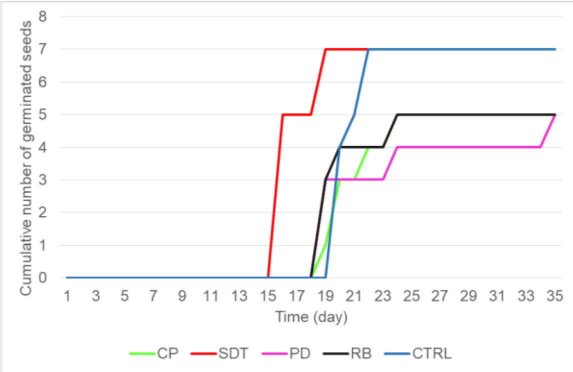

Fig. 5. Latency time after sowing

In the meantime, the latency times ranged from the 16th day to the 20th day. Herein the shortest delay of germination was observed in the substrate containing sawdust and it took 20 days in the control (Fig. 5). Moreover, the duration of germination varied from 2 days to 16 days. All seeds which have germinated appeared after 2 or 3 days outside the substrate edges respectively for the substrate of sawdust and the control. It took 5 days in the mixture of cocoa pod + topsoil and rice bran + topsoil. Then the germination spread on 16 days in the substrate made of poultry dung + topsoil. Breaking seed dormancy is an essential step in germination. This break is sometimes characterized by the destruction of the seed coat, which can be induced by environmental factors such as the substrate. The results show that the dormancy of seeds was removed between the 2nd and the 6th week. Ouattara *et al.* (2005) qualified the germination of a seed in this delay to be quick. That means, there was probably a quick germination of *S. comorensis* seeds. Conversely the germination of *S. comorensis* seeds was not so very quick comparatively to Diawara *et al.* (2020) point of view who considered a maximum delay of 7 days for a very fast germination. Additionally, the delay of the germination of *S. comorensis* seed looked better than *S. senegalensis* which ranged between 17 to 30 days whatever the seeds were treated or not (Hien *et al.*, 2023). Anyway, the addition of sawdust to topsoil has improved this germination comparatively to the others agricultural inputs by allowing a good humidification of the medium and making water safely available to the seeds. Better water availability is essential to swell the seeds and activate the enzymatic processes involved in germination.

Growth parameters

The results are presented in Table 2. They showed that there was not a significant difference (P>0.05), whatever the substrate used, in the number of leaves, the number of nodes visible on the stem and the diameter of collar of the seedlings. The average number of leaves varied between 6.09±0.45 and 7.47±0.53, it was about 7.65±0.37 and 7.15±0.43 for the number of nodes, while the collar diameters averages varied between 3.19±0.1mm and 3.30±0.09mm.

Table 2. Growth parameters data performed with one-way ANOVA test in R software

Substrates	Number of leaves (±SEM)	Number of nodes ((±SEM)	Seedlings' height (cm±SEM)	Collar diameters (mm±SEM)
SDT	7.47 ± 0.53a	7.65±0.37a	28.45 ± 1.06a	3.30 ± 0.09a
RB	6.65 ± 0.53a	7.15±0.43a	23.70 ± 1.25b	3.19 ± 0.10a
CP	6.15 ± 0.53a	7.15±0.43a	22.17 ± 1.25b	3.22 ± 0.10a
PD	6.09 ± 0.45a	7.42±0.43a	25.70 ± 1.25ab	3.28 ± 0.10a
CTRL	6.64 ± 0.45a	7.42±0.37a	23.64 ± 1.06b	3.27 ± 0.09a
p value	0.298	0.885	0.00653 **	0.94

Values are means of thirteen readings performed across the thirteen weeks of observation. Means with different superscripts in the same column are significantly different (P<0.05).

The highest value in collar diameter was obtained with the seedlings bred in the sawdust-based substrate (3.30 ± 0.09mm), followed by those obtained in the poultry dung substrate (3.28±0.10mm) and the control (3.28±0.10mm). The addition of any agricultural input to topsoil had no effect on the number of leaves, the number of nodes, and the radial growth of the seedlings. Generally, a node appears at the point of the leaf insertion. During the trials the observations have shown that the leaves of *S. comorensis* appear in pairs and in opposition at the same node. Therefore, they could be a correlation between the number of the leaves and the number of the nodes. This could explain the non-significance of the results obtained both for the number of leaves and the number of nodes.

However, there was a significant difference in the seedling's height ($p<0.05$) between those bred in the sawdust-based substrate and the others except with those obtained in the poultry dung substrate. Average values were, 28.45±1.06 cm, 25.70±1.25 cm and 23.64±1.06 cm respectively in the sawdust-based, the poultry dung and the control. The sawdust-based substrate significantly improved the height growth of the seedlings more than the control and the other substrates except for the substrate containing poultry dung. The number of nodes did not change with the height of the seedlings, indicating that some internodes were longer than others. In fact, *S. comorensis* is a liana plant species whose stem has positive phototropism. Therefore, depending on light intensity, the stem meristem would tend to produce a longer internode than when there is less light. These occurrences would mean that sawdust and poultry dung have also helped to lengthen the internodes. These substrates also enhance the soil fertility, making minerals more available for the roots. The safe and easy growth of plants which substrate is enriched with sawdust-based compost has recently been mentioned. That compost contained poultry dung as one ingredient and was suggested to be a natural fertilizer to plants. It offers good temperature and facilitates minerals (phosphorus, nitrogen and potassium) availability to plants for their nutrition (Tokpa *et al.,* 2024). The presence of poultry dung emphasizes how animal inputs was potentially good to enhance soil fertilization. Indeed, the animal origin-based substrate has been notified in previous study to improve height growth of seedlings (Logbo *et al.,* 2022). But the fact that the sawdust-based substrate looked better than the control and not the substrate made of poultry dung and topsoil bring at considering sawdust suitable for soil enrichment and help *Saba comorensis* growth and development.]

CONCLUSION

The domestication of *S. comorensis* in nursery condition has been made possible with unpulled fresh seeds without any treatment. Althought the germination rate with the most of the substrates tested didn't exceed that of the control, the sawdust-based substrate had the same potential to topsoil (58%). Conversely, the sawdust-based substrate favorized quick germination of unpulled seeds, just from the 16th days after sowing comparatively to the control that made it effective at the 20th day. Then, two months after sowing, the seedlings obtained grew up better and significantly in sawdust-based substrate than in the control.

Whereas, there was no significant difference regarding the number of leaves, the number of nodes and the collar diameters whatever the substrate employed. These outcomes bring at recommending the mixture of topsoil with sawdust to favorize the germination and the seedlings growth of *S. comorensis* seeds without need to remove the pulp before. Therefore, the regeneration of this indigenous plant species can be assured efficiently. That is an advantage for the conservation of the biodiversity and to provide welfare to human. Further investigations should be necessary to determine the effect of the variation in percentage of the inputs composing the substrates. Probably a comparative study on the germination of unpulled seeds and simple seeds could be examined too. Then the contribution of vigorous seedlings bred in nursery to agroforestry system and carbon sequestration should also be determined.

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