

Article

Growth and Physiology of *Senegalia senegal* (L.) Britton Seedlings as Influenced by Seed Origin and Salinity and Fertility Treatments

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Abstract: Multipurpose trees such as *Senegalia senegal* are widespread in arid and semi-arid lands that have natural or induced saline soils and poor soil fertility. Such environmental problems impact growth and have the potential to influence plant physiological adaptations. Identifying superior genotypes better adapted to these environmental stresses will be of great importance for tree selection for reclamation of degraded drylands. The main objective of this study was to examine the growth performance, and physiological and morphological adaptations to salinity, and fertility treatments of different *Senegalia senegal* families. We used five families (DB16, DB14, K4B19, K17B19, NB1) selected from 60 families of a *Senegalia senegal* progeny trial in Dahra, Senegal. Seedlings were grown under greenhouse conditions by watering all plants for three weeks and then stopping all watering for three more weeks. In a randomized complete block design, a two-level factorial combination was used for salinity (zero and 183.1 mM NaCl added) and fertility (zero and 100 kg/ha N-P-K added) treatments. A significant family \times salt \times fertilizer interaction was found for all biomass parameters (leaf dry matter, stem dry matter, root dry matter, and leaf area). The fertilizer application resulted in a significant increase of total biomass of all families, ranging from 63% to 237% for NB1 and K17B19, respectively. In contrast, salt only decreased total biomass of NB1 and K17B19 increased growth. Despite similar net photosynthetic rates before treatment started, fertilizer and salinity induced different effects between families. Prior to drought stress, fertilizer did not affect photosynthesis of DB16, while salt significantly decreased stomatal conductance of all families. DB16 and NB1, despite significant differences of stomata size and density, significantly decreased transpiration, and thereby increased their intrinsic water use efficiency. Under drought, relative growth rate was significantly decreased. Given that genotype differences were found, these families and salinity and fertilizer treatments need to be explored in field trials.

Keywords: *Senegalia senegal*; salt tolerance; fertilizer; drought

1. Introduction

In recent years, frequent drought occurrences and anthropogenic pressures on forest resources in arid and semi-arid lands have heavily impacted species composition [1]. The natural vegetation becomes sparse and progressive soil degradation develops, which accelerates desertification and induces salinization [2–4]. Drought and salt tolerant species are seeking for restoration of such affected lands. *Senegalia senegal* is a drought tolerant species, widely used to control desertification in arid and semi-arid lands. It is particularly exploited because it is the source of many products, including gum arabic which is traded on both local and international markets. Recent studies have revealed its potential tolerance to salinity stress [5–7]. However, salt tolerance among local *Senegalia senegal*

provenances has not yet been investigated. Salt-affected lands in Senegal are estimated at about 1.7 million hectares [8]. Due to increasing population, it is likely that, in the future, tree crops and fuel wood plantations will increase on degraded lands in general and particularly on salt affected soils.

In arid and semi-arid areas, salt stress is often combined with water limitations in the context of poor soil management. Afforestation/reforestation plans are often proposed solutions for soil improvement of degraded lands. However, plantation failures can occur when inappropriate species are selected. An understanding of plant response to salinity is of great importance for reclamation of salt affected lands. Yet, strategies to improve salt and/or drought tolerance in such ecosystems require an understanding of tree physiological mechanisms that links their growth and adaptation. Growth characteristics and photosynthesis efficiency are traditionally used to select higher quality family or genotypes more adapted to hostile environments. Stomatal response is among one of the most important factors influencing photosynthetic capacity under limiting environmental factors [9]. Different stomatal characteristics may account partially for different stomatal resistance that consequently impact the photosynthetic rate [10]. However, little attention has been given to the leaf physiology of *Senegalia senegal*, and particularly stomata characteristics. Under water stress, stomatal density is known to impact leaf photosynthesis and water use efficiency [11,12]. Reduced stomatal density can be advantageous under saline stress [13,14]. Drought and salinity stress both induce a reduced photosynthetic rate through stomata and mesophyll restriction [15,16]. Furthermore, selection of adapted genotypes based on leaf traits and water use efficiency to such environments are highly recommended.

This study investigates adaptive features in *Senegalia senegal* from different families which may allow them to grow better in both saline and dry conditions. The main objective is to examine physiological and morphological changes among *Senegalia senegal* provenances subjected to salinity and fertility treatments. We tested the hypothesis that salinity tolerance and fertilizer effect on the physiology and biomass accumulation of *Senegalia senegal* seedlings are influenced by seed origin. We expected that leaf gas exchanges, growth and biomass accumulation will decrease under the effect of salinity whereas the fertilizer may increase those potentials. We are interested in seeking genotypes that perform best in terms of dry matter accumulation and intrinsic water use efficiency under well-watered and then drought conditions, and whether stomatal characteristics explain potential changes among families and environmental conditions.

2. Materials and Methods

2.1. Plant Material

Senegalia senegal families from five randomly selected mother trees (seed origin) were used in this study. Seeds were harvested in January 2015 from each of the 5 different trees in a 20-year-old *Senegalia senegal* progeny trial in Dahra, Senegal. Ngane21B1, Kidira4B19, and Diamenar27B14 maintained a relatively high gum production over 4 successive years while Kidira17B19 and Diamenar27B16 displayed low gum yields for the same period (Table 1). Each tree label represents a mother tree, and families within the same name and different numbers belong to the same provenance.

Table 1. Families and mean annual gum yields (g) by tree of the mother trees from which seeds in this study were harvested. (-) indicates that the tree died.

Provenance	Families	Tree Label	2013 Gum Yield	2014 Gum Yield	2015 Gum Yield	2016 Gum Yield
Diamenar	Diamenar27B16	DB16	29.3	9.7	0	-
Diamenar	Diamenar27B14	DB14	899.5	791.7	386.1	758.3
Kidira	Kidira4B19	K4B19	555.3	551.8	583.3	990.3
Kidira	Kidira17B19	K17B19	131.3	62.1	22.2	51.7
Ngane	Ngane21B1	NB1	522.2	476.4	945.2	1103.5

2.2. Growth Conditions, Treatment, and Experimental Design

The experiment was conducted from June to October 2015 in a greenhouse where the temperature was maintained above 25 °C and ventilated with box fans when temperatures went above 32 °C. Light was extended using sodium vapor lights to maintain a 16-h photoperiod. Seeds were chemically scarified with concentrated sulfuric acid (98%) in order to perforate the tough seed coat and then rinsed with water to remove the acid before germinating them in shallow flats filled with Promix BX as a growth medium. Once emerged, young germinals were transplanted individually into small tubes (SC10 super cell, 3.8 cm diameter × 21 cm deep, Stuewe and Sons, Tangent, OR, USA) containing Promix and kept well-watered for about six weeks. They were then transplanted into 2.3-liter plastic pots (Treepots, 30.5 cm height and 10.2 cm width, IGC Greenhouse Megastore, Danville, IL, USA) containing either soil substrate (a 2:1 mix of Promix BX and washed sand) or soil substrate with salt added. A factorial combination of salinity and fertilizer treatments was then established.

The salinity treatment consisted of mixing 1070 g of sodium chloride with 100 kg of soil substrate. The salt treatment used was based on an early study which revealed a significant decrease of about 50% on biomass of *Senegalia senegal* seedlings [17]. The average corresponding electrical conductivity of the saline soil was 7.525 dSm⁻¹, whereas the electrical conductivity of a control soil was 0.31 dSm⁻¹. For the measurement of electrical conductivity, a soil suspension was prepared in distilled water at a ratio of 1:2. The suspension was shaken and allowed to stand overnight [17]. Thereafter, electrical conductivity of the supernatant solution was determined with an electrical conductivity meter (2265FS/2265FSTP, Spectrum Technologies, Inc., Aurora, IL, USA). The fertilization treatment consisted of applying 2.3 g of N-P-K (10-10-10) in each pot to give the equivalent fertilizer supply of 100 kg/ha. The experiment was designed as a randomized complete block with five replications of the 2 by 2 factorial treatments (no salt/no fertilizer (control), salt only, fertilizer only, and salt plus fertilizer) making a total of 100 experimental units.

When treatments started, all plants were kept well-watered for three weeks, and then the drought stress treatment was started where all watering was stopped in all treatments for three more weeks. This procedure was adopted to mimic field conditions (alternation between a wet season and a dry season) under which *Senegalia senegal* is naturally subjected. The induction of drought in this experiment is not considered as a treatment factor, but a common condition subjected to all experimental units. Prior to drought stress, shallow trays were placed beneath pots that received the salt treatment (the salted and salt-fertilized) in order to recover any salt discharge which occurred when pots were watered. Any recovered water was then poured back into each tree pot. This step was to maintain the same salt concentration in each pot prior drought stress.

2.3. Stomatal Density and Stomatal Size Measurements

Stomatal density and size were assessed at the end of the experiment before seedlings were harvested by using a stereo zoom microscope SMZ 1500 (Nikon Corporation, Chiyoda-ku, Tokyo, Japan) equipped with a digital camera (Nikon digital sight). A colorless nail polish was coated on the abaxial and adaxial leaflet surfaces from the newest fully mature leaf, then leaflets were mounted on glass slides. Images were taken on both leaf faces at 70× magnification.

Stomatal density was expressed as the number of stomata per mm² and stomatal size reflected the length in micrometers between the junctions of the guard cells for each stoma. Two leaflets per face and per plant were sampled and 2 subsamples of 0.01 mm² were measured on each leaflet face. The number of stomata for each sample was counted under a photomicroscope system with a computer attachment.

2.4. Leaf Gas Exchanges and Chlorophyll Content Measurements

Measurements of net photosynthetic rate (A), stomatal conductance (G_s), and transpiration (E) of a fully expanded leaf per plant were made using a portable infrared gas analyzer (LI-6400, Lincoln, NE, USA). Environmental conditions in the leaf cuvette were set to 2000 μmol m⁻² s⁻¹ photosynthetic

photon flux density (PPFD), 398 $\mu\text{mol s}^{-1}$ reference CO_2 concentration, 25 °C block temperature, and a flow rate of 400 $\mu\text{mol s}^{-1}$. Gas exchange was measured weekly from day 0 (when planted into large pots with fertilizer and salt) for the 6 weeks of the experiment. The intrinsic water use efficiency (WUE_i) was calculated as the ratio of photosynthetic to transpiration rates. Total chlorophyll content (mg/g) was estimated at the same time in intact leaves using a portable chlorophyll meter CCM-300 (Opti-Sciences Inc., Hudson, NH, USA). Three leaflets per plant were randomly chosen from new fully mature leaves and an average taken for analysis.

2.5. Relative Growth Rate and Biomass

The relative growth rates of height (RGH, $\text{cm cm}^{-1}\text{day}^{-1}$) and diameter (RGD, $\text{mm mm}^{-1}\text{day}^{-1}$) were determined [18], where $\text{RGR} = (\ln X_2 - \ln X_1)/(t_2 - t_1)$, t is the number of days, X is either plant height or diameter, $\ln X$ is the natural log of height or diameter.

All plants were harvested at the end of the experiment. We manually washed soil from roots and then separated leaf, stem, and root from each other. Root area from each plant was measured by using WinRHIZO Software (version 2005b Pro, Regent Instruments Inc., Québec, Canada) and an EPSON Expression 1680 scanner (Epson America Inc., Long Beach, CA, USA). The area of leaves for each plant was determined with Photoshop software CS6 (version 13.0.1, Adobe Systems Inc., Mountain View, CA, USA). Different plant parts were then dried at 65 °C to a constant weight before weighing them to determine dry matter.

2.6. Statistical Analysis

Data were analyzed using JMP software (version 12, SAS Institute, Cary, NC, USA). Normality and constant variance were examined across all data. We used a log (natural) transformation of the root/shoot ratio for analysis to meet assumptions of analysis of variance (ANOVA). Also, an outlier identified from specific leaf area dataset was omitted in the analysis. A full factorial model analysis was conducted to test treatments and family effects on all measured variables twice, once under well-watered conditions (prior drought) and then again under water stress conditions (drought). Student's t -test and Tukey-Kramer Honestly Significance Difference were used for separation of means tests.

3. Results

3.1. Prior Drought Conditions

3.1.1. Leaf Gas Exchanges and Chlorophyll Content

Prior to drought stress, a family \times fertilizer, and family \times salt interaction was significant for A , G_s , E , and WUE_i (Table 2). No interaction effect was found to be significant for total chlorophyll content, which was similar among families at the starting treatment ranging from 264.7 mg m^{-2} (K17B19) to 330.0 mg m^{-2} (in K4B19). However, main effects of salt ($p < 0.0001$) and fertilizer ($p < 0.0001$) (Table 2) were found on total chlorophyll content. Salt and fertilizer increased chlorophyll from 275.3 to 314.20 mg m^{-2} and from 281.2 to 309.1 mg m^{-2} , respectively.

With the exception of DB16, fertilizer significantly increased A (Figure 1a,b). G_s and E were also generally increased with fertilizer, but were only significantly increased in a few families (Figure 1a). Since both A and E increased with fertilizer, the WUE_i was generally unaffected by fertilizer. However, KB19 displayed higher WUE_i . The salt treatment significantly decreased G_s in all families and E was lower in all families, but only significantly decreased in DB16, K4B19 and NB1. A was lower in the salt treatments in all families, but only significantly decreased in K4B19. WUE_i was increased in all families, but only significantly in DB16 and NB1 (Figure 1b).

Table 2. Analysis of variance (ANOVA) results for the response of different *Senegalia senegal* families to salinity and fertility for leaf gas exchanges (net photosynthetic rate (A), stomatal conductance (G_s), transpiration (E), and intrinsic water use efficiency (WUE_i)), relative growth of height (RGH) and diameter (RGD), and chlorophyll content.

Sources of Variation	df	p-Value						
		RGH	RGD	A	G_s	E	WUE_i	Chlorophyll Content
Salinity	1	0.5807	0.2407	0.0025	<0.0001	<0.0001	<0.0001	<0.0001
Fertility	1	<0.0001	0.0023	<0.0001	0.0008	0.0133	0.1899	<0.0001
Family	4	0.0004	0.3738	0.9632	0.0375	0.3877	0.0054	0.0922
Family \times salinity	4	0.6415	0.7013	0.622	0.0188	0.0494	0.079	0.3469
Family \times fertility	4	0.7818	0.1669	0.0302	0.1636	0.0265	0.0246	0.4621
Family \times salinity \times fertility	4	0.1288	0.6976	0.8522	0.5166	0.9227	0.8606	0.5800

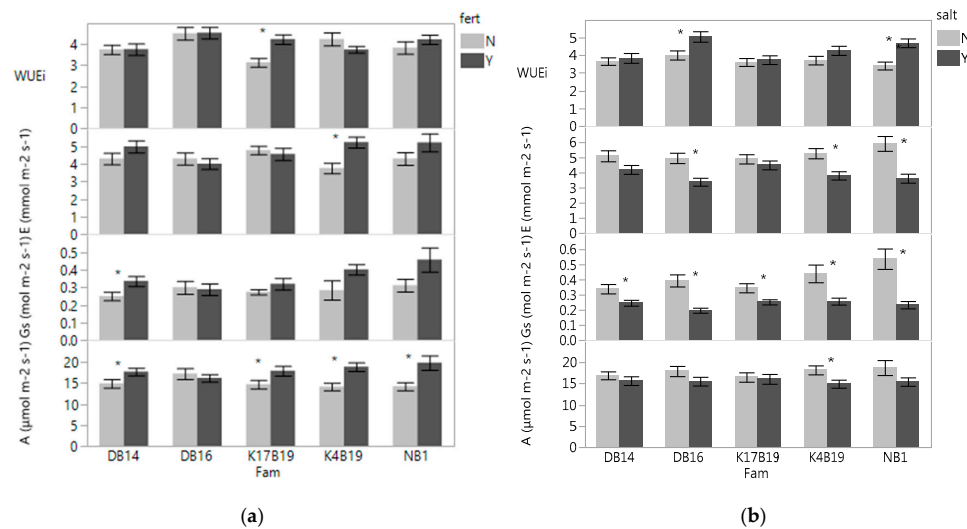


Figure 1. The effect of fertilizer (a) and salt (b) treatments on net photosynthetic rate (A), stomatal conductance (G_s), transpiration (E), and intrinsic water use efficiency (WUE_i) of *Senegalia senegal* families. Asterisk marks on bars mean significant effect of fertilizer (a) and salt (b) at $p < 0.05$.

3.1.2. Relative Growth Rate

Relative growth rate of height of *Senegalia senegal* seedlings was significantly different among families (Table 2, Figure 2) varying between 1.18% for NB1 and 0.80% for K17B19. Significantly, NB1 had the highest RGH in comparison with the rest of the families, which did not differ significantly.

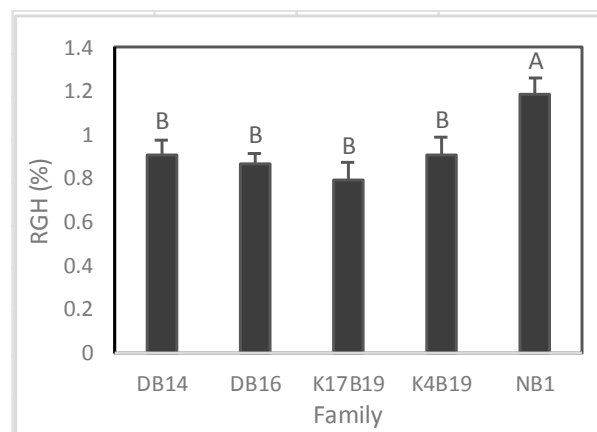


Figure 2. Variation of relative growth rate of height (RGH) among *Senegalia senegal* families. Different letters on bars mean significant differences among families at $p < 0.05$.

Salinity had no significant effect on the relative growth (Table 2), whereas the fertilizer significantly increased RGH and RGD by 43% and 24%, respectively (Figure 3).

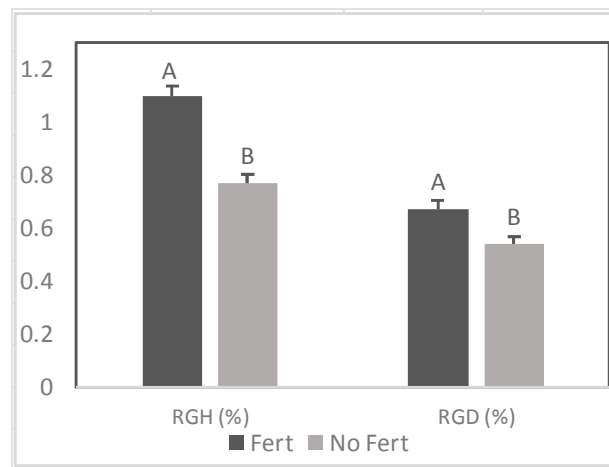


Figure 3. Effect of fertilizer (Fert) on relative growth rate of height (RGH) and diameter (RGD) among *Senegalia senegal* seedlings. Different letters on bars mean significant differences between fertilized (Fert) and no fertilized (No Fert) at $p < 0.05$.

3.2. Under Drought Conditions

3.2.1. Stomatal Density and Size

At the end of 3 weeks of drought, we found the presence of stomata on both abaxial and adaxial leaf surfaces and stomatal density and length did not differ significantly between leaf surfaces. Hence, stomatal density was considered to be the average of abaxial and adaxial surfaces. No family by treatment interactions were found to be significant in stomatal density and stomatal size (Table 3). However, salinity significantly increased stomatal density from 82 to 89 stomata per mm^2 , and a significant effect of family was found on both stomatal length and distribution (Figures 4 and 5a,b).

The Diamenar provenance (DB16) had the highest stomatal density but was only significantly greater than Ngane NB1 (Figure 5a). Ngane displayed significantly larger stomata than all other provenances and significantly lower stomatal density than the Diamenar provenance (DB14 and DB16) (Figures 4 and 5).

Table 3. Analysis of variance (ANOVA) results for the response of different *Senegalia senegal* families to salinity and fertility for stomatal density and size, total biomass, specific leaf area (SLA), root/shoot, leaf gas exchanges (net photosynthetic rate (A), stomatal conductance (G_s), transpiration (E), and intrinsic water use efficiency (WUE_i)), relative growth of height (RGH) and diameter (RGD), and chlorophyll content following a drought period.

Sources of Variation	df	<i>p</i> -Value											
		Stomatal Density	Stomata Size	Total Biomass	SLA	Root/Shoot	RGH	RGD	A	G _s	E	WUE _i	Chlorophyll Content
Salinity	1	0.0158	0.2604	<0.0001	0.0059	<0.0001	0.0435	0.6073	0.452	<0.0001	<0.0001	<0.0001	<0.0001
Fertility	1	0.6565	0.91	<0.0001	0.6169	<0.0001	0.623	0.0012	0.2827	0.0142	0.0057	<0.0001	<0.0001
Family	4	0.0091	<0.0001	<0.0001	0.0159	0.023	0.0421	0.0019	0.4944	0.7874	0.3642	<0.0001	0.2614
Family × Salinity	4	0.2459	0.5518	0.0634	0.9215	0.2997	0.2108	0.4326	0.0438	0.1426	0.5021	0.233	0.6956
Family × fertility	4	0.7811	0.0733	0.6723	0.2896	0.1454	0.1914	0.5758	0.9961	0.4089	0.514	0.2803	0.5889
Family × Salinity × fertility	4	0.5933	0.6437	0.0002	0.2343	0.7375	0.0925	0.1375	0.4873	0.1361	0.3306	0.0410	0.7770

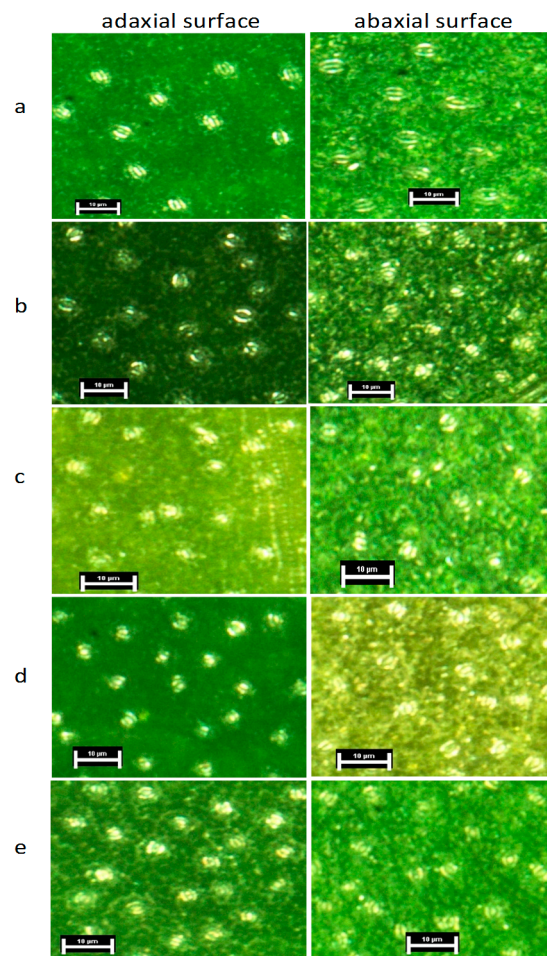


Figure 4. Stomata on the abaxial and adaxial leaf surfaces of 12-week-old *Senegalia senegal* seedlings. NB1 (a), K4B19 (b), K17B19 (c), DB14 (d), DB16 (e).

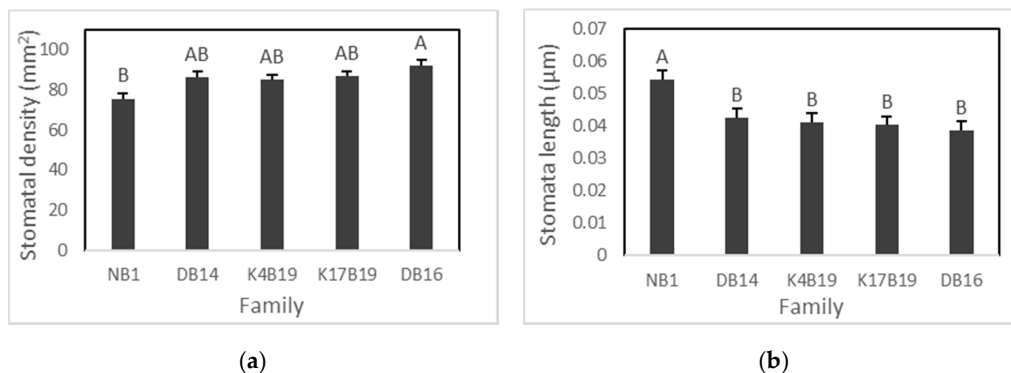


Figure 5. Variation of stomatal density (a) and size (b) among *Senegalia senegal* families. Different letters on bars mean significant differences among families at $p < 0.05$.

3.2.2. Leaf Gas Exchanges

There was a significant salt \times family \times fertilizer and salt \times family interactions for WUE_i and A , respectively (Table 3). The magnitude of the effect varied with family (Figure 6). Gas exchange rates were lower after the drought stress and the salt treatment generally still decreased G_s and E , and increased WUE_i .

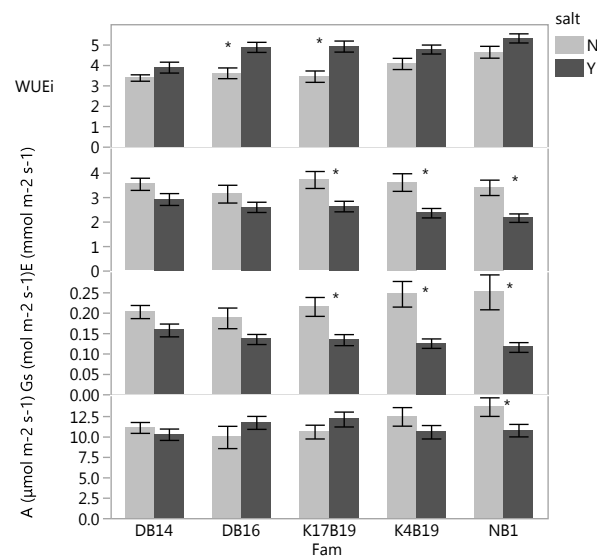


Figure 6. Effect of salt on net photosynthetic rate (A), stomatal conductance (G_s), transpiration (E), and intrinsic water use efficiency (WUE_i) of *Senegalia senegal* families under drought stress. Asterisk marks on bars mean significant effect of salt at $p < 0.05$.

3.2.3. Biomass and Relative Growth Rates

There was a significant family \times salt \times fertilizer interaction for all biomass parameters (Table 4). K17b19 was the only family which increased biomass in response to the salt treatment. Total weight, leaf area, and root weight in this source were increased by 51%, 17%, and 47%, respectively. Ng21B1 growth was significantly decreased by the salt (Table 4). No other family had growth changed by the salt treatment. Growth was generally increased (more than 100%) in all families in response to added fertilizer (Table 4). Salt generally had a negative impact on this fertilizer growth enhancement.

Table 4. The effect of salt and fertilizer treatments on growth parameters of *Senegalia Senegal* families.

Variable	Treatment	Families				
		DB14	DB16	K4B19	K17B19	NB1
Leaf (g)	Control	1.4 \pm 0.47Aa	1.7 \pm 0.28Aab	2.3 \pm 0.61ABab	1.5 \pm 0.17Aa	3.7 \pm 0.40Bb
	Salt	1.4 \pm 0.24Aa	1.6 \pm 0.12Aa	1.7 \pm 0.18Aa	2.1 \pm 0.16Aab	1.6 \pm 0.17Aa
	Fert	4.2 \pm 0.11Ab	4.9 \pm 0.45Ac	4.2 \pm 0.33Abc	4.8 \pm 0.41Ac	5.3 \pm 0.30Ab
	Salt \times fert	2.9 \pm 0.09BCab	2.3 \pm 0.21Aab	3.7 \pm 0.36ABbc	3.3 \pm 0.30ABCbc	4.2 \pm 0.32Cb
Stem (g)	Control	1.3 \pm 0.40Aa	1.9 \pm 0.34Aa	1.7 \pm 0.47Aa	1.2 \pm 0.16Aa	2.7 \pm 0.23Aab
	Salt	1.3 \pm 0.23Aa	2.0 \pm 0.11Ba	1.8 \pm 0.14ABa	2.1 \pm 0.15Bab	1.5 \pm 0.17ABa
	Fert	3.9 \pm 0.24Ab	5.3 \pm 0.65Ac	4.8 \pm 0.62Ab	5.0 \pm 0.52Ac	5.2 \pm 0.25Ac
	Salt \times fert	3.0 \pm 0.17ABab	2.5 \pm 0.21Aab	4.0 \pm 0.35Bb	3.3 \pm 0.31ABbc	4.2 \pm 0.35Bbc
Root (g)	Control	2.0 \pm 0.35ABab	2.7 \pm 0.17ABab	2.9 \pm 0.45ABa	1.7 \pm 0.25Aa	3.3 \pm 0.29Bab
	Salt	1.5 \pm 0.30Aa	2.4 \pm 0.22ABab	2.6 \pm 0.38Ba	2.5 \pm 0.25ABab	1.7 \pm 0.07ABa
	Fert	3.9 \pm 0.36Ac	5.4 \pm 0.40Ac	5.2 \pm 0.34Ac	5.1 \pm 0.68Ac	5.2 \pm 0.20Ac
	Salt \times fert	1.8 \pm 0.19Aab	1.7 \pm 0.15Aa	3.3 \pm 0.14Bab	2.0 \pm 0.22Aab	3.6 \pm 0.41Babc
Leaf area (cm ²)	Control	242.2 \pm 111.3Aa	279.9 \pm 57.1Aa	366.9 \pm 106.9Aa	295.1 \pm 52.9Aa	551.5 \pm 61.6Aab
	Salt	263.2 \pm 58.7Aa	237.7 \pm 22.4Aa	380.6 \pm 90.6Aa	346.0 \pm 72.1Aab	226.2 \pm 50.5Aa
	Fert	891.6 \pm 80.6Ac	909.9 \pm 113.8Ac	686.8 \pm 111.3Aab	818.0 \pm 134.8Ac	756.1 \pm 106.1Ab
	Salt \times fert	435.1 \pm 74.0Aab	323.9 \pm 38.4Aab	560.8 \pm 118.0Aab	522.5 \pm 49.2Aabc	546.6 \pm 92.7Aab
Total dry matter (g)	Control	4.6 \pm 1.16Aa	6.2 \pm 0.75ABa	6.8 \pm 1.41ABab	4.4 \pm 0.49Aa	9.7 \pm 0.76Bb
	Salt	4.2 \pm 0.71Aa	6.0 \pm 0.22ABa	6.2 \pm 0.63ABa	6.7 \pm 0.53Bab	4.8 \pm 0.38Aa
	Fert	12.0 \pm 0.57Ab	15.6 \pm 1.39Ab	14.1 \pm 0.90Acd	14.9 \pm 1.51Ac	15.7 \pm 0.43Ac
	Salt \times fert	7.8 \pm 0.30ABab	6.5 \pm 0.45Aa	11.0 \pm 0.77BCbc	8.6 \pm 0.80ABb	12.0 \pm 0.99Cbc

For each variable, values in each column (between treatment) with different small letters are significantly different ($p < 0.05$ Tukey test). Values in each line (between families) with different capital letters are significantly different ($p < 0.05$ Tukey test). Values represent the mean of five replicates \pm standard error.

The main effects of family and salt treatments had a significant influence on specific leaf area (SLA) and root/shoot ratio. NB1 had the lowest SLA and root/shoot ratio while the other families did not differ significantly (Figures 7 and 8).

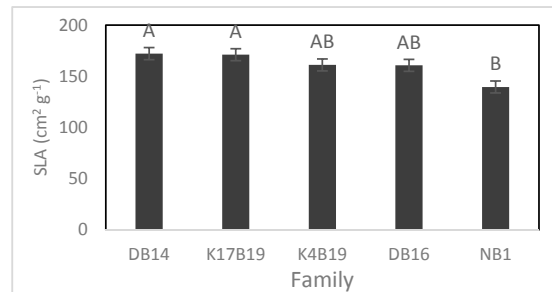


Figure 7. Variation of specific leaf area (SLA) of *Senegalia senegal* families. Different letters on bars mean significant differences among families at $p < 0.05$.

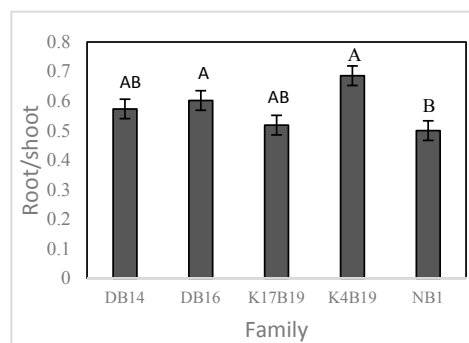


Figure 8. Variation of root/shoot ratio among families of *Senegalia senegal* seedlings. Different letters on bars mean significant differences among families at $p < 0.05$.

Both SLA and root/shoot ratio were significantly reduced by the salt, decreasing from 169.7 to 152.2 cm² g⁻¹ and 0.61 to 0.47 in controls versus salt treated. The root/shoot ratio also decreased from 0.66 to 0.43 in controls versus fertilized plants.

There was a positive effect of fertilizer application on RGD prior to and during the drought stress period (Figures 3 and 9). In contrast, fertilizer did not increase RGH during drought (Figure 9).

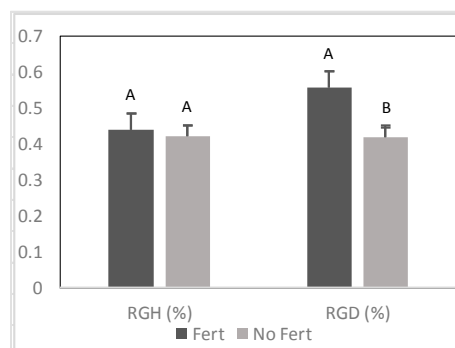


Figure 9. The effect of fertilizer (Fert: fertilized, No fert: no fertilized) on the relative growth rate of diameter (RGD) and relative growth rate of height (RGH) of *Senegalia senegal* seedlings during drought stress. Different letters on bars mean significant differences among families at $p < 0.05$.

A significant difference among families was found on RGH (Figure 10), whereas RGD was similar between families. Prior to the drought, Ng21B1 had significantly greater RGH than all other families. During drought, RGH was greatly reduced and K17B19 displayed the highest RGH, but it was only significantly higher than DB16 (Figure 10).

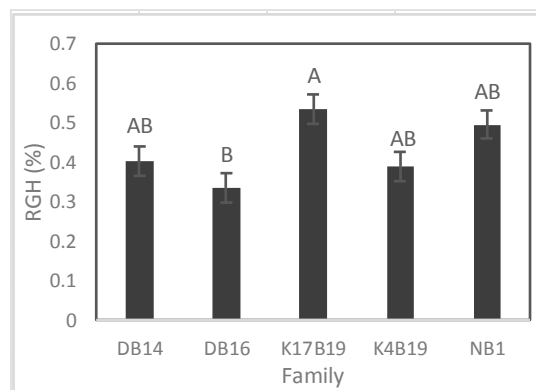


Figure 10. Variation of relative growth rate of height (RGH) between *Senegalia senegal* families) and during drought stress. Different letters on bars mean significant differences among families at $p < 0.05$.

4. Discussion

This study was the first attempt to look for growth performance and physiological adaptation under salt, and fertility conditions among different *Senegalia senegal* families collected in Senegal. Results of this study confirmed that photosynthetic capacities of *Senegalia senegal* seedlings are similar among families in non-limiting water conditions [19]. However, significant differences in stomatal density and size, SLA, root/shoot ratio, and RGH may be indicative of different responses to environmental stress among the tested families. The significant salinity by family interaction on dry matter accumulation found in this study implies that some *Senegalia senegal* families may perform better on salt affected drylands than on non-salty drylands [20]. NB1 had significantly reduced growth with salt addition, whereas K17B19 grew better in the salt treatment. An earlier study showed a significant decrease of *Senegalia senegal* shoot and root dry weights in response to the same salinity level used in our study [21]. However, they used a mixture of seeds collected from one coastal area and did not examine families, so it is possible they utilized a susceptible source such as our NB1.

The significant decrease of total biomass observed in NB1 reveals its higher sensitivity to salt stress than the other genotypes tested in this study. Despite originating from more saline areas, NB1 tended to be more sensitive to salinity, a finding similar to that which was reported in an earlier study which revealed that topodemes from areas subjected to salinity are more salt tolerant than those from non-salted areas [22]. In contrast, salt tolerance in *Senegalia tortilis* might be attributed to genetic factors that may be site independent [23]. *Senegalia senegal* is known as a salt tolerant species [5–7] and this is confirmed for most of our sources. Further, results on relative growth rate revealed a positive effect of salinity on RGH, which is significantly increased by 21% under drought ($p = 0.0435$). Our results suggest potential salt tolerance improvement of *Senegalia senegal* through genotype selection. Genetic variation for salt tolerance has been reported for many other species [23–27]. Surprisingly, K17B19 grew better in saline conditions, increasing total biomass by 51%, compared to non-saline conditions, even though the increase was not significant. Increased growth of plants under saline soils may be due to specific genes that control cell growth and leaf function [26]. K17B19, K4B19, DB14, and DB16 may have inherent ability to control water loss and improve ionic balance to their favor, therefore maintaining growth and biomass under salinity [28]. Recently, a sodium hydrogen antiporter gene (NHX1) has been identified in the *Senegalia senegal* tonoplast cells, which through an overexpression may confer capacity to tolerate salinity stress [5,29].

Unexpectedly, stomatal density and total chlorophyll content were significantly increased by salinity. Past studies found salinity to negatively affect stomatal density and chlorophyll content [14,23,30–33]. The presence of salt increased WUE_i and decreased SLA (Figures 1b and 7). Reduced SLA (thicker leaves) usually have more mesophyll cells with a higher density of chlorophyll and proteins per unit leaf area, hence a higher photosynthetic capacity than thinner leaves. Our results confirmed the negative association between WUE_i and SLA reported by [34], who suggested lower SLA as a selection criterion for enhanced WUE_i . Our study revealed that *Senegalia senegal* response to salt depended on genotypes and whether or not drought was induced. The increased WUE_i in DB16 and NB1 in response to salt when water was not a limiting factor may be attributed to the significant decrease of transpiration relative to the assimilation rate through stomata closure (Figure 1b). The same response was observed on K17B19 under drought conditions. The increased WUE_i likely is due to high photosynthesis, or low transpiration, or both [35,36]. However, under drought stress, the higher WUE_i in response to salt without a significant change in stomata conductance observed in DB16 might be influenced by other traits such as mesophyll conductance. Besides stomatal control, inherent differences in mesophyll CO_2 fixation capacity may influence the rate of photosynthesis and accordingly WUE_i [37,38]. It is reported that enhancing mesophyll conductance/stomata conductance ratio can improve WUE_i [37,39]. Moreover, notable effects of salinity and drought stress on mesophyll conductance have also been reported in *Vitis vinifera* [40]. Stomatal conductance was decreased in saline conditions by 39% prior to drought and by 37% during drought, averaged across all families. Still, influence of stomatal closure on A inhibition and regulation of stomatal conductance are strongly determined by both mesophyll and stomatal resistance to CO_2 diffusion [41].

As expected, the fertilizer significantly increased the total biomass and biomass partition of all families. A positive effect of nutrient supply on *Senegalia senegal* growth has been revealed in an earlier study [42]. The significant increase in total biomass was likely due, in part, to the significant increase in the net photosynthetic rate under fertilizer conditions in most families (Figure 1a). However, the increased biomass partitioning was higher in leaves and stems than in roots, inducing a lower root/shoot ratio (Figure 8). It is reported that, in young seedlings, nutrient addition favored shoot growth more than root growth [38,43,44]. Our results confirm that fertilizer supply influences not only plant dry matter, but also biomass partitioning.

Trends in growth observed in seedling plants, showing lower RGH for DB16, were similar to what was found in their parent trees grown in the field which displayed lower height for DB16 (396 cm) compared to other genotypes. In other families, height varied from 510 cm for K4B19 to 590 cm for K17B19 [19]. We assume that height and RGH are highly heritable traits among *Senegalia senegal* genotypes. According to Dvorak et al. [45], heritability of growth traits for some forest trees does not change significantly between early and mature growth stages. With the exception of NB1, all sources (and in particular K17B19) used in this study would be suitable for testing on salt impacted soils. However, it would be important to test these findings in field conditions with more salt and fertilizer levels. Also, gum yield for such potential adapted genotypes would be important to take into account in the future to assess expected profit from growing *Senegalia senegal* in marginal and harsh dry lands.

5. Conclusions

This study testing the physiological and morphological adaptation of *Senegalia senegal* seedling under salinity and fertility conditions provided valuable information for improving *Senegalia senegal* tolerance to harsh environments through genotype selection. Our results revealed significant differences among *Senegalia senegal* genotypes in their leaf morphology. Large stomata size with low density was observed in NB1, which tended to be more sensitive to salinity despite lower specific leaf area. Photosynthetic capacities and growth rate performances showed significant interactions between families and salinity or fertility treatments. Diamenar and Kidira provenances in general and particularly the K17B19 family might be suitable for afforestation of salt affected drylands. All genotypes have notably increased biomass under the fertilizer effect. Our hypothesis that at least one

family would be better adapted to salinity or fertilizer effect could be supported. However, despite the importance of intraspecific variation in changing environmental conditions, more research is needed to test findings in field conditions and to determine salt and fertilizer thresholds for optimized growth of *Senegalia senegal* in arid lands.

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