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Effect of Substrates on the Mycorrhization and Growth of *Saba senegalensis* under Semi-Controlled Conditions

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Abstract

Saba senegalensis is a wild edible fruit plant species with a high economic potential which can be used to fight food insecurity in rural areas and to reduce poverty. Domestication programs are being carried out to boost production. However, no studies have been done to determine the optimal soil properties for growing *S. senegalensis*. This study was carried out to determine the effects of the physical and chemical properties of different substrates on the mycorrhization and growth of *S. senegalensis* under semi-controlled conditions. *S. senegalensis* seeds were grown for 4 months in the nursery using five substrates: S1 (1/2 sand + 1/2 potting soil), S2 (1/3 sand + 2/3 potting soil), S3 (2/3 sand + 1/3 potting soil), S4 (potting soil) and S5 (sand). The intensity of mycorrhization was highest for plants grown on substrates with a lowest clay, silt, and nutrient content S3 (29.5%) and S5 (23.5%) respectively. Substrates with much higher clay and silt content stimulated better the growth of *S. senegalensis* than substrates with lower silt clay and nutrient content. In the context of domestication, the quality of the substrates could be used to stimulate the mycorrhization and the growth of *S. senegalensis* and thus quickly produce vigorous plants.

Keywords

Mycorrhizal Intensity, Nursery, Physicochemical Properties, Wild Fruit

1. Introduction

Saba senegalensis is a forest fruit species belonging to the order Gentianales and

the family of Apocynaceae. The genus *Saba* includes three (3) species *S. senegalensis*, *S. comorensis*, and *S. thompsonii* endemic in Africa [1]. In Senegal, two species have been described so far: *S. senegalensis* and *S. comorensis* [2]. *S. senegalensis* or *Vahea senegalensis* A. DC. or *Landolphia senegalensis* is a large woody liana that can reach 30 m in height, with a diameter hardly exceeding 15 cm [3]. The plant is used for the treatment of certain diseases and is a source of vitamins; it contains sterols and triterpenes with anti-inflammatory activity [4]. The fruit is a large ovoid berry, which can reach 10 cm in length to 8 cm in width. The berry made of a yellowish, edible, pulpcontains 80% of water, 18.5% of carbohydrate and 0.048% of vitamin C [2] [5]. In Senegal, *S. senegalensis* fruits sales revenues are significant and account for 1/3 to 2/3 of farmers' households' income [6]. Programs aimed to increase *S. senegalensis* fruits production through its domestication are being promoted. Grafting of *S. senegalensis* for domestication and to shorten the age of fruiting was already successfully conducted [7]. However, there are no studies to determine the optimal growth conditions of *S. senegalensis* or to improve the growth of seedlings through mycorrhization in the nursery for its domestication.

Mycorrhizal symbiosis is a reciprocal benefit association between plant and fungi, which results in the formation of mycorrhizae [8]. Two main types of mycorrhizae have been described in the tropics: vesicular and arbuscular mycorrhizae (AM), also called endomycorrhizae and ectomycorrhizae [9] [10]. The presence of vesicles, arbuscules, and intracellular hyphae in roots are characteristic of colonization by AM fungi. Within this symbiotic relationship, the fungus feed on photosynthetates provided through plant exudates (carbohydrates, vitamins); in return, the fungus absorbs the mineral elements (P, Fe, Cu, water) and transfers them to the host plant. In addition to mineral nutrition, mycorrhizal symbiosis plays a major role in the rhizosphere of forest tree species [11] [12] [13]. Mycorrhizal symbiosis improves plants' tolerance to abiotic stresses [14], reduces the incidence of disease [15] and allows the accumulation of nutrients [16].

The establishment of mycorrhizal symbiosis and subsequently mycorrhizal dependency depends on several factors including fungus species, plant species, environmental conditions, edaphic properties such as the physical and chemical characteristics of the soil, the presence of other microorganisms, etc. Plant mycorrhizal dependency expresses the extent to which symbiosis is likely to increase plant biomass given environmental conditions [17]. In previous research, [18] showed nomycorrhizal dependency of *S. senegalensis* toward fungi *Rhizophagus irregularis* (previously known as *Glomus intraradices*). [19] showed that there was a morphological variability of *S. senegalensis* fruits depending on their geographical provenance. Soil physical and chemical characteristics in those geographic areas were different. *Saba senegalensis* predominated in areas with texture ranging from sandy-loam to sandy-clay-loam [19]. It is unknown if soil physical and chemical characteristics are related to the geographical distribution of *S. senegalensis*. In the context of domestication, it would be important to de-

termine to what extent soil substrate influence the growth and mycorrhization of *S. senegalensis*.

The objective of this study was to determine the effects of different substrates on *S. senegalensis* mycorrhization and growth under semi-controlled conditions. We hypothesized that substrates with higher clay and silt content would stimulate more mycorrhizal symbiosis and plant growth than substrates with lower silt and clay content. Sandy substrates which are poorer in nutrients will not be able to provide an optimal environment for the development of the fungi and the establishment of the mycorrhizal symbiosis.

2. Material and Methods

2.1. Preparation of Substrates

Substrates used in this study consist of a mixture of sand and potting soil at different proportions: S1 (1/2 sand +1/2 potting soil), S2 (1/3 sand + 2/3 potting soil), S3 (2/3 sand + 1/3 potting soil), S4 (potting soil) and S5 (sand). The physical and chemical characteristics of these substrates were determined at the French Research Institute for Development in Dakar (IRD) and were shown in Table 1. The mixture of soil and potting soil resulted in a sandy texture for S1, S3 and S5 substrates and a sandy-loam texture for S2 substrate.

2.2. Plant Material

Seeds of *S. senegalensis* were collected from ripe fruits originated from the South of Senegal. The seeds were then prepared for sowing by mixing them with water to remove the pulp.

2.3. Arbuscular Mycorrhizal Fungi and Inoculum Production

The arbuscular mycorrhizal fungus *Rhizophagus irregularis* was used in this study. The inoculum was composed of hyphae, spore and root fragments and was

Table 1. Physical and chemical characteristics of substrates.

	Substrates				
Characteristics	**S1**	**S2**	**S3**	**S4**	**S5**
Clay (%)	3.45	4.90	2	8.75	0.70
Silt (%)	4.35	6.65	2.30	13.40	0.70
Fine sand (%)	1.45	2	2.65	3.50	0.95
Medium sand (%)	43.25	42.35	43.25	40.20	54.10
Coarse sand (%)	47.50	44.10	49.80	34.15	43.55
Total carbon (%)	12.13	17.27	7.92	35.53	2
Total nitrogen (%)	1.38	1.45	0.45	1.98	0.21
Assimilable P (ppm)	49.47	44.42	47.45	57.04	39.88
Texture	Sandy*	Sandy-loam	Sandy	Sandy-loam	Sandy

*Classification triangle textural USDA.

provided by CM laboratory in Dakar. That inoculum was produced by cultivating tomato plants with the original strain for 3 months. The colonized roots were removed, cut into small fragments mixed with the culture substrate and air-dried [20].

2.4. Experimental Design and Monitoring

Plastic bags with a volume of 1.5 L were filled individually with five (05) types of substrates: S1, S2, S3, S4, and S5. For each type of substrate, 8 bags were grouped as a block. The blocks were then repeated 5 times for a total of $8 \times 5 \times 5 = 200$ units. Within each bag, a seed of *S. senegalensis* extracted and prepared on the same day was sown. The seeds were then watered daily. After 3 weeks of growth, plants were inoculated with 20 g of mycorrhizal inoculum. The plants were kept in the nursery and watered at the same daily frequency.

2.5. Parameters Studied

After four months of culture, the height of the plants was measured. Plants were subsequently removed from the plastic bags, aboveground and below ground biomasses were collected and prepared for analysis. The following parameters were evaluated: frequency and intensity of mycorrhization, root collar diameter and Plant biomass (aboveground and belowground biomasses).

Mycorrhizal frequency and intensity were evaluated on *S. senegalensis* roots using a method by [21]. Roots were collected and cut into 1 cm fragments. The fragments were placed in flasks containing 10% potassium hydroxide (KOH) solution and then heated at 115˚C for 10 minutes in a water bath Memmert. Root fragments were then washed three (03) times with tap water. In order to lighten them, root fragments were put to soak in a solution of hydrogen peroxide for 15 to 20 minutes. After washing with tap water, the fragments were acidified with 1% hydrochloric acid (HCl) and then put in flasks containing a red dye composed of 63 ml of glycerol, 65 ml of water, 875 ml of lactic acid and 0.1 g of fuchsin acid. The red dye allows clear visualization of vesicles, arbuscules and hyphae during microscopic observation of root fragments. Twenty fragments were observed at once under a microscope Labomed LX-300 Binocular Microscope. The frequency (F) and intensity (I) of roots colonization by mycorrhization were calculated according to [22] formula.

$$F\ (\%) = (\text{number of colonized roots})/(\text{total number of roots}) \times 100$$

$$I\ (\%) = (\text{length of colonized roots})/(\text{total length of roots}) \times 100$$

2.6. Data Analysis

The software XLSTAT software (version 2014, Addin-soft) was used to analyze the data. Plant height and diameter, biomass, frequency and intensity of mycorrhization were analyzed using ANOVA. Mean values were compared using Tukey' HSD test (honestly significant difference) at a significance of $p < 0.05$. Data were also further analyzed using principal component analysis (PCA)

and a correlation matrix of the different substrates with the parameters was established.

3. Results

3.1. Mycorrhizal Frequency and Intensity

Mycorrhizal frequency was quite high regardless of the substrate with values ranging from 88.3% to 96.7% (Table 2). Higher mycorrhizal frequencies were observed on roots from substrates S3 and S5 with respectively 96.7% and 96.3%. The lowest frequency was observed on substrate S2 with 88.3%. The sandy soils S3 and S5 which had at least 2/3 of sand presented the highest mycorrhizal frequency. S3 and S5 are very poor in phosphore, this could have influenced mycorrhizal frequency.

With regards to the intensity of mycorrhization, it was higher on the substrate S3 (Table 2). Plants grown on S3 had a significantly higher mycorrhizal intensity 29.5% than plants grown in all other substrates. Plants from substrates S1 and S2 had the lowest mycorrhizal intensity with respectively 16.8% and 18.7%. The higher sand content in addition to low organic matter content may have triggered higher mycorrhizal activities. In substrates with more potting soils, this might have hindered mycorrhizal activities and colonization. Contrary to the high frequency of mycorrhization, the intensity of mycorrhization was quite low for all substrates (less than 30%).

3.2. Height, Root Collar Diameter and Plant Biomass

Plants grown on substrates S1, S2 and S4 which contain at least 50% of potting soil were statistically taller than plants grown on S3 and S5 substrates (Table 3). S3 plants, however, were significantly taller than plants of the S5 substrate. Plants from substrates S3 and S5 had significantly shorter height but higher mycorrhizal intensity. The potting soil which is richer in nutrients may have impacted positively plant height but negatively mycorrhizal intensity.

Root collar diameters of plants grown on substrate S2 were significantly greater

Table 2. Mycorrhizal frequency and Intensity of *S. senegalensis* roots after 4 month of growth in nursery.

	Mycorrhization	
Substrates	**F (%)**	**I (%)**
S1	91.7 a*	18.7 a
S2	88.3 a	16.8 a
S3	96.7 a	29.5 c
S4	91.7 a	20.3 ab
S5	96.3 a	23.5 b

*Values in the same column followed by the same letter are not significantly different at a level of $p < 0.05$ (Tukey HSD test).

Table 3. Height, root collar diameter and plant biomass of *S. senegalensis* grown on different substrates in nursery during 4 months.

	Growth Parameter		
Substrates	**Average height (cm)**	**Average root collar diameter (cm)**	**Average plant biomass (g)**
S1	23.5 c*	0.31 a	55.01 d
S2	23.8 c	0.36 b	32.09 b
S3	19 b	0.32 ab	36.63 bc
S4	25 c	0.33 ab	42.34 c
S5	16.5 a	0.34 ab	26.33 a

*Values in the same column followed by the same letter are not significantly different at a level of $p < 0.05$ (Tukey HSD test).

than those of plants grown on substrate S1, respectively 0.36 cm vs. 0.31 cm. There was no difference compared to the plants grown on substrates S3, S4 and S5. The substrate composed of potting soil alone (S4 substrate) or sand alone (S5 substrate), did not stimulate any optimal growth. On the other hand, mixtures of substrates containing between 50% and 66% of potting soil were best in stimulating plant growth (Table 3). The mixture of substrate may have the right balance of nutrients to stimulate plant growth. For plant biomass, after 4 months of growth, it was significantly more important for substrate S1, 55.01 g. Plants from substrate S5 had the lowest weight 26.33 g due probably to the lowest nutrients content of the substrate.

3.3. Correlation of Parameters

The PCA analysis of the parameters explained 91% of the variability. The correlation of all the parameters showed two separate groups. In the first group, there was a strong positive correlation of substrates S4, S2 and S1 with the height and biomass of *S. senegalensis*. In the second group, substrates S3 and S5 had a weak correlation with the height and biomass, but a positive and strong correlation with the intensity of mycorrhization. The intensity of mycorrhization was strongly correlated with the substrate S3 (Figure 1). Plant height and biomass showed a stronger correlation between them but also with substrate S4 composed only of potting soil. The parameter height was strongly but negatively correlated with the mycorrhizal intensity.

4. Discussion

The low intensity in our study could be explained by a weak mycorrhizal dependency of *S. senegalensis* towards *Rhizophagus irregularis*. In a previous study, [23] showed that *S. senegalensis* was not able to form any mycorrhizal symbiosis. The same authors also showed that *S. senegalensis* had no mycorrhizal dependency toward *Glomus aggregatum* and *Rhizophagus irregularis*. However, this low intensity could also be linked to the physical and chemical characteristics of

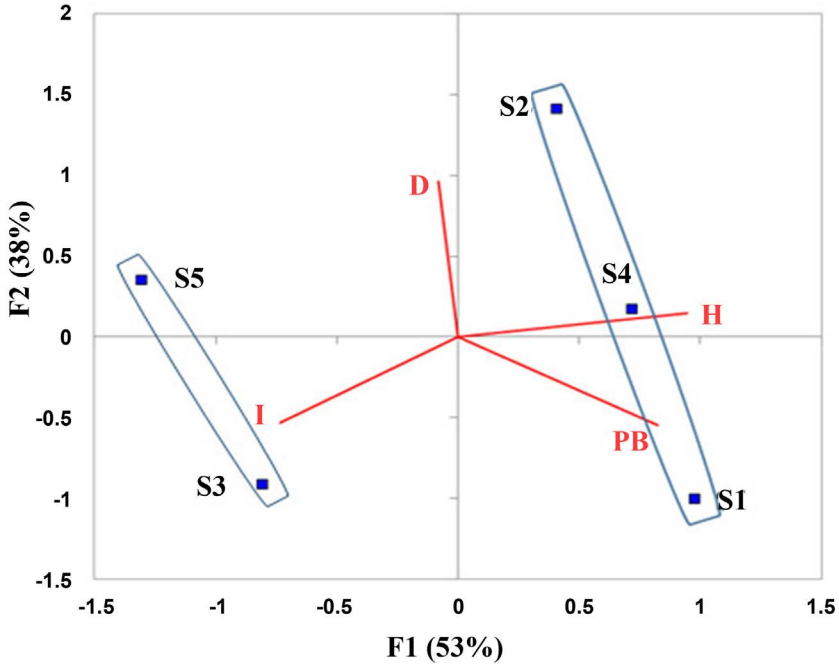

Figure 1. Correlation matrix of the variables evaluated on *S. senegalensis* plants grown on substrates S1, S2, S3, S4 and S5. D = Diameter, H = Height, I = Mycorrhizal intensity, PB = Plant Biomass.

the substrates. Indeed, PCA analysis showed that mycorrhizal intensity was higher for plants grown on substrates S3 and S5. S3 and S5 substrates had the lowest clay and silt content (**Table 1**). In addition, chemical analysis showed that S3 and S5 also had the lowest phosphorus and nitrogen level. One of the primary roles of mycorrhizal symbiosis was to provide the host plant with soil phosphorus in the event of a deficit [24] [25]. Moreover, the mycorrhizal intensity may also depend on the availability of soil nutrients [26]. Our results indicated that mycorrhizal intensity was lower with plants grown on nutrient-rich substrates S2, S4 compared to poorer substrates S3 and S5. [27] reported similar results with higher mycorrhizal colonization at low P level than at high P level for *Poncirus trifoliate* seedlings. The high presence of certain nutrients such as P may induce a decrease in the intensity of mycorrhization [28]. The establishment of mycorrhizal symbiosis is often hindered when the soil is fertile [29] [30] [31]. Nevertheless, [32] [33] and [34] showed that there was no correlation between mycorrhizal intensity and the physical-chemical properties of the soil. For [35], when the soil is poor in certain nutrients such as N, P and K, mycorrhizal intensities will be lower for plants grown there.

Growth of *S. senegalensis* plants was greater on soils richer in clay and silt S4, S2 and S1 than their counterparts. [3] showed that *S. senegalensis* grew best on soils rich in clay able to retain water. The weak plant growth noted on the substrate S5, which has a much higher sand content, may be related to the low nutrient content. Indeed, substrate S5, in addition to having a low clay and silt content, had also low nutrient content compared to the other substrates. Corre-

lation of parameters also showed that substrates were more positively correlated with the height of plants than the diameter (**Figure 1**). This phenomenon is characteristic of lianas. Indeed, studies carried out by [5] in Senegal in the Niokolo Koba National Park (PNNK), showed that *S. senegalensis* produces long but small diameter branches. This may be an adaptive strategy that would allow them to grow quickly and make the most of their support. In our analysis, there was a negative correlation between the intensity of mycorrhization and the height of plant biomass. This agrees with results by [33] who find that *Glomus intraradices* reduced shoot growth in plants growing in phosphorus-rich soil. [26] and [36] found that plant growth was not necessarily related to the degree of mycorrhizal fungi colonization of their roots. In our study, mycorrhizal intensity was higher on substrate with poorer nutrient content, S3 and S5. Since *S. senegalensis* has a weak to no mycorrhizal dependency and, in addition, the substrate was poor in nutrients, the stimulation of plant growth will not be as high compared to plants grown in substrates with higher nutrient content. Therefore, despite the highest intensity of mycorrhization for plants grown in substrates S3 and S5, their growth was not stronger than those of plants grown in other substrates. [35] showed that in semi-arid areas, a high intensity of mycorrhization was not necessarily related to better plant growth. With regards to our hypothesis, substrates with the highest clay and silt content stimulated better *S. senegalensis* growth. But, for the establishment of mycorrhizal symbiosis, substrates with the highest sand content and the lowest nutrient content were more efficient in increasing mycorrhizal intensity.

In this study *S. senegalensis* ability to established efficient mycorrhization was not tested. This may be the main limitation of this study as the results should have allow to compare plants with mycorrhizae vs. plant without mycorrhizae in order to assess and separate the real impact of the mycorrhization vs the impact of the substrate. Nonetheless, the design of this study allowed a characterization of the substrate and the mycorrhization effects on *S. senegalensis* growth which is very important for agroecosystem programs.

5. Conclusion

Substrates had influenced the growth and mycorrhization of *S. senegalensis* differently depending on their physical and chemical properties. Substrates with a lower clay and silt content but also with lower nutrient content, in particular phosphorus and nitrogen, stimulated better plant mycorrhization. *S. senegalensis* growth (height, root collar diameter and plant biomass) was strongly influenced by soil texture with much higher growth for substrates with higher clay and silt content. The sandy-loam substrate stimulated the growth of *Saba senegalensis* and decreased the frequency and the intensity of mycorrhization of *Saba senegalensis* in opposite to the sandy substrate. These results are significant and could be used to improve the growth of *S. senegalensis* in a semi-controlled environment. For domestication, the sandy-loam substrate could be used to pro-

duce more vigorous plants and improve production quickly. However, when the substrate is sandy, mycorrhizal inoculation would be recommended. This constitutes an important step toward the domestication of *S. senegalensis*. It would be interesting to test other mycorrhizal strains and to determine the optimal nutrient requirements of *S. senegalensis* for regeneration.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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