

# Original Research Article

## Effects of soil water availability on leaf traits and branching patterns in *Acacia tortilis* (Forsk.) Hayne subsp. *raddiana* (Savi) Brenan, *Balanites aegyptiaca* (L.) Del. and *Ziziphus mauritiana* Lam. seedlings originated from the Sahel

### ABSTRACT

**Aims:** The aim of this study was to compare the leaf traits and branching patterns in *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* seedlings in response to water stress, ~~watered then put under water stress~~ [M1].

**Study design:** ~~The s~~Seeds used in this experiment were collected on from species growing in their natural range in the Sahel, ~~then~~ germinated and grown in a common garden experiment.

**Place and Duration of Study:** Seeds were transferred at around 400 km south of the seed origin, where the climate is less harsh climate. ~~They were after germinated and~~Seedlings were planted in the experimental site, on an area of 1300 m<sup>2</sup>, between October 2002 and October 2004 [M2].

**Methodology:** The seedlings were watered ~~3/7~~three days a week from the beginning of the experiment until July 2004. ~~The reafter, seedlings were y were assessed measured at three dates. The first measurement was carried out in at~~ 11 months, ~~old seedlings, the second measurement in 16 months old, and the third in at~~ 24 months after the seed germination date. In the assessment, We we measured the length ( $L_{GU}$ ) and the number ( $N_{GU}$ ) of growth units, the number of nodes ( $N_{node}$ ), and leaves ( $N_{leaf}$ ), and the single ( $A_{leaf}$ ) and total ( $A_{foliage}$ ) leaf areas [M3].

**Results:** Following the water stress,  $N_{leaf}$  and  $A_{leaf}$  only decreased in *Acacia tortilis*, to tightly control the transpiration.  $A_{leaf}$  increased in *Balanites aegyptiaca* and *Ziziphus mauritiana*, due to persistent leaves flushed before the water stress.  $A_{foliage}$ ,  $N_{GU}$  and  $N_{node}$  were generally consistent, while  $L_{GU}$  decreased in *Balanites aegyptiaca*. The defoliation in dry season despite the watering suggested an endogen control, allowing the species to escape harsh conditions in their natural range [M4].

**Conclusion:** The study do not support the hypothesis according to which species naturally coexisting are likely to display similar trend in leaf traits and branching patterns in response to drought [M5]. The study has been limited to three weeks of water stress and deserves to be extending to provide more insight into traits pattern for a longer periods of water stress .

**Keys words:** drought, growth units, leaf area, phenology, tropical area.

### 1. INTRODUCTION

The Sahel *sensu* stricto [M6] is one of the driest savanna ecozones with high inter-annual rainfall variability ~~of annual rainfall~~, ranging from 200 to 400 mm, high potential evaporation of about 2000 mm year<sup>-1</sup> and ~~a groundwater usually deeper~~ groundwater of more than 30 m [1]. The rainy season spans from July to September, and the dry season from October to June. The mean annual maximum temperature is between 35-37°C with a peak up to 48°C, and the minimum between 13-15°C [2]. Rainfall is one of the main limiting factors, explaining the highly variable recruitment potential generally occurring in semi-arid savannas [3]. Leaf traits are associated with resistance to drought [4] and can be used to assess species vulnerability to water shortage.

*Acacia tortilis* (Forsk.) Hayne, *Balanites aegyptiaca* (L.) Del., and *Ziziphus mauritiana* Lam. are multipurpose tree species naturally occurring in the Sahel, with leaf traits adapted to frequent water stress. They are used by the local population as fodder for livestock and ~~for personal consumption food~~ and herbal medicine medication. The genus *Acacia* is particularly known to produce commercial gum, generating substantial income for local population [5]. In search for the sustainability for these species in the context of advance aridification due to climate change, there is a growing interest to better know the functional and morphological traits allowing these species to cope with drought regularly occurring in these areas. Their phenology is closely linked with climate including drought and can be used to provide insight into species reaction-response to drought.

Several studies on mature Sahelian native species ([6, 7 and 8][M7]; [7]; [8]) focused on qualitative phenology based on visual assessment of leafing patterns in relation with climate. Hence, it has been shown a semi-evergreen pattern of *Acacia tortilis* ([7]; [9]), and an evergreen pattern of *Balanites aegyptiaca* (L.) Del. ([9]; [10]). *Ziziphus mauritiana* Lam. is sometimes considered as semi-evergreen ([8]; [9]), or deciduous [11][M8]. The leaf flush is generally noted at the end of the dry season, when the water availability at the soil surface is completely depleted for *Acacia tortilis* ([7]; [9]), *Balanites aegyptiaca* [8] and *Ziziphus mauritiana* ([8]; [9]). While *Acacia tortilis* [7] and *Ziziphus mauritiana* ([8]; [12]) have their maximum leafing in the earlier rainy season and lasts until the middle of the dry season, *Balanites aegyptiaca* remains green all year round [8]. Water stress reduces the duration of full canopy stage for *Acacia tortilis* [7], while we did not find such an evidence in *Balanites aegyptiaca* and *Ziziphus mauritiana*. These patterns were described in mature trees and may not necessarily be translated into seedlings because the direction of the responses may be changing according to the species ontogeny. Water stress significantly reduced leaf area in *Acacia tortilis* seedlings [13], leaf area and leaf weight in *Balanites aegyptiaca* [14], and leaf area in *Ziziphus sp.* [15], suggesting that stress conditioning enhances adaptive traits for drought tolerance [14]. However, there ~~are still~~ are uncertainties about the comparative traits patterns in response to drought ~~between-among~~ *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana*. The aim of this study was to compare their leaf traits and growth unit patterns related to soil water availability. Because these three species naturally coexist in the same area, we hypothesized that they may have similar trend in leaf traits and branching patterns in response to water stress, which allow them to adapt to similar habitats and form the niche differentiation [16][M9].

This study ~~will-was~~ be conducted on seedlings grown in a common garden experiment, after their seeds were collected from adult trees growing in their natural range in the Sahel. Because the environmental conditions of experimental site is uniform for all the species, we will be able to compare the leafing patterns between species, and to provide insight into leafing strategy allowing these species to cope with water shortage. A favorable soil water status will be simulated by watering the seedlings, followed by an induced water stress.[M10]

## 2. MATERIAL AND METHODS

### 2.1 Study site and climate data

#### Site description

*Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* naturally occur in the Sahel ecozone of Senegal. Their seeds were collected near Souilène village (16°20'N, 15°25'W) located 20 km south of Dagana where ~~is located there~~ is a weather station ~~that is~~ used as reference for the zone (Fig. 1). The dry season spans from October to June, and the rainy season from July to September. Annual rainfall averages 280 mm. The dry season can be subdivided into a hot dry season in May with average maximum and minimum temperatures ~~respectively~~ of 41 and 22.8°C ~~respectively~~, and a cool dry season in January with ~~respective~~ maximum and minimum temperatures ~~respectively~~ of 31 and 14.8°C (annual average = 28.8°C). The soils are sandy with a deep water table [7].

#### Seeds c

The collected seeds were germinated and their seedlings transferred in the experimental site located in the Institut de Recherche pour le Développement (IRD) of Bel-Air in Dakar 17°33'N, 14°55'W, at about 400 km south of the seed origin (Fig. 1)[M11].

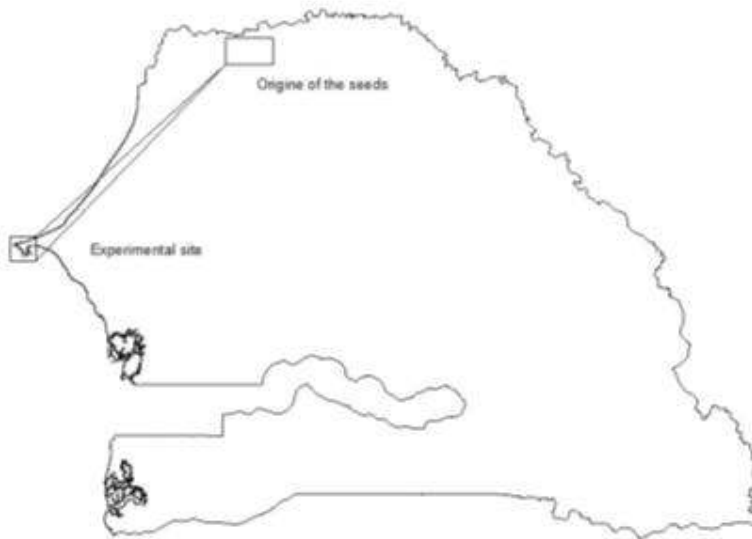


**Fig. 1. Origin of the seeds of *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* from their natural stands coexisting in the same area, and location of with their seedlings planted in the experimental site approximately, at about 400 km south of the seeds origin.**

Soils are tropical ferruginous, non-washed [17], slightly basic (pH = 8.35) resting on sandy sedimentary substratum, and contain 0.92% of organic matter, 0.53% of organic carbon and 0.46% of nitrogen [18]. The cold Canary current and the maritime trade winds moderate the climate [1]. The study site is characterized by a dry season from November to May and a rainy season from June to October [M12]. Based on average data climate calculated from 1981 to 2010 provided by Direction Nationale de la Météorologie de Dakar-Yoff, total annual rainfall is 376.3 mm. In dry season, lowest temperatures are in January-February (20.7°C), and highest in September-October (27.7°C). [M13]

## 2.2 Experimental design and measurements

In order to obtain a uniform and high germination rate, pretreatments were performed on seeds before sowing for germination. The seeds of *Acacia tortilis* were pre-treated with sulphuric acid for 60 minutes and then soaking in cold water for 10 to 15 minutes (ref.). Seeds of *Balanites aegyptiaca* and *Ziziphus mauritiana* seeds were pre-treated by removing the impermeable seed coats and first got rid of their removed and pre-treated seeds impermeable seed coats, then soaking in cold water for 24 hours. The seeds were consecutively treated with sulphuric acid for 5 minutes then soaked in cold water for 48 hours [M14]. In August 2002, the pretreated seeds were germinated in plastic bags filled with sand and soil mixture, at a rate of one seedling per plastic bag in a nursery located in the Centre National de Recherches Forestières (CNRF / ISRA, Dakar). One month following the germination and the nursery acclimation phase (September 2002, end of rainy season), the seedlings were transplanted in the experimental site of [IRD] [M15].

In an open area of 1300 m<sup>2</sup> (65 m × 20 m), the experimental design (Fig. 2) was originally implemented to conduct many researches including the current study. [M16] In an orientation East-West, 12 *Balanites aegyptiaca* seedlings separated by a distance of 1.30 m were planted in a first row. This first row of seedlings was followed by other parallel rows extending in south direction separated each other by a distance of 2 m. [M17] In total, Hence, from North to South, five consecutive rows of *Balanites aegyptiaca* were followed by 7 rows of *Ziziphus mauritiana*, an additional row of *Balanites aegyptiaca*, and finally 8 rows of *Acacia tortilis*. Overall, 96 seedlings of *Acacia tortilis*, 72 seedlings *Balanites aegyptiaca*, and 84 seedlings of *Ziziphus mauritiana* were planted in the experimental site (Fig 2), for a total number of 252 seedlings. Because competition can influence growth ([19]; [20]) the experiment was designed to minimize competition between seedlings.

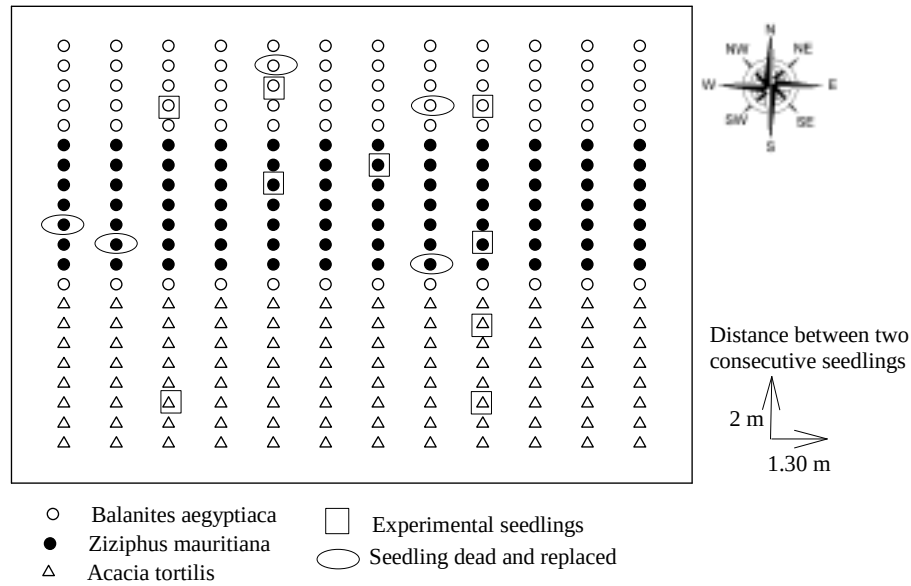


**Fig. 2. Experimental design in an open area located in the Institut de Recherche pour le Développement (IRD) of Bel-Air, Dakar 17°33'N, 14°5'W. Two month old seedlings of *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* were directly transplanted in the soil, after germination and acclimation in a nursery.**

In order to build models to estimate leaf areas, leafy twigs were randomly and destructively sampled randomly in three individuals different from the measured seedlings. The sampled twigs were stored in small refrigerated containers until they were brought to the laboratory where the leaf areas were measured using a photo-planimeter Delta-T Devices LTD, Cambridge, England. Linear regression models were consecutively performed then used to estimate the areas of the leaves followed in the non-destructive measurements.

On 3 month old seedlings, non-destructive measurements were carried out from October 2002 to October 2004, on 3 seedlings per species randomly selected randomly per species. In tree architecture model, when an episode of continuous twig elongation is followed by an interruption of growth, it produces a morphological A linear branch on a leafy axis was used a morphological marker delimiting of a growth unit (GU) easily discernible on a leafy axis as for example a linear branch [21]. A growth unit is constituted of a succession of nodes and internodes and is defined by its order in the branching ramification pattern. Hence, along a given leafy axis, we defined a GU of order 1 as the one closer to the main stem. It bears a GU of order 2, followed by a GU of order 3 and so on, until the tip of the leafy axis. In the followed seedlings, all the different orders of growth units (GU) were numbered and marked from the top of the branches and progress downwards until the main stem. The seedlings were watered 3/7 days from the beginning of the experiment until July 2004 (beginning of rainy season) when the watering was withheld, making the rains the only source of water provision, until the end of the experiment on at month 26 months from the germination date. The seedlings were measured at three different dates. The first measurement was carried out in 11 month old seedlings ( $M_{11\_wet}$ ), the second measurement in 16 month old ( $M_{16\_wet}$ ), and the third one in the last week of October 2004, i.e. 24 months after the seed germination date ( $M_{24\_dry}$ ).

In every measurement date, the number of nodes and leaves of the GU were counted. Since *Balanites aegyptiaca* has simple leaves and *Ziziphus mauritiana* is made up only of two leaflets, hence the leaf length and the maximum width perpendicularly to the central rib were measured. *Acacia tortilis* has compound leaves several leaflets so that the number of leaflets was also counted. Single leaf area was estimated using the regression models obtained by the destructive sampling, to derive an average value for the GU. The total number of leaves and their area was obtained by adding the leaves of all the GU of the corresponding seedling. Total length of GU was obtained by summing up all the GU of the seedling.

## 2.3 Statistical analyses

A two-way analysis of variance was performed using the GLM procedure of the SAS software package version 9.3, according to the model:

$$Y_{ijk} = \beta_0 + \beta_1 S_i + \beta_2 GU_{ij} + \beta_3 M_{ijk} + \beta_4 SM_{ijk} + \epsilon_{ijk}$$

Where Y is the dependent variable and represents the total number of leaves, average single leaf area, total leaf area, total number of GU, total GU length, and total number of nodes per seedling. The subscripts i, j, and k represent are respectively for the species, the-GU, and the-month respectively. The fixed effects of the model are represented by S, GU, and M respectively for the species, GU, and month.  $\beta_0$  is the intercept,  $\beta_1$  to  $\beta_4$  the estimates for the fixed effects, and  $\epsilon_{ijk}$  is the error term ( $\epsilon_{ijk} \sim N(0; \sigma^2)$ ). Interaction between S, M and GU was not considered due to low replicate of GU ending with insufficient degree of freedom. An effect was considered significant at  $p < 0.05$  based on a type III F-test on the fixed effects. When a fixed effect was significant, paired-comparisons between species, and month were performed with a t-test ( $p < 0.05$ ) using the LSMEANS statement and PDIF option of SAS software.

## 3. RESULTS AND DISCUSSION

### 3.1 Climate of the study site

In 2002, 2003, and 2004, monthly mean maximum temperatures were around 28°C and usually occurred in rainy season while monthly mean minimum temperatures (21°C) were noted in the dry season around January and February (Fig. 3). Also, monthly mean vapor pressure displayed a minimum in the dry season ( $18 \times 10^{-1}$  hPa) and a peak in the rainy season ( $30 \times 10^{-1}$  hPa). In 2002, total annual rainfall was 151 mm but the seedlings were transplanted in the late rainy season, exactly the first week of October when was recorded only 4 mm of rain before the end of the rainy season. In 2003 the rainy season spanned from June to October with total amount of rainfall of 385 mm. In 2004, 234 mm of rainfall were recorded between June and October when dropped a last significant rain of 4 mm. The seedlings consecutively remained almost 3 weeks without additional water.

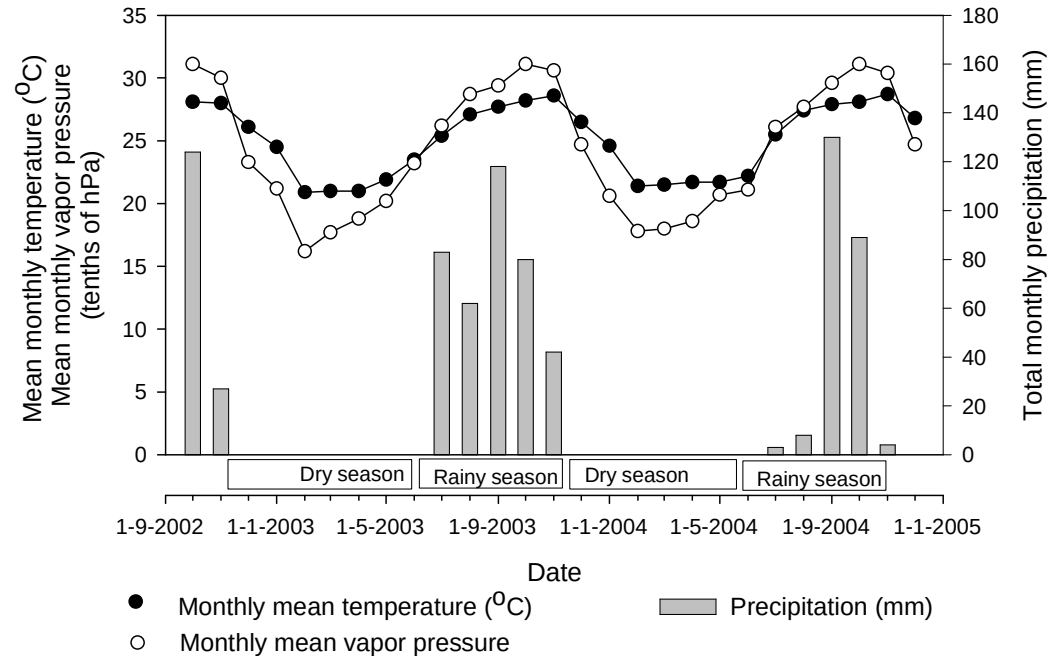


**Fig. 3. Total monthly rainfall, and average monthly temperature and vapor pressure of the experimental site in the Institut de Recherche pour le Développement, Dakar 17°33'N, 14°55'W. The reference weather station is located in Dakar-Yoff, at about 12 km far as a crow flies from the experimental site. Data came from the Monthly Climatic Data for the World 2002–04 (WMO-NOAA, 2002–04[M22]).**

### 3.2 Leaf area estimation models

The linear regression models predict well the single leaf area for *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* (Table 1).

**Table 1.** Linear model of assessment of the single leaf area ( $A_{\text{leaf}}$ ,  $\text{cm}^2$ ) of 24 month old seedlings of *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana*.

| Species                     | Prediction model of $A_{\text{leaf}}$                                             | N   | R <sup>2</sup> | p           |
|-----------------------------|-----------------------------------------------------------------------------------|-----|----------------|-------------|
| <i>Acacia tortilis</i>      | $0.164 \times (L_{\text{leafstalk}}) + 0.164 \times (N_{\text{leaflet}}) - 0.523$ | 228 | 0.61           | $p < 0.001$ |
| <i>Balanites aegyptiaca</i> | $1.040 \times (L_{\text{leaf}}) + 1.999 \times (W_{\text{leaf}}) - 4.639$         | 109 | 0.84           | $p < 0.001$ |
| <i>Ziziphus mauritiana</i>  | $1.493 \times (L_{\text{leaf}}) + 3.437 \times (W_{\text{leaf}}) - 5.759$         | 130 | 0.96           | $p < 0.001$ |

**Note:** N = total number of leaves,  $L_{\text{leafstalk}}$  = length of the leafstalk (cm),  $N_{\text{leaflet}}$  = number of leaflets,  $L_{\text{leaf}}$  = leaf length (cm) and  $W_{\text{leaf}}$  = leaf width (cm).

The R<sup>2</sup> were 0.61, 0.84, and 0.96 in *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana*, respectively. These high R<sup>2</sup> associated with significant p-values indicated a good prediction capability of the models.

### 3.3 Total number of leaves per seedling

The species ( $F = 30.32$ ,  $p < 0.001$ ) and the month ( $F = 5.06$ ,  $p = 0.014$ ) had significant effects on the total number of leaves ( $N_{\text{leaf}}$ ) while the interaction species  $\times$  month was not significant (Table 2).

**Table 2.** Two-way ANOVA of the effects of Species, GU, Month, and the interactions Species  $\times$  Month on the leaf and growth unit traits in a semi-controlled experiment on seedlings of *Acacia tortilis* (Forsk.) Hayne subsp. *raddiana* (Savi) Brenan, *Balanites aegyptiaca* (L.) Del., and *Ziziphus mauritiana* Lam.

| Source                                    | df | Anova SS | Mean square | F value | p > F   |
|-------------------------------------------|----|----------|-------------|---------|---------|
| Number of leaves per seedling             |    |          |             |         |         |
| Species                                   | 2  | 25610.40 | 12805.20    | 30.32   | < 0.001 |
| GU                                        | 6  | 2770.94  | 461.82      | 1.09    | 0.392   |
| Month                                     | 2  | 4174.05  | 2087.02     | 4.94    | 0.015   |
| Species $\times$ Month                    | 4  | 668.57   | 167.14      | 0.40    | 0.810   |
| Average single leaf area                  |    |          |             |         |         |
| Species                                   | 2  | 9.10     | 4.55        | 6.38    | 0.006   |
| GU                                        | 6  | 4.11     | 0.68        | 0.96    | 0.471   |
| Month                                     | 2  | 18.05    | 9.02        | 12.65   | < 0.001 |
| Species $\times$ Month                    | 4  | 11.69    | 2.92        | 4.10    | 0.011   |
| Total leaf area per seedling              |    |          |             |         |         |
| Species                                   | 2  | 215.40   | 107.70      | 0.09    | 0.911   |
| GU                                        | 6  | 3050.17  | 508.36      | 0.44    | 0.848   |
| Month                                     | 2  | 8655.11  | 4327.55     | 3.72    | 0.038   |
| Species $\times$ Month                    | 4  | 6663.05  | 1665.76     | 1.43    | 0.252   |
| Total number of growth units per seedling |    |          |             |         |         |
| Species                                   | 2  | 772.76   | 386.38      | 3.87    | 0.0313  |
| GU                                        | 6  | 8737.37  | 1456.23     | 14.58   | < 0.001 |
| Month                                     | 2  | 35.55    | 17.77       | 0.18    | 0.838   |
| Species $\times$ Month                    | 4  | 675.04   | 168.76      | 1.69    | 0.177   |
| Total length of growth units per seedling |    |          |             |         |         |
| Species                                   | 2  | 6968.01  | 3484.01     | 6.53    | 0.004   |
| GU                                        | 6  | 51363.02 | 8560.50     | 16.05   | < 0.001 |
| Month                                     | 2  | 12193.38 | 6096.69     | 11.43   | < 0.001 |
| Species $\times$ Month                    | 4  | 1176.29  | 294.07      | 0.55    | 0.700   |

|                                    |   |          |         |       |         |
|------------------------------------|---|----------|---------|-------|---------|
| Total number of nodes per seedling |   |          |         |       |         |
| Species                            | 2 | 2058.50  | 1029.25 | 12.03 | <0.001  |
| GU                                 | 6 | 14840.63 | 2473.44 | 28.92 | < 0.001 |
| Month                              | 2 | 3300.21  | 1650.11 | 19.29 | < 0.001 |
| Species × Month                    | 4 | 194.56   | 48.64   | 0.57  | 0.688   |

**Note:** GU = growth unit

For *Acacia tortilis*,  $N_{\text{leaf}}$  were 96, 66 and 63 in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively (Fig. 4). Significantly lower  $N_{\text{leaf}}$  were noted in  $M_{18\_wet}$  ( $p = 0.05$ ) and  $M_{26\_dry}$  ( $p = 0.03$ ) relatively to  $M_{11\_wet}$ , while no difference was noted between  $M_{18\_wet}$  and  $M_{26\_dry}$ . For *Balanites aegyptiaca* and *Ziziphus mauritiana*  $N_{\text{leaf}}$  hardly varied for the duration of the experiment. *Acacia tortilis* had all the time significantly higher  $N_{\text{leaf}}$  than *Balanites aegyptiaca* ( $p < 0.001$ ) and *Ziziphus mauritiana* ( $p < 0.001$ ). The water stress reduced  $N_{\text{leaf}}$  by 5% in *Acacia tortilis* and 83% in *Balanites aegyptiaca*, whereas an increase of 62% was noted in *Ziziphus mauritiana*. However, *Acacia tortilis* still had, after shading its leaves, more than 49%  $N_{\text{leaf}}$  compared to the two other species [M23].

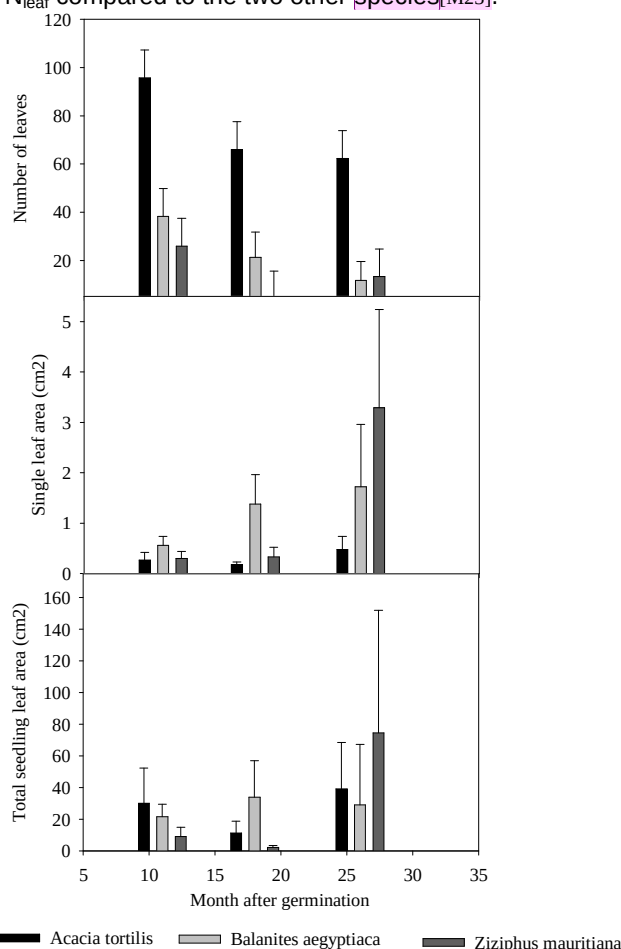


**Fig. 4.** Number of leaves, single leaf area, and total leaf area followed on seedlings from their germination to 26 month old in *Acacia tortilis*, *Ziziphus mauritiana*, and *Balanites aegyptiaca* in a semi-controlled experiment. Seedlings were watered from the start of the experiment until month 23; when the watering was withheld to simulate water stress for the rest of the experiment ended on month 26.

Error bars represent standard errors [M24].

### 3.4 Average single leaf area

The species, the month and the interaction between species × and month had significant effects (Table 2) on the average single leaf area ( $A_{\text{leaf}}$ ) as shown in Table 2 [M25]. *Acacia tortilis* displayed a quite constant  $A_{\text{leaf}}$  of about  $0.31 \text{ cm}^2$  in average over the three measurement dates (Fig. 4). For

*Balanites aegyptiaca*,  $A_{\text{leaf}}$  were 0.56, 1.38 and 1.72 for  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively. Relatively to  $M_{11\_wet}$ , significant increase of  $A_{\text{leaf}}$  was noted in  $M_{26\_dry}$ , ( $p = 0.008$ ), but no difference was observed between  $M_{18\_wet}$  and  $M_{26\_dry}$ . For *Ziziphus mauritiana*,  $A_{\text{leaf}}$  were 0.30, 0.33 and 2.29 for  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively. The water stress led to a significant increase of  $A_{\text{leaf}}$  in  $M_{26\_dry}$  relatively to both  $M_{11\_wet}$  ( $p < 0.001$ ) and  $M_{18\_wet}$  ( $p < 0.001$ ). The three species showed similar  $A_{\text{leaf}}$  in  $M_{11\_wet}$ . In  $M_{18\_wet}$ , only *Acacia tortilis* displayed significantly lower  $A_{\text{leaf}}$  than *Balanites aegyptiaca* ( $p = 0.031$ ). Following the water stress, *Acacia tortilis*  $A_{\text{leaf}}$  significantly decreased relatively to *Balanites aegyptiaca* ( $p = 0.008$ ), and *Ziziphus mauritiana* ( $p < 0.001$ ). *Balanites aegyptiaca* also had significantly lower  $A_{\text{leaf}}$  than *Ziziphus mauritiana* ( $p = 0.05$ ) after the water stress.

### 3.5 Total leaf area of seedlings

Only the month had a significant effect (Table 2) on the total seedling leaf area ( $A_{\text{foliage}}$ ). The average  $A_{\text{foliage}}$  were respectively 26.92 and 28.24 cm<sup>2</sup> in *Acacia tortilis* and *Balanites aegyptiaca*, with no significant fluctuations over the three measurement dates (Fig. 4). For *Ziziphus mauritiana*,  $A_{\text{foliage}}$  were 9.05, 2.21 and 74.57, in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively. A significant increase was noted in  $M_{26\_dry}$  relatively to  $M_{11\_wet}$  ( $p = 0.013$ ). Overall,  $A_{\text{foliage}}$  did not significantly vary between species.

### 3.6 Total number of growth units per seedling

The species and the GU had significant effects on the total number of GU ( $N_{\text{GU}}$ ) (Table 2).  $N_{\text{GU}}$  slightly decreased in *Acacia tortilis* and increased in *Balanites aegyptiaca*, and *Ziziphus mauritiana*, but the effects of water stress were not significant.  $N_{\text{GU}}$  had average values of 10 in *Acacia tortilis*, 21 in *Balanites aegyptiaca*, and 14 in *Ziziphus mauritiana*. The three species showed no significant difference in  $M_{11\_wet}$ . Following the water stress, *Acacia tortilis* had significantly lower  $N_{\text{GU}}$  than *Balanites aegyptiaca* ( $p = 0.005$ ) and *Ziziphus mauritiana* ( $p = 0.037$ ), while all other differences between species remained were not significant.

### 3.7 Total length of growth unit per seedling

The species, GU, and month had significant effects (Table 2) on the total length of GU ( $L_{\text{GU}}$ ).  $L_{\text{GU}}$  was in average 21.67 cm in *Acacia tortilis* with no significant variations (Fig. 5). For *Balanites aegyptiaca*,  $L_{\text{GU}}$  were 25, 79, and 47 in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively.  $L_{\text{GU}}$  significantly increased from  $M_{11\_wet}$  to  $M_{18\_wet}$  ( $p < 0.001$ ), and decreased from  $M_{18\_wet}$  to  $M_{26\_dry}$  ( $p = 0.024$ ). For *Ziziphus mauritiana*,  $L_{\text{GU}}$  were 9, 54, and 26 for  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively. A significant increase was noted between  $M_{11\_wet}$  and  $M_{18\_wet}$  ( $p = 0.005$ ). In  $M_{11\_wet}$ ,  $L_{\text{GU}}$  did not vary between the three species. However, *Acacia tortilis* had significantly shorter GU than *Balanites aegyptiaca* in both  $M_{18\_wet}$  ( $p = 0.004$ ) and  $M_{26\_dry}$  ( $p = 0.04$ ).

### 3.8 Total number of nodes per seedling

Significant effects of species, GU and month were noted in the number of nodes ( $N_{\text{node}}$ ). For *Acacia tortilis*,  $N_{\text{node}}$  were 25, 43 and 44 in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively (Fig. 5), with a significant positive shift in  $M_{18\_wet}$  ( $p = 0.019$ ) and  $M_{26\_dry}$  ( $p = 0.01$ ) compared to  $M_{11\_wet}$ . *Balanites aegyptiaca* had 6, 36 and 36 nodes in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , with significant increase in  $M_{18\_wet}$  ( $p < 0.001$ ) and  $M_{26\_dry}$  ( $p < 0.001$ ) relatively to  $M_{11\_wet}$ . For *Ziziphus mauritiana*,  $N_{\text{node}}$  were 7, 25, and 26 in  $M_{11\_wet}$ ,  $M_{18\_wet}$  and  $M_{26\_dry}$ , respectively. However, relatively to  $M_{11\_wet}$ ,  $N_{\text{node}}$  significantly increased in  $M_{18\_wet}$  ( $p < 0.008$ ) and  $M_{26\_dry}$  ( $p < 0.006$ ). *Acacia tortilis* had significantly lower  $N_{\text{node}}$  than *Balanites aegyptiaca* ( $p = 0.05$ ) in  $M_{18\_dry}$ . Following the water stress, *Acacia tortilis* showed significantly lower  $N_{\text{node}}$  compared to *Balanites aegyptiaca* ( $p = 0.005$ ) and *Ziziphus mauritiana* ( $p = 0.037$ ).

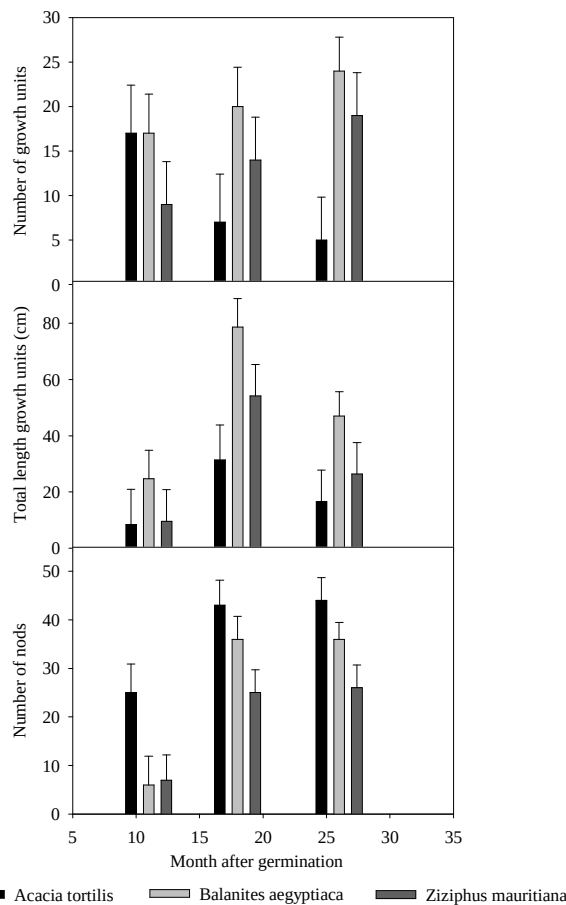


**Fig. 5. Number of growth units, their total length, and the number of nodes followed on seedlings from their germination to 26 month old in *Acacia tortilis*, *Ziziphus mauritiana*, and *Balanites aegyptiaca* in a semi-controlled experiment. Seedlings were watered from the start of the experiment until month 23; when the watering was withheld to simulate water stress for the rest of the experiment ended on month 26.**

Error bars represent standard errors.

This study aimed to understand how leaf traits and branching patterns of *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* seedlings are linked with soil water availability kept experimentally favorable, then depleted to create conditions of water stress. Two main information can be drawn from this study. The first one pertains to the patterns displayed within one species, and the second one lays on the difference between species in their response to drought.

### 3.9 Variation within species

Despite the favorable soil water status due to the watering, all the species shed their leaves, even slightly, in the dry season, but not translating into total leaf area reduction. In their natural range, a systematic defoliation also occurred in hot dry season for *Acacia tortilis* [7], and *Ziziphus mauritiana* [9]. *Balanites aegyptiaca* also shed its leaves in hot dry season but this defoliation overlapped with the next leaf flush [9]. In addition, a systematic defoliation of *Acacia tortilis*, grown in the Negev where it was introduced, occurred when soil water status was locally favorable, corresponding to the hot dry season of their origin in Africa [22]. The defoliation at least for *Acacia tortilis* may therefore be related to an endogen control, and it is probable that the same mechanism played for *Balanites aegyptiaca* and *Ziziphus mauritiana*, as well. In their natural range where drought is real at this time of the year, this could be seen as an escape strategy because maintaining a lower foliage in hot dry season is conducive to some extent to tight regulation of leaf transpiration. Hence, soil water availability is seldom, if ever, to be the only variable explaining leaf shading in dry season.

*Balanites aegyptiaca* and *Ziziphus mauritiana* increased their growth unit lengths in the middle of the dry season in response to favorable soil water availability while *Acacia tortilis* showed a stronger seasonality of its GU elongation. This also indicates that *Balanites aegyptiaca* and *Ziziphus mauritiana* elongate their twigs in dry season to be able to respond to soil water experimentally increased in this period. Following the water stress, the GU length only decreased for *Balanites aegyptiaca* confirming suggesting the active phase of GU elongation in dry season and the plastic behavior to water stress of this species. Based on GU patterns, *Ziziphus mauritiana* displayed higher tolerance to stress as shown by its consistent growth GU despite the water stress. The greater number of GU and nodes in *Acacia tortilis* consecutively to the water stress resulted in reduced internode distances, ending up with a greater number of nodes per unit of leafy axis. The study illustrates the statement according to which, measurement of shoot or twig is an effective way to assess fine scale plant response to environmental stimuli [23].

### 3.10 Variation between species

The water stress was followed by a marked decrease in single leaf area for *Acacia tortilis*, but an increase for *Balanites aegyptiaca* and *Ziziphus mauritiana*, however not resulting in significant variation in total leaf area. However, the slight defoliation generally observed, was enough to maintain a consistent total leaf area despite the significant change in single leaf area in the three species. *Acacia tortilis* had generally more leaves than *Balanites aegyptiaca* and *Ziziphus mauritiana* all the time, even before the stress was applied. Earlier studies have reported leaf area decrease due to water stress in *Acacia tortilis* [13], *Balanites aegyptiaca* [24] and in the genus *Ziziphus* [15]. Water stress has been shown to reduce leaf area through reduced leaf initiation, leaf size and leaf production rate [13]. We also observed in *Acacia tortilis* a reduced leaf production rate, even if it was not significant. The advantage of having higher number of leaves associated with lower size single leaf areas is to allow more plasticity in leaf surface area regulation. Small leaves ensure for convective heat loss that improves cooling in hot, dry conditions, and a tight control of transpiration [25]. Unexpectedly, *Ziziphus mauritiana* had greater single leaf areas following the water stress. *Balanites aegyptiaca* also positively reacted to water stress, but in comparison with first measurement date and not the second. To be able to increase their single leaf area while maintaining a consistent total leaf area following the water stress, *Balanites aegyptiaca* and *Ziziphus mauritiana*, have to shed proportionally their leaves to balance their single leaf area increase. After the rainy season of 2004 with 234 mm of rainfall, the seedlings remained 3 weeks without additional water in soil. *Balanites aegyptiaca* and *Ziziphus mauritiana* may have kept their leaves flushed during this previous rainy season or sooner while the seedlings were still watered. Three weeks of water stress seemed to be too short for *Balanites aegyptiaca* and *Ziziphus mauritiana* to shed their leaves, showing that they can withstand water stress, for this duration longer, by keeping their leaves than *A. tortilis* [M33].

### 3.11 What can we learn so far?

*Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* naturally occur in the same range characterized by harsh climate conditions with frequent drought episodes. They have sclerophyllous leaves and can withstand severe water stress. But unlikely to the hypothesis in which we stated that *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* may have similar trend in leaf traits and branching patterns in response to water stress because they naturally coexist, the current research does not support such evidence. [M34] Two clear patterns emerge from this study. *Acacia tortilis* bears more leaves which are also smaller than those of their counterparts, as a strategy to cope with soil water depletion [M35]. *Balanites aegyptiaca* and *Ziziphus mauritiana* can withstand water stress with increased single leaf area, because they might have kept their greater size leaves flushed whether during the previous rainy season or earlier when the seedlings were still watered. In addition, *Balanites aegyptiaca* decreased its GU length following the water stress. Overall, larger plasticity in response to drought was observed in *Acacia tortilis* compared to *Balanites aegyptiaca* and *Ziziphus mauritiana*. In addition, within a given species, the same seedlings were followed during the course of the experiment, at different ages. However it is unlikely that the age of the seedlings influenced the leaf size, since individual leaf area was shown to have a modest increase with plant size [26]. In a typical common garden experiment, many provenances are grown in a common area to study the phenotypic variability. In our experiment, the seeds which have been germinated were rather collected in a restricted area where the species naturally coexist. The differences in leaf traits and branching patterns may therefore not be attributed to a genetic variability leading to a phenotypic divergence within the species.

#### 4. CONCLUSION

In the Sahel, a semi-arid tropical area of Africa where were collected the geminated seeds used in this experiment, soil water availability is the main environmental driving factor. *Acacia tortilis*, *Balanites aegyptiaca* and *Ziziphus mauritiana* naturally coexist in these areas and the understanding of how they cope with water shortage is essential to implement guidelines for a more efficient assisted recruitment. This study provides to these purposes important information on leaf traits and branching patterns related to soil water availability. In contrast with what we hypothesized, species naturally coexisting do not necessarily displayed similar trend in leaf traits and branching patterns in response to drought. Although straightforward, the study was limited to three weeks of water stress. Perspectives for future studies suggest extending the drought period.

#### REFERENCES

1. Le Houérou HN. Definition, geographical limits, contacts with other ecoclimatic zones. In: The grazing land ecosystems of the African Sahel, Springer Berlin Heidelberg; 1989.
2. Lawesson JE. Sahelian woody vegetation in Senegal. *Vegetatio*. 1990; 86: 161-174.
3. Couteron P, Kokou K. Woody vegetation spatial patterns in a semi-arid savanna of Burkina Faso, West Africa. *Plant Ecol*. 1997; 132(2): 211-227.
4. Deines JM, Hellmann JJ, Curran TJ. Traits associated with drought survival in three Australian tropical rainforest seedlings. *Aust. J. Bot.* 2011; 59(7): 621-629.
5. Ngaryo FT, Goudiaby VA, Dagbenonbakin GD, Agbangba EC, Diatta S, Akpo LE. Modeling the production of Arabic gum (*Acacia senegal* (L.) Willd) based on the proper tapping characteristics in the semi arid Sahel of Chad. *International Journal of Science and Advanced Technology*. 2011; 1(8): 24-30.
6. Grouzis M, Sicot M. A method for the phenological study of browse populations in the Sahel: the influence of some ecological factors. In: *Browse in Africa, the current state of knowledge*, Le Houérou éd. ILCA, Addis Abeba; 1980.
7. Do FC, Goudiaby VA, Gimenez O, Diagne AL, Diouf M, Rocheteau R, Akpo LE. 2005. Environmental influence on canopy phenology in the dry tropics. *Forest Ecol. Manag.* 2005; 215: 319-328.
8. De Bie S, Ketner P, Paasse M, Geerling C. Woody plant phenology in the West Africa savanna. *J. Biogeogr.* 1998; 25(5): 883-900.
9. Seghier J, Do CF, Devineau JL, Fournier A., 2012. Phenology of Woody species along the climatic gradient in West Tropical Africa. In: *Phenology and Climate Change*, Dr. Xiaoyang Zhang (Ed.), In Tech; 2012.
10. Akpo LE. Phenological interactions between tree and understory herbaceous vegetation of a sahelian semi-arid savanna. *Plant Ecol.* 1997; 131(2): 241-248.
11. Mishra RK, Upadhyay VP, Bal S, Mohapatra PK, Mohanty RC. Phenology of species of moist deciduous forest sites of Similipal biosphere reserve. *Lyonia*. 2006; 11(1): 5-17.
12. Grice C. Ecology in the Management of Indian Jujube (*Ziziphus mauritiana*). *Weed Science*. 1998; 46(4): 467-474.
13. Otieno DO, Schmidt MWT, Adiku S, Tenhunen J. Physiological and morphological responses to water stress in two *Acacia* species from contrasting habitats. *Tree Physiol.* 2005; 25(3): 361-371.
14. Elfeel AA, Warrag EI, Musnad HA. Response of *Balanites aegyptiaca* (L.) Del. seedlings from varied geographical source to imposed drought stress. *Discov. Innov.* 2007; 184: 319-325.
15. Arndt SK, Clifford SC, Wanek W, Jones HG, Popp M. Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. *Tree Physiol.* 2001; 21(11): 705-715.
16. Xu F, Guo W, Xu W, Wei Y, Wang R. Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves? *Prog. Nat. Sci.* 2009; 19(12): 1789-1798.

- 401 17. Diagne Y. Boisements et paysages dans la ville de Dakar. Mémoire de maîtrise, UCAD, FLSH,  
402 Dakar, 1988. French.
- 403 18. Ngaryo FT, Akpo LE, Goudiaby, VA, Badiatte AK, Logbo J, Diouf M. Estimation de la surface  
404 foliaire de jeunes plants de trois essences sahéliennes : *Acacia tortilis*, *Balanites aegyptiaca* et  
405 *Ziziphus mauritiana* en conditions semi naturelles (Dakar, Sénégal). Annales de l'Université de  
406 N'Djaména. 2007 ; Série C N°2 : 27-41. French.
- 407 19. Goudiaby V, Brais S, Grenier Y, Berninger F. Thinning effects on jack pine and black spruce  
408 photosynthesis in Eastern boreal forests of Canada. *Silva Fennica*. 2011; 45(4): 595-609.
- 409 20. Goudiaby V, Brais S, Berninger F, Schneider R. Vertical patterns in specific volume increment  
410 along stems of dominant jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*) after thinning.  
411 *Can. J. For. Res.* 2012; 42(4): 733-748.
- 412 21. Barthélémy D, Caraglio Y. Plant architecture: A dynamic, multilevel and comprehensive approach  
413 to plant form, structure and ontogeny. *Ann. Bot.* 2007; 99:375-407.
- 414 22. Halevy G, Orshan G. Ecological studies on *Acacia* species in the Negev and Sinai. II. Phenology  
415 of *Acacia raddiana*, *A. tortilis* and *A. gerrardii negevensis*. *Israël J. Bot.* 1973; 22: 120-138.
- 416 23. Naydenov K, Tremblay F, Bergeron Y, Goudiaby V. Effect of active charcoal on the primary growth  
417 of jack pine seedlings, Influence du charbon actif sur la croissance primaire des plantules de pin gris.  
418 *Can. J. For. Res.* 2006; 36(3): 761-767.
- 419 24. Elfeel A, Warrag E, Musnad H. 2007. Response of *Balanites aegyptiaca* (L.) Del. seedlings from  
420 varied geographical source to imposed drought stress. *Discov. Innov.* 2007; 184:319-325.
- 421 25. Yates MJ, Anthony Verboom G, Rebelo AG, Cramer MD. Ecophysiological significance of leaf size  
422 variation in *Proteaceae* from the Cape Floristic Region. *Funct. Ecol.* 2010; 24(3): 485-492.
- 423 26. Price CA, Wright IJ, Ackerly DD, Niinemets U, Reich PB, Veneklaas EJ. Are leaf functional traits  
424 'invariant' with plant size and what is 'invariance' anyway? *Funct. Ecol.* 2014: 1-14.