

Morphological and Physiological Responses of *Senegalia senegal* (L.) Britton Provenances to Drought, Salinity, and Fertility

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Abstract

Increasing drought and salinity tolerances in economically important trees adapted to dry land areas is key challenge for maintaining the socioeconomic welfare of dry land areas. Strategies to improve drought and salt tolerance must examine the tree physiological mechanisms that link to the trees survival and growth. This study examined physiological adaptive traits allowing *Senegalia senegal* to grow better in both saline and dry lands. We conducted two greenhouse experiments and one field study to characterize growth, photosynthetic capacity, water use efficiency (WUE) and gum arabic yield potential among different *Senegalia senegal* provenances. In the first experiment, we tested early growth and photosynthetic response of seed sources to a cyclic drought treatment. The second greenhouse study examined seed source response to drought, salinity and fertility conditions. Gum yield assessment, growth and WUE of mature trees were determined from a field trial. In general, results showed a high intra genetic variability of *Senegalia senegal* on gum yield, biomass accumulation and growth. Ngane provenance presented superior growth characteristics as both mature trees and seedlings and exhibited a more conservative water use strategy under drought. Both greenhouse studies revealed similar photosynthetic capacity among *Senegalia*

senegal genotypes when conditions are not limiting. However, when factors such as salinity, fertility and severe drought are involved, different physiological and morphological responses appear and at times this was dependent on seed source. But, at moderate drought stress (chapter 2), no drought by provenance interaction was found. Results of chapter 3 revealed that Ngane has larger stomata with low density in comparison with Diamenar and Kidira provenances. With the exception of Ng21B1, all seed sources displayed similar adaptations to salt stress in term of biomass accumulation. Fertilizer increased total biomass of all seed sources from 63% to 213% for Ng21B1 and K17B19, respectively. However, salinity reduced the fertilizer effect on biomass increment. Leaf gas exchanges were affected by salinity and fertilizer within various responses among seed sources. Results of chapter 4 revealed that gum yield was found to be positively correlated with tree height, crown width, stem volume index and crown area index. Ngane and Diamenar appeared the best provenances in term of annual gum yield per hectare. Diamenar had a higher survival rate than Ngane. This finding reveals the need to consider the tradeoff between tree survival rate and individual tree gum yield in *Senegalia senegal* stands. In addition to being more sensitive to salinity, Ngane also seems to be more susceptible to low soil pH in terms of survival, but this result needs to be tested further.

This study suggests that improvement of gum arabic production can be possible through genetic selection. But, for the best adapted genotypes, research should explore new genetic combination and investigate physiology and genetic diversity. Moreover, the findings on the relationship between soil pH and tree survival rate suggests a need for care in selecting appropriate sites for *Senegalia senegal* stands. Therefore, silviculture practices as well as genetic selection appear critical in improving gum arabic production.

General audience abstract

Senegalia senegal is a small tree with many uses and the main gum arabic producing species. It is a deciduous tree which grows mainly in arid and semi-arid lands. Since the major drought years of the 1970s and 1980s, a substantial decline of natural stands of the species and the low gum arabic productivity have affected rural incomes in the Sahel where trees such as *Senegalia senegal* contributes significantly to rural livelihood. Moreover, saline soils are increasing in arid and semi-arid regions where the amount of rainfall is insufficient for substantial evapotranspiration. Therefore, investigating more drought and salt tolerant species will be appropriate in the context of extreme climate events such as drought. However, strategies to improve tolerance need an understanding of physiological mechanisms that link tree growth and adaptation. This study was looking for adaptive characteristics allowing *Senegalia senegal* to grow better in saline and dry lands while producing more gum arabic. We conducted two greenhouse experiments and one field study to characterize growth, photosynthetic capacity, water use efficiency (WUE, the amount of carbon fixed in photosynthesis per unit water transpired) and gum arabic yield potential among different *Senegalia senegal* provenances. In the first experiment, we tested growth and biomass allocation to root and shoot of seven *Senegalia senegal* provenances and their photosynthetic responses to moderate drought treatment. The second greenhouse study was to test five seed sources responses to drought, salinity and fertility conditions. Gum yield assessment, growth and WUE of mature trees were determined in the field study. Globally, results obtained showed a high intra genetic variability of *Senegalia senegal* on gum arabic yield, biomass accumulation and growth. Ngane provenance presented superior growth characteristics as both mature tree and seedling and exhibited a higher WUE under drought in comparison with the other provenances used in this study. Both greenhouse studies

revealed similar photosynthetic capacity among provenances when conditions are not limiting. However, when factors such as salinity, fertility and drought are involved, different physiological and morphological responses appear depending on seed source. But, no drought by provenance interaction was found under drought treatment. Results of chapter 3 revealed that Ngane has larger stomata with low density in comparison with Diamenar and Kidira provenances. Except Ng21B1, all seed sources displayed similar adaptations to salt stress in term of biomass accumulation. Fertilizer has increased total biomass of all seed sources from 63% to 213% for Ng21B1 and K17B19, respectively. However, salinity may reduce the fertilizer effect on biomass increment. Results of chapter 4 revealed that gum yield was found to be positively correlated to tree height and crown width. Ngane and Diamenar appeared as the best provenances in term of annual gum yield per hectare. In addition to be more sensitive to salinity, Ngane seems also to be more susceptible to low soil pH in term of survival. Moreover, the finding on the positive relationship between soil pH and tree survival rate concede a great importance of soil conditions in *Senegalia senegal* viability. So, in this study it appears critical for the improvement of gum arabic sector to consider both biological component and environmental factors. To seek for best adapted genotypes, research should explore new genetic combinations and investigate physiology and genetic diversity.

Dedication

This thesis is dedicated to my daughter Leila, my parents, my spouse and my siblings for their love and support.

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I. Chapter 1: Introduction and Literature Review

1.1.Introduction

Although drought events have always occurred in natural ecosystems, successive drought crises during the last decades in the Sahel have seriously induced ecological problems causing loss of biodiversity in many localities (Brandt *et al.*, 2014; Diouf and Lambin, 2001; Gonzales, 1997; Gonzales, 2001; Sadio , 2000). For example, in the north-western part in Senegal, changes in woody species composition were reported, revealing a loss of 21 species in about 50 years (Gonzales, 2001). The decline of rainfall coupled with low soil quality and increase of rural population density are pointed out as the most determinant factors of deforestation, which in turn favors desertification and soil salinization (Domries, 1991; Hastenrath,1991; Jamil *et al.* 2011; Shukla, 1990; Vincke *et al.*, 2009). Over 6% of the world's land are affected by salinity (Manchanda and Garg, 2008). In fact, arid and semi-arid regions are more exposed to the phenomenon of salinization because of low soil leaching and high evapotranspiration rate. The decline of natural stands of many valuable species have affected incomes for many households in the northern Senegal where forests and trees such as *Acacia* species and especially *Senegalia senegal* contribute significantly to rural livelihoods (Gaudiose, 2013; Behnke and Mortimore, 2016). To attenuate impacts of such constraints on natural and valuable forest resources, it is critical to integrate selected species/genotypes into reforestation programs to improve and sustain restoration of ecosystems stability. In Senegal, where poverty remains high in rural areas (Sonneveld *et al.*, 2010) maintaining forest resources potential and enhancing rural livelihood appears to be a major challenge.

Senegalia senegal is a good choice for planting in these rural, dry land areas. It is drought and salt tolerant, and provides a wide variety of economic and environmental services. However, selecting the best genotypes with physiological and morphological drought adaptations would improve biological and economic success. Therefore, better understanding of the drought and salinity tolerance for this species is critical before embarking into reforestation and domestication programs. Investigating physiological traits related to growth in stressful conditions is a good approach to screen for suitable genotypes. Traits such as leaf level physiology, stomatal regulation and water use efficiency (WUE, the amount of carbon fixed in photosynthesis per unit water transpired) are desirable traits used in plant breeding programs to select for more drought tolerant species. Investigating more drought-resistant *Senegalia* trees which are able to bear higher gum yield will be both ecologically and economically appropriate. Therefore, identifying populations with more than one desirable trait appears crucial. This study will investigate physiological mechanisms of *Senegalia senegal* in response to drought, salinity, and fertility conditions while assessing intraspecific variation and growth traits related to gum yield.

The primary goal of this research was to investigate gum yield variation among *Senegalia senegal* provenances and determine their physiological responses to drought, salinity and fertility conditions. The specific objectives are 1) to examine functional traits in seedlings of *Senegalia senegal* related to growth, biomass allocations and photosynthetic capacity during drought, 2) to determine morphological and physiological responses of different *Senegalia senegal* provenances as seedlings under drought, salinity and fertility conditions, 3) and to explore relationships between gum yield, growth traits and carbon isotope composition among provenances grown in the field in order to better improve gum yield prediction.

A lack of reliable data on gum arabic yield has complicated the relationship between plant physiological status and gum productivity. To establish gum yield predictions, we first assessed gum yield by tree for four years, and then determined where variations lie among provenances and families. The approach undertaken was to examine physiological traits under natural conditions (field studies) with those tested in semi-controlled conditions (greenhouse studies). To reach our goals, the study is guided by three research projects.

First, a progeny trial was used to examine intra-specific variation in WUE and key growth and morphological traits (height, basal diameter, crown width, tree volume and leaf area index) and determine if these traits are related to gum yield. This field study will contribute to elucidate factors underlining gum yield productivity. Second, to develop a further understanding of the physiology of *Senegalia senegal*, we conducted two greenhouse experiments which investigated various provenances responses to environmental stress. Not only did we wish to elucidate physiological traits related to seedling performance but also to determine if any genotype by environmental interactions exist. In the first experiment, we compared growth and physiological response of seven provenances under moderate drought stress in order to determine how drought stress effects growth, biomass allocation, and photosynthetic traits of *Senegalia senegal* seedlings. The third study examined how drought, salinity, and fertility treatments affect morphological and physiological traits among *Senegalia senegal* provenances. Growth characteristics as well as physiological and morphological features were determined.

1.2.Literature review

1.2.1. Taxonomy and species description

Senegalia senegal (L.) Britton is a deciduous tree or shrub which grows widely across the Sahelian belt from Senegal in the west to Somalia in the east between the Sahara Desert and north of the equator (Figure 1. 1). *Senegalia senegal* trees are also found in southern Africa, India and Pakistan (Cossalter, 1991). *Senegalia senegal* was first described as *Mimosa senegal* by Linnaeus in 1753 from a specimen likely collected in Senegal. In 1806, Willdenow described it as *Acacia senegal*. Recently, a global revision of the species has retained the genus *Acacia* only for Australian species whereas African species have all been renamed and *Acacia senegal* is now known as *Senegalia senegal* (L.) Britton (Kyalangalilwa *et al.*, 2013). The tree is in the family Fabaceae and sub-family Mimosoideae. Four varieties exist within the species (Figure 1): *senegal*, *kerensis*, *leiorharchis*, and *rostrata* (Brenan, 1983). Variety *senegal* is most valued for gum arabic quality and quantity (Lelon *et al.*, 2010) and the only variety found in Senegal (Fagg and Allison, 2004). *Senegalia senegal* leaves are photosensitive, closing leaflets at night and opening them during the day. Leaf orientations are controlled by a specialized organ called pulvinus located at the basal part of each leaflets (Uehlein and Kaldenhoff, 2008). The root system consists of a deep tap root with numerous lateral roots making up to 40% of the total tree biomass (Pearce, 1988).

Senegalia senegal is highly tolerant to rainfall and temperature variations. It grows mainly in sandy soils with annual rainfall mostly between 200-600 mm, and the best sites have a pH between 5 to 8 (Von Maydell, 1986). The mean annual temperature in its natural range is between 28 to 30 degrees centigrade (Adamson and belt, 1974; Cossalter, 1991; Pearce, 1990). The species is an important source of fodder for herbivores especially during dry periods (Mallet

et al., 2003; Fagg and Allison, 2004), improves soil fertility through nitrogen fixation, serves as a windbreak and contributes to dune fixation (Cossalter, 1991, Barbier, 1992; Wickens *et al.*, 1995). The tree also provides fuel wood, local construction materials and is highly suitable for use in agroforestry systems (Lott *et al.*, 2003). Furthermore, *Senegalia senegal* is the main species in the world producing the internationally traded gum arabic and it is grown primarily for gum production.

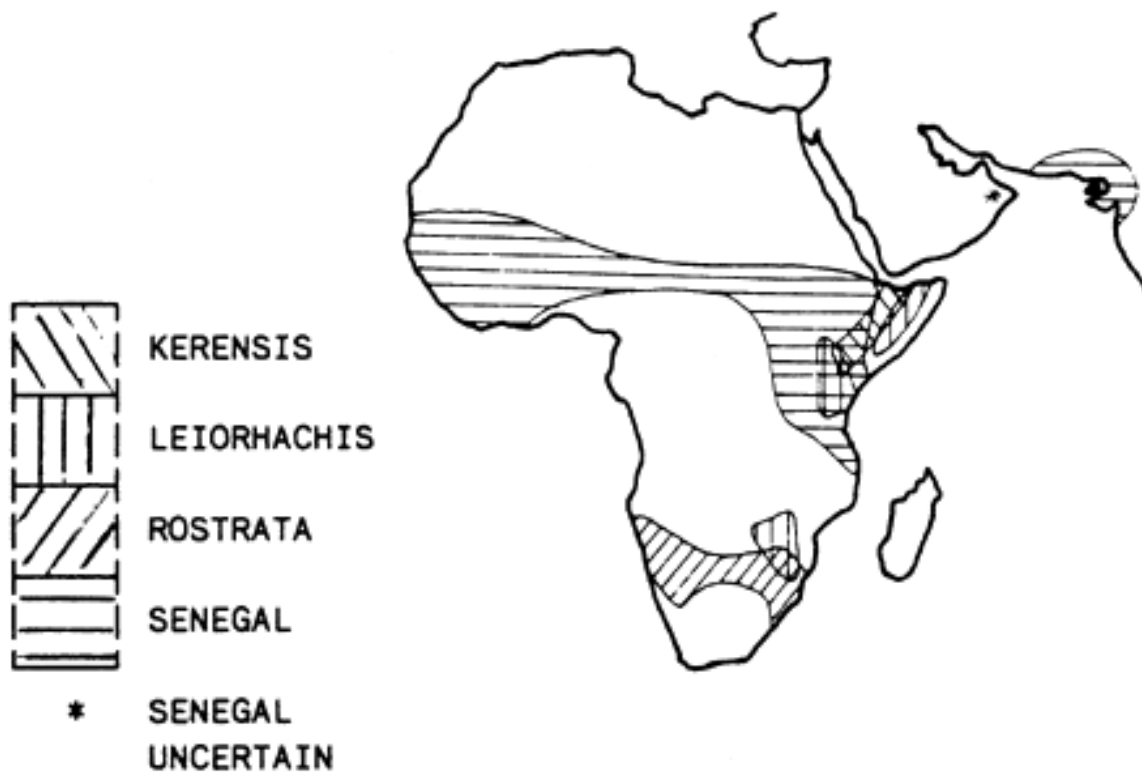


Figure 1.1: *Senegalia senegal* natural range and the distribution of its varieties.

Source: Framond (1990)

1.2.2. *Gum arabic: production and use*

Gum arabic is an exudate that consists of a mixture of polysaccharides, oligosaccharides, and glycoproteins obtained from the stems and branches of *Senegalia senegal* or *Acacia seyal*

(Anderson and Stoddart, 1966; FAO, 1999). It is odourless, flavourless, and non-polluting and thus is considered generally safe (GRAS) for use in food and non-food industries as an emulsifier, thickener and stabilizing agent in beverages, frozen dairy products, confectionery, syrups and as binding agent in tablets, pills, throat pastilles and cough drops (Pearce, 1988; Wang, 1993). World gum arabic demand is growing rapidly and no substitutes match it for quality and cost (Domeisen, 2000). Only 5% of world commercial gum are from *Acacia seyal* and sold as a separate product known as gum tahla (Leakey *et al.*, 1996). The remaining 95% is from *Senegalia senegal*. Sudan dominates the world export market, providing 70% to 80% of gum supply (Fadl and Elsheikh, 2011; Iqbal, 1993). Nigeria is the second largest producer, and Senegal belongs among the small producers whose exports total less than 5% of world exports (Iqbal, 1993; DEFCC, 2005; ITC, 2008 cited by Madjuwamaria, 2012). From 1966 to 1978 Senegal was the second largest producer after Sudan, but since then has seen its gum market significantly decline and remain low (Seif el Din *et al.*, 1996, Majuwamaria, 2012).

Gum production, which occurs seasonally, is not only governed by water stress, but is also linked to plant age and size of the tree. After age four to five years, *Senegalia senegal* becomes mature enough to be tapped for gum arabic, but between seven to twelve years of age are the most productive (CNI, 2008; Iqbal, 1993). The tapping treatment is done by using a special tool locally named “Daba” in Senegal or “Sonki” in Sudan. The tool is pushed through the bark to peel off an approximately 4 cm wide strip of bark to a length of 30 cm to 1 m on stems and branches (Harmand *et al.*, 2012). This process mimics natural wounds on stems and branches which are necessary conditions for the tree to exude gum in order to protect wounds against infections and other external factors (Fagg and Alison, 2004; Giffard, 1966). Gum yield is also a function of temperature, amount of precipitation, wind intensity, tapping date and intensity

(Ballal *et al.*,2005; Larson and Bromley, 1991; Pearce, *et al.* 1990). The gum yield of *Senegalia senegal* may be as high as 2 kg/tree (Iqbal, 1993; Sall, 1997; ITC, 2008). However, it is reported that some trees do not produce gum at all (Fagg and Allison, 2004). Better gum quality is often associated with higher price. High quality gum droplets should be a pale white to orange-brown color, globular and more than 3 cm in diameter (Majawamaria, 2012). Sudanese gum arabic is generally accepted as being of highest quality in the world market, but Senegal's gum is internationally recognized to be of good quality (Majawamaria, 2012; Mallet, *et al.*, 2003).

1.2.3. Drylands ecosystems: pressure and challenges

The term dryland represents arid, semi-arid and dry sub-humid lands characterized by an arid index (precipitation/potential evapotranspiration) varying between 0.03 and 0.75. Potential evapotranspiration, determined by Penman method, is a measure of the ability of the atmosphere to remove water through evaporation and transpiration under the control of atmospheric humidity, solar radiation, and wind. (FAO, 1993; Cervigni and Morris, 2016). Dryland affects about 40% of the world terrestrial surface. Approximately 55% of Africa's land are either arid or semi-arid, characterized by a short growing season (2 to 4 months) within low annual rainfall varying between 100 to 600 mm (UNEP, 2013). Projections are that by 2050 dry affected lands in west and east Africa may increase up to 40 % (Cervigni and Morris, 2016). The region of west Africa has experienced significant changes in land cover, ranging from deforestation to desertification and important loss of plant biodiversity (Zheng and Eltahir, 1997). An increased frequency of drought occurrences over the past decades were noticed and models predict further drought impacts. In Senegal, land forests cover an area of 8,513,000 ha for which only 5% are planted forests, while deforestation is estimated at 0.47% per year (UNEP, 2013). Furthermore,

Senegal has been listed as an African country where half of forest resource genetics are threatened (Bozzano *et al.*, 2014).

A major consequence of increasing population is an over exploitation of forest trees for domestic uses such as fuelwood, charcoal, fodder, and construction materials. Further, multipurpose tree species such as *Senegalia spp.* which have the most economic value and utility, are more often the victim of overharvesting (Bozzano *et al.*, 2014). In sub-Saharan Africa, drought stress is the most limiting factor of plant productivity and survival especially in arid and semi-arid ecosystems (Khan *et al.*, 2010). In Senegal, desertification has induced a shift in vegetation cover from areas of low rainfall to areas of higher rainfall, suggesting many species have low drought resistance (Gonzales, 2001). Furthermore, planted trees are becoming the most important source of timber and non-timber forest products. Thus, there is a great interest in improving reforestation of degraded lands in Senegal. But, failure of newly planted tree seedlings has limited the success of afforestation of dry lands largely because of poor drought tolerance (Marshall and Maki, 1946; Kramer, 1986). The challenge is to use local species with more desirable traits for environmental and economic purposes. Exotic tree species are often suggested (Turnbull, 1999; Wishnie *et al.*, 2007) and used in reforestation programs because of their fast growth ability under water limited conditions, but indigenous multipurpose tree species are more socially preferred.

In the sylvo-pastoral zone of Senegal, species providing timber and non-timber forest product are important to the economic livelihood of farmers. Recently, farmers and companies have shown interest in growing *Senegalia senegal* because of the revenue that can be generated from gum arabic production. However, a lack of good management strategies and genotypes has

limited the success of gum arabic production. Building sustainability on an integrated management system with better genotypes may improve productivity and adaptability.

1.2.4. Water use efficiency and Carbon isotope discrimination

With increasing worry about frequent drought occurrences in arid and semi-arid areas, there is a high interest in improving WUE of drought adapted species. WUE refers to the ratio of biomass produced to the rate of transpiration. It is the measure of a plant capacity to convert water into biomass. High WUE enables plants to produce more dry matter for the same quantity of water. Plants with higher WUE may extend the growing season when a long dry period occurs which can confer an ecological advantage in dryland ecosystems (Aroca, 2012; Nobel, 1991). The conservative water is favourable when a long drought stress occurs, enabling plants to extend the growing season and allocate more biomass to their components under water deficit condition whereas plants reflecting lower WUE under drought conditions and presenting a higher water use requirement may optimize growth when conditions are more favorable (Blum, 2005). However, the association between drought tolerance and WUE is not straightforward. The relationship is further complicated by other physiological mechanisms such as carbon allocation patterns, osmotic adjustment, gas exchange patterns, hydraulic architecture, hormonal regulation and plant phenology that may be involved to drought tolerance mechanism.

An excellent tool for study plant water use efficiency is stable carbon isotope discrimination. Carbon has two stable isotopes, ^{12}C and ^{13}C , with natural abundances in the atmosphere of 98.9 and 1.1 %, respectively. The ^{13}C isotope has a heavier molecular mass than ^{12}C . The isotope ratio $^{13}\text{C}/^{12}\text{C}$ is higher in the atmosphere than in plant tissues, indicating that plants discriminate against ^{13}C during photosynthesis. Isotope values in plant material are governed by physical and

biochemical processes, and environmental conditions. In C₃ plants, the isotopic ratio varies mainly due to carbon dioxide (CO₂) diffusion and enzymatic processes (Farquhar *et al.*, 1989). Discrimination occurs mainly because the heavier carbon isotope ¹³C diffuses more slowly than the lighter one ¹²C, and also because the enzymes involved in photosynthetic carbon fixation, particularly rubisco, discriminates between the two isotopes. As a result of this discrimination, determination of carbon isotope composition ($\delta^{13}\text{C}$, expressed in ‰) in plant material gives information on plant physiology and environmental conditions. In particular, $\delta^{13}\text{C}$ is highly correlated with plant water use efficiency (WUE) and thus has been proposed as a method for assessing WUE in C₃ plants. The $\delta^{13}\text{C}$ can be used to estimate isotope discrimination following this equation.

$$\Delta = (\delta_a - \delta_p) / (1 - \delta_p) \text{ (expressed as high } \delta^{13}\text{C} \text{ or low } \Delta^{13}\text{C)}$$

Where δ_p is the isotope composition of the plant material, δ_a is that of the air.

1.2.5. Physiological adaptation to drought and salinity

Drought is the absence or insufficient rainfall or irrigation for a period of time sufficient to induce a soil water deficit (Jaleel *et al.*, 2009; Taiz and Zeiger, 2014). Plants have evolved different mechanisms to endure drought stress. Water deficit affects plants differently depending on the water stress level, species, genotypes and the stage of growth (Chaves *et al.*, 2002; Jaleel *et al.*, 2009). In dryland areas, woody plants have portions of their life cycle during drought stress and therefore cope with drought stress through complex adaptive mechanism involving both biomass partitioning, osmotic adjustment, stomatal and non-stomatal regulation, WUE, and hormonal balance. Deciduous tree species such as *Senegalia senegal* utilize avoidance mechanisms during the dry season in order to minimize water loss. Also, their deep tap root

favors water uptake (Deans *et al.*, 1993). This strategy to prevent water loss and increase the rate of water uptake is known as desiccation postponement, an adaptive strategy for effective water saving. In arid environments where long and severe droughts prevail, desiccation postponement may be important for survival and longer term productivity. Drought tolerant species typically minimize water loss through stomata regulation which plays an important role in photosynthesis acclimation to water stress (Levitt, 1980). Drought induces stomatal closure which decreases photosynthesis because both CO₂ uptake and water loss by transpiration take place through stomatal aperture (Aroca, 2012). However, the relationship between photosynthesis and transpiration is non-linear (Raschke, 1979; Cowan, 1982; Nobel, 1983). Stomatal closure causes a proportionally greater decrease in transpiration than photosynthesis, thereby increasing WUE (Fischer & Turner; 1978; Kramer, 1983). Although differences in stomatal size, and density may be important in increasing stomatal resistance to water loss (Kozlowski, 1972; Tschaplinski, *et al.*, 1984), a reduction in stomatal conductance is likely the most important means of avoiding dehydration. Plants with higher WUE postpone drought stress by effectively using the available water efficiently thereby extending the growing season. When the stress becomes severe enough, dehydration postponement adaptations results in leaf abscission and a more extensive and deeper root system.

In dryland areas, not only water deficit but also salt stress causes poor plant survival and productivity with similar plant physiological effects to drought stress (Jaleel *et al.*, 2007). Salinity is known to induce water stress in plants by limiting water uptake due to low osmotic potential (Carrow and Duncan, 1998). However, under severe salt stress, the physiological responses involve osmotic and ionic adjustments. Osmotic adjustment, the active accumulation

of solutes during stress lowers water potential, and the resulting increase in water uptake helps to maintain cell turgor above the critical level for stomatal closure.

1.2.6. Senegalia senegal adaptation to drought

Senegalia senegal is known to be a drought tolerant species that grows under arid and semi-arid areas where water availability is the main factor limiting survival, growth and productivity. Physiological mechanisms associated with drought stress in *Senegalia senegal* have been reported in many studies asserting that *Senegalia senegal* develops morphological and physiological adjustments under water stress (Brenan, 1983). Merine *et al.* (2015) reported that *Senegalia senegal* is an opportunistic water user which grows rapidly when water availability is favorable. This may explain why *Senegalia senegal* exhibits high transpiration rate despite arid conditions (Ullmann, 1985). Therefore, *Senegalia senegal* grow mostly during the rainy seasons (Ballal, 2002). Further, Gebrekistos *et al.*, (2011) suggested that *Senegalia senegal* is an opportunistic water use tree which exhibit lower WUE under drought in comparison with other drought tolerant species such as *Acacia tortilis* and *Balanites aegyptiaca* which are more performant in terms of growth during drought. This implies that *Senegalia senegal* is less tolerant to drought than *Acacia tortilis* and *Balanites aegyptiaca*. However, according to Raddad and Luukkanen (2006) variation of water use strategy among *Senegalia senegal* provenances may depend on environmental conditions. They reported that *Senegalia senegal* provenances from clay soils grow faster and accumulate more biomass than those from sandy soils under less water stress. Moreover, plant water status and soil water regime are believed as being main predictors of plant phenology. However, day length rather than rainfall amount was correlated with *Senegalia senegal* flowering. The effects of temperature variations and vapor pressure

deficit (VPD) on leaf phenology should be explored further to seek for eventual plasticity of leaf phenology in response to temperature and VPD variations. Increases in root/shoot ratio may also be an important morphological adaptation in this species. Higher survival rate of *Senegalia senegal* seedlings was positively correlated with root/shoot ratio and earlier leaf shedding on the onset of drought (Bhimaya and Kaul, 1966). Gray *et al.* (2013) assessed leaf carbon isotope discrimination of *Senegalia senegal* and found a significant difference among provenances which imply a possible drought tolerance improvement in this species. This finding confirmed Raddad and Lukkanenn (2006) who tested provenances from contrasting conditions.

In Senegal, genotypes based approach has not yet been undertaken to improve drought tolerance of *Senegalia senegal*. Nonetheless, most reported findings concede possibilities to improve drought tolerance and eventually gum arabic yield through genotype selection. Within the context of climate change, frequent drought occurrences and increasing salinity in drylands ecosystems, it appears important to select plus trees in order to improve the performance of plantations. Therefore, our current study will particularly focus on growth and physiological adaptation to drought and salinity at intraspecific level of *Senegalia senegal*.

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2. Chapter 2: Effect of drought on the physiology and early growth of 7 *Senegalia senegal* provenances

Abstract

Senegalia senegal is a multipurpose tree mostly valued in dryland ecosystems for its ability to tolerate drought and to contribute economically to local households. Decline of this species in its natural range has been observed and was mainly attributed to harsh environmental conditions such as frequent drought occurrences. The objective of this study was to assess the effect of water stress on growth, biomass allocation and photosynthetic capacity of *Senegalia senegal* at seedling stage. Seeds from seven provenances harvested in Senegal were grown under greenhouse conditions. Seedlings were subjected to three cyclic droughts by watering them when average soil moisture content dropped to 4.7%, 2.7%, and 2.1% during the first, second, and third dry-down cycles, respectively. At the end of the experiment, height, diameter, leaf area, root area, specific leaf area, and biomass of leaf, stem and root were determined. Net photosynthetic rate, stomatal conductance, transpiration and chlorophyll content were measured before treatment began and at each dry-down cycle. No drought by provenance interaction was found for any traits measured in this study. Ngane had a lower root/shoot ratio. Allometric analysis revealed that Ngane seemed to allocate less biomass to roots than shoots. This finding reveals no size dependency on above and belowground biomass allocation on *Senegalia senegal* seedlings. Ngane developed superior growth traits as well as biomass production, despite similar net photosynthetic rate with other provenances before treatment started. Overall, drought stress significantly reduced biomass, height, diameter and leaf gas exchanges. Total biomass was reduced by 28.5%, whereas root/shoot ratio was increased by 25%. Based on

similar responses to water limitation of photosynthetic performance and biomass allocation of the seven *Senegalia senegal* provenances, it would be auspicious to test more severe drought stress levels.

Key words: *Senegalia senegal*, provenance, drought, biomass, leaf gas exchanges.

2.1. Introduction

Senegalia senegal is a multipurpose tree and the main gum Arabic producing species distributed throughout the arid and semi-arid lands in Africa. It is known to be adapted to a wide range of soils types, soil pH and climate (Von Maydell, 1986) and has a reputation for a high tolerance to water stress (Gaafar *et al.*, 2006). However, water stress can result in heavy mortality such as during severe droughts in the 1970s where 80% of gum forests in West Africa were lost (Coulibaly 1988 cited by Fagg and Allison 2004; Wate, 1979). Loss of this forest type is further accentuated by low natural regeneration of this species due to a high mortality following seed germination (Seif el din, 1973). In semi-arid areas such as Senegal, artificial regeneration is also difficult since seedlings planted to the field face a long dry period after only a short growing season (July-September). Therefore, plant establishment and growth are very critical in the early developmental stage of such species.

In response to water limitations, plants are able, through physiological regulations, morphological adjustments, and changes in allocation to modify their growth and often become more drought tolerant. Usually, drought tolerant species allocate more resources to root growth than shoot growth for their survival in dry environments (Aroca, 2012). Root to shoot ratio is crucial and may confer adaptability to plants in water limited habitats. However, the relationship between root/shoot ratio and drought tolerance remain unclear (Liu *et al.*, 1999; Zollinger *et al.*, 2006). Changes in allocation can result from a faster increase of root growth than shoot growth, a reduction of shoot growth without change in root growth, or a less restricted root growth (Espinoza *et al.*, 2013; Li, 1998; McMichael and Quisenberry, 1991; Zollinger *et al.*, 2006). Studies have reported that biomass partitions to organs are controlled by mechanisms inherent to species/genotypes, influenced by the ability of plants to cope with environments (Aroca, 2012;

Haugen, 2008; Hausmann *et al.*, 2005; Otieno, 2005). Allometric analysis (Ledig *et al.* 1970) to determine how biomass allocation patterns change in response to water stress may help elucidate patterns of morphological plasticity in genotypes which may be related to productivity.

Water management in water limited ecosystems is especially important to the survival, growth, and productivity of tree species (Kozlowski *et al.*, 1991). Stomatal closing and leaf shedding are among drought avoidance strategies to limit water loss, but these reduce photosynthesis and suppress growth (Taiz *et al.*, 2015). *Senegalia senegal* is native to dry lands, but studies examining eco-physiological adaptation (Gaffar, 2005; Gray, 2013; Isaac, 2011; Lassouane, 2013; Siam, 2011; Vassal, 1992) are still scarce. Siam (2011) reported that higher intrinsic water use efficiency induced by strong stomatal control would be a selective trait for more drought tolerant *Senegalia senegal* genotypes. Indeed, stomatal regulation plays an important role in photosynthesis acclimation to water stress (Xu and Zhou, 2008). Physiological factors related to drought tolerance are likely to differ between genotypes and this information can be used to improve management of *Senegalia senegal* in the semi-arid lands of the sahelian area. In this area, *Senegalia senegal* is considered an endangered tree species due to consistent severe aridity (Poupon and Bille, 1974). Therefore, a better understanding of drought effects on physiological and morphological adaptation is crucial and will aid in assessment of genotypes for reforestation purposes. This study examines the growth and physiological response of 7 *Senegalia senegal* provenances to drought stress. Our specific objectives are to: 1) determine if *Senegalia senegal* genotypes differ in their leaf gas exchanges, morphological adjustments and biomass accumulation in response to drought, and 2) determine if leaf gas exchange is related to *Senegalia senegal* growth response to drought.

2.2. Material and methods

2.2.1. Plant material and growth

The study was conducted in a greenhouse during August 2014 to May 2015. The greenhouse was kept above 25 °C and ventilated with box fans when temperatures went above 32 °C. Temperatures generally stayed between 25 °C and 35 °C. Light was extended using sodium vapor lights in order to maintain a 16-hour photoperiod. Seeds of seven *Senegalia senegal* provenances were obtained from National Institute for Forestry Research of Senegalese Institute for Agriculture Research in Senegal in 2014 (Table 2. 1). Seeds were chemically scarified with concentrated sulfuric acid (98%) for 10 minutes in order to remove the tough seed coat. After being rinsed with water to remove the acid, treated seeds were germinated into shallow flats filled with Promix BX (Premier Tech Horticulture, Quakertown, PA). Once emerged, young germinals were transplanted individually into small pots (SC10 super cell, 3.8 cm diameter x 21 cm deep, Stuewe and Sons, Tangent, OR) containing Promix BX and grown for 3 months. Following this initial growth, they were transplanted into a 2.3 liters plastic pots (Treepots, 30.5 cm height and 10.2 width, IGC Greenhouse Megastore, Danville, IL) filled with a 2:1 mix of Promix BX and washed sand. Seedlings were initially kept well-watered and fertilized with 2.5 g slow release Osmocote (14-14-14, N-P-K Scotts Miracle-Gro Marysville, Ohio) and grown for 1 more month.

2.2.2. *Experimental design and treatments*

The experimental design was a randomized complete block with 5 replications, 2 treatments (control-well watered and moderate drought stress) and 7 provenances, providing a total of 70 experimental units. As *Senegalia senegal* drops leaves during severe drought, we used a moderate drought stress in order to avoid leaf shedding. The moderate drought treatment was designed to mimic field conditions during the wet season where discontinued occurrence in drought and/or rainfall events occur. Therefore, we adopted a dry cycle treatment where plants were not watered until the soil volumetric moisture content, averaging initially 28%, dropped to 4.7%, 2.7%, and 2.1%, at the first, second, and third dry down cycles, respectively. In total, the stressed plants experienced three dry cycles and three re-watering cycles.

Table 2.1: *Senegalia senegal* seed sources, locations, and mean annual site rainfall (or the closest meteorological stations) of the 7 provenances used in this study. Diamenar, Diery Birane, Ngane, Ranerou and Velingara were collected in their sites of origin while Daiba and Kidira were obtained in Dahra from a provenance trial established in 1994.

Provenances	Longitude (N)	Latitude (W)	Altitude (m)	Mean annual rainfall (mm)
Daiba	13° 06' 92"	15° 21' 95"	28	458
Diamenar	15° 52' 5"	15° 59' 7"	10	284
Diery Birane	15° 23' 5"	15° 23' 7"	45	301
Kidira	12° 13' 89"	14° 27' 23"	39	505
Ngane	16° 12' 3"	14° 12' 6"	2	712
Ranerou	13° 57' 4"	15° 17' 4"	35	359
Velingara	14° 41' 5"	15° 00' 8"	25	721

2.2.3. *Measurements and harvest*

Height and diameter of all plants were measured at the end of the experiment. Leaf gas exchange was measured on the newest fully mature leaf using a portable infrared gas analyzer (LI-6400 Lincoln, Nebraska, USA). Environmental conditions in the leaf cuvette were set at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD), 398 $\mu\text{mol mol}^{-1}$ reference CO_2 concentration, 25 °C block temperature, and a flow rate of 400 $\mu\text{mol sec}^{-1}$. Photosynthetic rate (A), transpiration (E), and stomata conductance (G_s) were measured immediately before treatments began, and then at the end of each dry cycle when the average soil moisture volumetric content was at a minimum prior to rewatering. Leaf area enclosed in the cuvette was determined using an EPSON expression 1680 scanner (EPSON America, Inc 45 Long Beach CA) at the end of the measurement period. Intrinsic water use efficiency (WUE_i) was estimated as A/E ratio. Soil volumetric water content across a depth of 0 to 12 cm was monitored twice a week using a HydroSense soil moisture meter (Campbell Scientific, Inc, Logan UT). Two compound leaflets were selected from each plant after measurements to assess a chlorophyll content index using a SPAD chlorophyll meter (SPAD-502 Minolta CO.LTD. Japan). As the leaflets were too small to fill the entire frame of the SPAD meter, a standard 2 mm leaf disk was removed using a Disposable Biopsy Punch with PLUNGER (Miltex, Inc. York, PA) and placed on the optical screen.

At the end of the experiment (84 days after the start of treatment) all plants were harvested. We manually washed soil from roots and separated different plant parts (leaf, stem, and root) and then dried them at 65°C to a constant weight. Before drying, root area and leaf area from each plant were measured by using WinRHIZO Software (version 2005b Pro, Regent

Instruments Inc, Quebec, Canada) and an EPSON Expression 1680 scanner (Epson America Inc., Long Beach, California).

2.2.4. Data analysis

Statistical analysis was conducted using JMP software (version 12). Analysis of variance was used to determine the effect of drought and provenance on the biomass and the physiology of *Senegalia senegal*. Data were tested for normality and constant variance in order to meet assumptions of ANOVA and regression analysis. A Box-Cox transformation was used whenever we needed to improve normality and homoscedasticity. Student's t-test and Tukey Tukey's Honestly Significant Difference test were performed to determine the significance for two means and several mean comparisons respectively at 0.05 probability level. We conducted an allometric analysis between natural log (ln) transformed growth variables. We used type II regression model to determine the slope in order to look for any allometric scaling relation between above ground and below ground biomass. An allometric relation is expressed as power function $Y_1 = \beta Y_2^\alpha$ (where Y_1 and Y_2 represent the two different variables, β and α are the scaling constant and exponent, respectively).

2.3. Results

After 3 months of growth, significant effects of drought on *Senegalia senegal* biomass, height, and diameter were found (Table 2. 2). All biomass components were reduced by the drought treatment ranging from a 16.3% reduction in root area to 38.7% reduction in leaf area. Shoot and root dry weight were reduced by 35% and 19% respectively and total biomass was reduced by 28.5%. Root/shoot ratio was increased by 25%. The allometric scaling coefficient

for shoot to root growth was significantly changed due to the drought treatment (0.93 versus 0.77). This indicates that the drought treatment caused a shift in allocation from shoots to roots, and that the change in root/shoot ratio is not just a size dependent shift in allocation. Ngane presented significantly both the lower root/shoot ratio (Table 2. 3) and the lower biomass allocation to root (0.65 against 0.87) than the other provenances.

There was no drought by provenance interaction in our findings; however, there was a significant provenance main effect for biomass components, height, and diameter (Table 2. 3). Total dry weight ranged from 8.7 g for Diamenar and Kidira to 17.5 g for Ngane. Root/shoot ratios did not differ between provenances, with the exception of Ngane which was much lower averaging only 0.6 compared to all the others which ranged from 1.7 to 1.4. Allometric analysis of Ngane in comparison with all other provenances suggests that the differences in root/shoot ratio is due to a shift in allocation from roots to the shoot in Ngane (allometric scaling coefficient is 0.65). Height, diameter, leaf area, leaf weight, stem weight, shoot weight, and total weight were significantly higher in Ngane provenance. Daiba displayed the highest root area (539.9 cm²) and root weight (6.7 g).

Analysis of variance of A, G_s, E, and WUE_i, revealed significant differences in drought, provenance, and the drought by provenance interaction (Table 2. 5). Before drought began, no significant differences of leaf gas exchanges were found between provenances (Table 2. 5, Figure 2. 1). However, at the second and third dry down periods, there was a significant drought by provenance interaction for A, G_s, and E (Table 2. 5).

Table 2.2: The effect of the drought treatment on *Senegalia senegal* growth parameters. Student's t-test comparison is given. Means with the same letters on each line are not significantly different at $P < 0.05$

Treatment ¹	Control	Drought
Root area (cm ²)	463.8±26.0 a	388.1±23.4 b
Leaf area (cm ²)	257.1±17.2 a	157.5±15.3 b
Root weight (g)	5.7±0.4 a	4.6±0.3 b
Leaf weight (g)	1.8±0.1 a	1.1±0.1 b
Stem weight (g)	3.7±0.4 a	2.4±0.3 b
Shoot weight (g)	5.5±0.5 a	3.6±0.4 b
Total weight (g)	13.0±0.9 a	9.3±0.7 b
Root/shoot	1.2±0.1 b	1.5±0.1 a
SLA (m ² /kg)	15.7±1.3 a	14.7±0.3 a
Chlorophyll content	2.1±0.1 a	1.9±0.1 a
Height (cm)	9.1±0.2 a	7.5±0.2 b
Diameter (mm)	41.3±1.8 a	33.5±1.4 b

The drought treatment was a total of 3 dry cycles where plants were not rewatered until soil moisture content reached 4.7%, 2.7%, and 2.1%, at the end of the three successive dry cycles respectively

Table 2.3: Provenance's effect on height, diameter and biomass on *Senegalia senegal* seedlings. LS Means Tukey honest significant difference (HSD) is given. Means followed by the same letters on each line are not significantly different at $p < 0.05$.

Source	Daiba	Diamenar	Kidira	Ngane	DieryBirane	Ranerou	Velingara
Root area(cm ²)	539.9±68.4 a	403.4±36.2 abc	358.6±31.1 abc	525.1±39.4 ab	353.9±37.3 bc	461.4±41.7 abc	339.6±31.3 c
Leaf area(cm ²)	216.6±31.8 b	156.4±17.1 b	196.6±36.1 b	330.8±36.8 a	147.6±24.3 b	235.1±25.3 ab	168.1±32.9 b
Root weight (g)	6.7±1.0 a	4.2±0.5 b	4.5±0.5 ab	5.3±0.5 ab	5.3±0.5 ab	5.4±0.4 ab	4.7±0.6 ab
Leaf weight (g)	1.5±0.3 b	1.1±0.2 b	1.1±0.2 b	2.6±0.3 a	1.1±0.1 b	1.5±0.2 b	1.2±0.2 b
Stem weight (g)	3.6±0.7 b	2.2±0.2 bc	2.0±0.3c	6.6±0.7 a	2.3±0.3 bc	2.7±0.2 bc	2.3±0.4 bc
Shoot weight (g)	5.1±0.9 b	3.3±0.4 b	3.1±0.4b	9.3±1.0 a	3.4±0.4 b	4.2±0.3 b	3.4±0.6 b
Total weight (g)	13.4±2.0 ab	8.7±0.9 b	8.7±1.1b	17.5±1.7 a	9.8±1.0 b	11.1±0.6 b	9.3±1.4 b
Root/shoot	1.4±0.2 a	1.4±0.1 a	1.5±0.1 a	0.6±0.1 b	1.7±0.1 a	1.4±0.2 a	1.5±0.1 a
Height (cm)	40.6±2.4 ab	31.6±1.5 bc	29.3±2.2 c	49.4±3.2 a	32.8±2.4 bc	40.4±1.8 ab	37.7±3.9 bc
Diameter (mm)	8.4±1.6 ab	8.0±1.8 b	8.3±1.4 ab	9.8±1.5 a	8.0±1.3 b	8.2±1.5 b	7.6±0.9 b

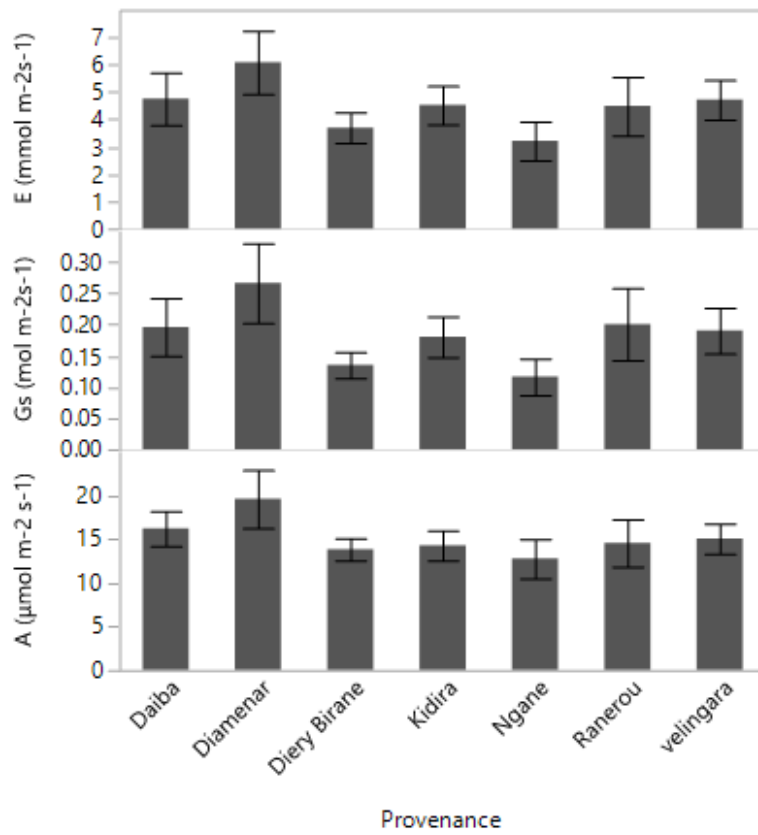


Figure 2.1: Net photosynthetic rate (A), stomatal conductance (Gs), and transpiration (E) of *Senegalia senegal* provenances prior to the start of the drought treatment. Bars show treatment means. Error bars are standard errors (\pm SE).

Table 2.4: Effects of block, drought, provenance, and drought-provenance interaction on A (net photosynthetic rate), Gs (stomata conductance), E (transpiration), WUEi (intrinsic water use efficiency) and (r_s) stomatal resistance of *Senegalia senegal* seedlings

Initial stage	Variable	Block	Drought	Provenances	Drought*Provenances
	A	*	ns	ns	ns
	Gs	**	ns	ns	ns
	E	**	ns	ns	ns
	WUEi	ns	ns	ns	ns
	r_s	***	ns	ns	ns
Dry Down Cycles¹					
First	A	ns	**	ns	ns
	Gs	ns	**	ns	ns
	E	ns	**	ns	ns
	WUEi	***	ns	ns	ns
	r_s	***	ns	ns	ns
Second	A	**	***	ns	**
	Gs	**	***	ns	*
	E	**	***	ns	*
	WUEi	ns	**	ns	ns
	r_s	ns	**	ns	ns
Third	A	ns	***	ns	**
	Gs	*	**	**	**
	E	**	**	**	**
	WUEi	**	ns	ns	ns
	r_s	*	ns	ns	ns

*P < 0.05, ** p < 0.01, ***p < 0.0001, ns (not significant)

¹ Dry down cycles correspond to the last days of each drying cycle before plants were rewatered

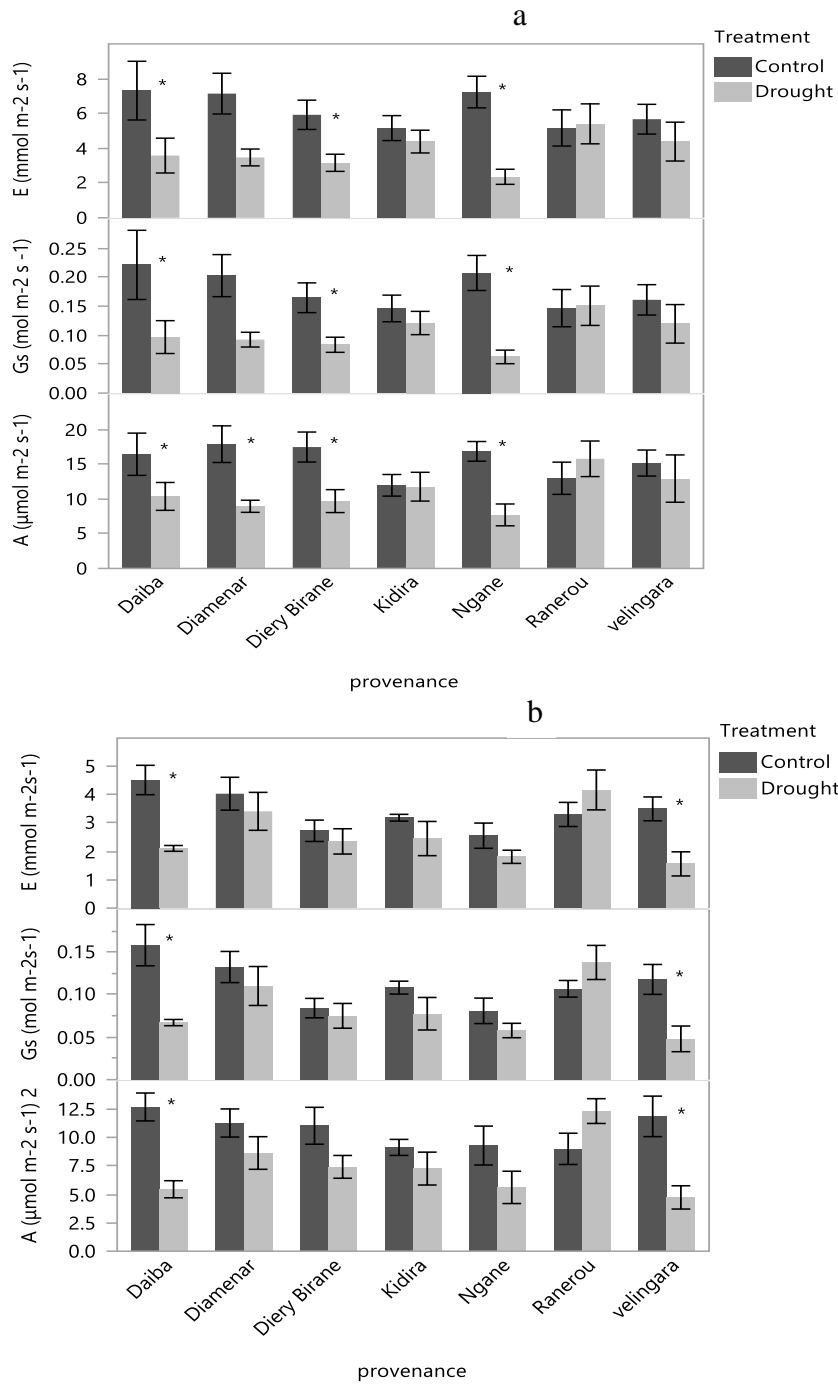


Figure 2.2: Photosynthetic rate (A), stomatal conductance (Gs), and transpiration (E) as effected by provenances and drought treatment at the second (a) and third (b) dry down cycles. Bars show treatment means. Error bars are standard errors (\pm SE). * indicate significant differences between treatments at $P < 0.05$ after Tukey HSD test.

Changes of A, G_s, and E between provenances under control and drought at the second and third dry down cycles are shown in Figure 2. 2a and b. In general, with the exception of Ranerou, all provenances had lower gas exchanges in the drought treatment. In the second drought cycle, Daiba, Diamenar, Diery Birane, and Ngane had significantly lower net photosynthetic rates and Daiba Diery Birane, and Ngane had lower G_s, and E (Figure 2. 2a). At the third dry down cycle, when drought stressed, Daiba and Velingara had significantly lower A, G_s, and E (Figure 2. 2b).

2.4. Discussion

As expected, all biomass components, height, and diameter of *Senegalia senegal* seedlings were considerably reduced by drought stress (Table 2. 2) similar to that reported by Merine *et al.* (2014). There was no drought by provenance interactions, hence no particular provenance is more or less tolerant to drought on the basis of our analysis of seedling growth. The higher root/shoot ratio we found in the drought treatment (1.50 against 1.19) is likely due to shifts in allocation to the root systems (Chapin 1991; Chiatante *et al.*, 2015; Espinoza *et al.*, 2013; Espinoza *et al.*, 2012; Harris, 1992). An increased root/shoot ratio in response to drought, likely to favor water uptake, has been frequently observed in many drought tolerant species (Lei *et al.* 2006; Liu *et al.* 2001). Ngane which accumulated a higher total biomass than the other provenances presented a lower root/shoot ratio that is likely due to a faster increase in top growth than in root growth. This finding corroborates an early study (Mustafa *et al.* 1996) which found that drought tolerance in term of WUE among *Senegalia senegal* provenances was positively associated with both higher dry matter accumulation and low root/shoot ratio. However, WUE_i was not different among provenances in our study. Possibly, the drought cycle treatment applied

may not be severe enough to induce different WUE between genotypes. Niklas (2005) stated that plant size may have a great influence on resource allocation as larger plants will have to invest a larger fraction of their biomass in corresponding tissues. Also, Coleman et al. (1994) argued that plant size is the primary cause of changes in resource allocation. That theory of size dependency on resource allocation does not seem to be applicable to our finding, especially the allocation between above and below ground biomass. Based on our allometric analysis, biomass allocations to root and shoot were statistically different ($P < 0.0001$) between Ngane (0.65) and the other provenances (0.87). Our results corroborate Coyne and Bradford (1985) that there is evidence of genetic variation in morphological adjustment.

Photosynthesis limitation as affected by water status depends on not only genotypes, but also drought intensification (Chaves et al. 2003). Significant drought by provenance interaction occurred when soil volumetric moisture content dropped to 2.7% and 2.1% at the second and third dry down cycles respectively. In fact, a significant positive correlation between net photosynthetic rate and soil moisture content was found in this study (data not shown). Aroca (2012) reported that a reduced soil moisture content may close stomata in order to minimize water loss while reducing net photosynthetic rate. Different responses to drought in (A), (G_s), and (E) between provenances (Table 2. 4, Figure 2. 2) revealed potential genetic variabilities between the tested provenances. At the second and third dry down cycles, Daiba, Diery Birane, Ngane, and Velingara had significantly reduced (G_s), (E), and (A) (Figures 2. 2a and b). It appears that under drought stress in these seed sources, stomatal conductance regulates water loss through transpiration thereby affecting net photosynthetic rate (Farquhar, 1982). Kidira and Ranerou physiology was less impacted by the drought treatments and may be less susceptible to drought stress. Moreover, the relative stomatal limitation, although not significant between

provenances varied from 52% to 44% for Velingara and Kidira, respectively. The decrease of A under drought among provenances is influenced by both stomatal and non-stomatal limitations. In fact, both stomatal and non-stomatal factors have been known to limit photosynthetic rate in plant leaves (Farquhar, 1982). However, little is known about the regulation of mesophyll conductance, which is crucial to understand the process of plant acclimation to water stress (Flexas *et al.*, 2008). Farooq *et al.* (2009) reported that although stomatal resistance may substantially limit A, non-stomatal limitations may be more important in some circumstances. Limited mesophyll response to drought was observed in other species such as *Gossypium hirsutum* and *Triticum aestivum* (Barbour, 2016), *Cedrus atlantica* (Grieu *et al.*, 1988, Guehl *et al.*, 1991), *Eucalyptus pauciflora* (Krishbaum, 1987), *Ricinus communis* (Neto *et al.*, 2014). These findings clearly reveal that both stomatal and mesophyll regulations are critical for the genotype by drought interaction in *Senegalia senegal*. It has been already shown in some woody species that drought tolerance can be attributed to stomatal and non-stomatal components (Ehleringer and Cook, 1984; Palladry, 2008). Siam (2011) revealed same evidences to different *Senegalia senegal* genotypes that displayed different tolerance strategies to drought.

Drought limitation to photosynthesis in *Senegalia* species has been reported in early studies (Aroca, 2012; Lassouane, 2013; Liu, 2011). We found stomatal limitations prior to drought to be 44.1% and 44.5% for control and drought respectively. However, drought significantly increased relative stomatal resistance (the opposition to transport of the rate of carbon dioxide entering and water vapor exiting through the stomata of a leaf) and WUE_i by both 13.3% at the second dry down cycle. This finding confirms Jones (2013) who stated a potential increase of WUE as G_s goes down which was the case in this study. However, A and internal leaf CO₂ concentration (C_i) were both significantly reduced by 43% and 18%, respectively implying that stomatal

responses are overall the most limiting factors to A (Farquhar and Sharkey, 1982). Chlorophyll concentration was not affected by water stress and did not differ between provenances (Tables 2 & 3). This result was consistent with the finding of Lassouane *et al.* (2013) who revealed no significant difference of chlorophyll content between controlled and drought stressed *Senegalia arabica*. However, Stomatal distribution would be of great importance to explore as it remarkably affects gas exchanges among species/genotypes (Nilson and Assmann, 2007).

Provenance differences in physiological traits have been reported in an earlier study by Raddad and Luukkanen (2006) who associated the origin of *Senegalia senegal* provenances with growth performance. Plants with a high rate of photosynthesis per unit of leaf area have been shown to produce more dry matter and grow more rapidly than plants with a low rate (Kramer, 1979). Indeed, Kramer and Kozlowski (1979) have shown interest in using CO₂ assimilation rate to determine growth potential of different genotypes. Our results revealed similar net photosynthetic rates, but significant growth responses and biomass allocation are found at provenance level. Ngane provenance, displaying the highest total weight, shoot weight, leaf area, height, and diameter, does not exhibit the highest A. Our study suggests the relationship between growth and physiological process is more complex than that suggested by Kramer and Kozlowski (1979). In this study, growth was not related to photosynthetic rate which in fact did not differ between provenances throughout the experiment. It is evident that photosynthetic capacity has a positive association with a biomass production, but there are studies which showed no or little association of photosynthetic capacity with growth in many species (King *et al.*, 2008; Pinheiro, 2007). Plant growth also involves phyto-hormones regulation and phases of cell division, enlargement and differentiation (Aroca, 2012) that were not investigated in this study.

2.5. Conclusion

Drought treatment significantly reduced growth, biomass allocations, and photosynthetic performance of *Senegalia senegal* seedlings. However, there was no drought by provenance interaction on any growth variables. Drought stress resulted in more biomass allocated to roots. This study shows that under moderate drought stress, no provenance displayed intrinsic features on WUEi, stomatal conductance, and growth traits. With leaf gas exchange, there was a drought by treatment interaction. Differences in photosynthetic capacities between provenances under drought were likely due to variation in both stomatal and non-stomatal control.

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3. Chapter 3: Growth and physiology of *Senegalia senegal* (L.) Britton seed sources as influenced by salinity, fertility, and drought.

Abstract

Dryland features are often associated with increasing salinity of soils, which are exasperated by poor soil fertility. Assessing genotype by environment interactions will be of great importance for tree selection for reclamation of degraded arid lands. The main objective of this study was to examine the growth performances and physiological and morphological adaptations to drought, salinity, and fertility treatments of different *Senegalia senegal* seed sources. We used five seed sources in this study selected from 60 families of a *Senegalia senegal* progeny trial in Dahra, Senegal. Seeds were grown under greenhouse conditions in a randomized complete block design with five replications. A two levels factorial combination was used for salinity and fertility treatments. The drought treatment consisted of watering all plant for three weeks and then stopping all watering for three more weeks. A significant seed source x salt x fertilizer interaction was found for all biomass parameters. The fertilizer application resulted in a significant increase of total biomass of all seed sources, ranging from 63% to 237% for Ng21B1 and K17B19, respectively. In contrast, salt only decreased total biomass of Ng21B1. Despite similar net photosynthetic rates before treatment started, fertilizer and salinity induced different effects between seed sources. Prior to drought stress, fertilizer did not affect photosynthesis of Dia27B16, while salt decreased significantly stomatal conductance of all seed sources. Dia27B16 and Ng21B1, despite significant differences of stomata size and density, significantly decreased transpiration, and thereby increased their intrinsic WUE. Under drought, relative growth rate was significantly decreased. More salinity and fertilizer levels need to be explored in

the future to detect optimum biomass, growth and photosynthetic rates under salt and fertilizer conditions.

Key words: *Senegalia senegal*, salt tolerance, fertilizer, seed sources, drought

3.1.Introduction

In recent years, frequent drought occurrences and anthropogenic pressures on forest resources in arid and semi-arid lands have heavily impacted species composition (Gonzales, 2001). The natural vegetation becomes sparse and progressive soil degradation develops, which accelerates desertification and induces salinization (UNEP, 2015). During years of drought, profits from traditional agriculture decrease and local populations often rely on forest resources to supplement their income (Bradley and Grainger, 2004). Estimates of deforestation in Senegal suggest a decrease of 9.4% in forest cover from 1990 to 2010 (UNEP, 2013). Reforestation programs and natural species regeneration lag far below resource exploitation (UNEP, 2013; UNEP, 2015). *Senegalia senegal* is among the most overused forest trees in such ecosystems. It is particularly exploited because it is the source of many products, including gum arabic which is traded on both local and international markets. *Senegalia senegal* is a drought tolerant species, widely used to control desertification in arid and semi-arid lands. Recent studies have revealed its potential tolerance to salinity stress (Fall, 2016; Pandey, 2007; Sambou, 2010). 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 (Sonneveld *et al.*, 2010). 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 seed sources or genotypes more adapted to hostile environments. Stomatal response is among the most important factors influencing photosynthetic capacity under limiting environmental factors. Different stomatal characteristics may account partially for different stomatal resistance that consequently impact photosynthetic rate. However, little attention has been given to leaf physiology of *Senegalia senegal*, and particularly stomata characteristics. Under water stress, stomatal density is known to impact leaf photosynthesis and water use efficiency (Masle *et al.*, 2005; Xu and Zhou, 2008). Reduced stomatal density can be advantageous under saline stress (Ouyang *et al.*, 2010, Reginato *et al.*, 2013). Drought and salinity stress both induce reduced photosynthetic rate through stomata and mesophyll restriction (Chaves *et al.*, 2011; Chaves *et al.*, 2009). 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* seed sources 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 drought, salinity and fertility treatments. We are interested in seeking ecotypes that perform best in terms of dry matter accumulation and intrinsic water use efficiency under those treatments, and whether stomatal characteristics explain potential changes among provenances and environmental conditions.

3.2. Material and methods

3.2.1. *Plant material*

Senegalia senegal seed sources 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 3. 1). Each seed source represents a family, and seed sources within same name and different numbers belong to the same provenance.

3.2.2. *Growth conditions, treatment, and experimental design*

The experiment was conducted from June to October 2015 in a greenhouse located on the Virginia Tech campus, Blacksburg, VA, USA. Inside the greenhouse, 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-hour 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 x 21 cm deep, Stuewe and Sons, Tangent, OR) containing Promix and kept well-watered for about six weeks. They were then transplanted into a 2.3-liter plastic pots (Treepots, 30.5 cm height and 10.2 width, IGC Greenhouse Megastore, Danville, IL) containing either soil substrate (a 2:1 mix of Promix BX and washed sand) or soil substrate with salt added. A factorial combination of a salinity and fertilizer treatments was then established.

Table 3.1: Families and mean annual gum yields (g) by tree of the mother trees from which seeds in this study were harvested. (-) means tree died.

Provenance	Families	Code	2013 Gum yield	2014 Gum yield	2015 Gum yield	2016 Gum yield
Diamenar	Diamenar27B16	Dia27B16	29.3	9.7	0	-
Diamenar	Diamenar27B14	Dia27B14	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	Ng21B1	522.2	476.4	945.2	1103.5

The salinity treatment consisted of mixing 1070g of sodium chloride with 100kg of soil substrate. The dose of salt used was based on an early study which revealed a significant decrease of about 50% on biomass of *Senegalia senegal* seedlings (Hardikar and Pandey, 2008). 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 (Hardikar and Pandey, 2008). Thereafter, electrical conductivity of the supernatant solution was determined with an electrical conductivity meter (2265FS/2265FSTP, Spectrum Technologies, Inc. Aurora, IL). 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 1000 kg N-P-K (10-10-10) per hectare. 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. Prior to drought stress, shallow pots were placed beneath treepots which contained salt (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 procedure was adopted to maintain the same salt concentration in each pot prior drought stress.

3.2.3. 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 X 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. Number of stomata for each sample was counted under a photomicroscope system with a computer attachment.

3.2.4. 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, Nebraska, USA). Environmental conditions in the leaf cuvette were set to 2000 μmols m⁻² s⁻¹ photosynthetic photon flux density (PPFD), 398 μmols mol⁻¹ reference CO₂ concentration, 25° C block temperature, and a flow rate of 400 μmols sec⁻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. NH,

USA). Three leaflets per plant were randomly chosen from new fully mature leaves and an average taken for analysis.

3.2.5. *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. The relative growth rate consists of mean relative growth rates or relative increment over a time period (Δt) (Blackman, 1919). In our study, we considered the rate of height and diameter increments before and then after drought stress treatment, corresponding to the growth rate (diameter and height increment) per day when plants are well watered and subjected to drought, respectively, 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 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, Quebec, Canada) and an EPSON Expression 1680 scanner (Epson America Inc., Long Beach, California). The area of leaves for each plant was determined with Photoshop software CS6 (version 13.0.1, Adobe Systems. Inc. Mountain View, California). Different plant parts were then dried at 65°C to a constant weight before weighing them to determine dry matter.

3.2.6. *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 assumption 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. (ANOVA) was performed to test treatments and seed source 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 HSD were used for separation of means tests.

3.3.Results

3.3.1. *Stomatal density and size*

We found the presence of stomata on both abaxial and adaxial leaf surfaces. Stomatal density and length did not differ between leaf surfaces. However, stomata were slightly more abundant on the adaxial surface. Hence, stomatal density was considered to be the average of abaxial and adaxial surfaces. No seed sources by treatment interactions in stomatal density and stomatal size were found to be significant. However, salinity significantly increased stomatal density from 82 to 89 stomata per mm², and a significant effect of seed source was found on both stomatal length and distribution (Figure 3. 1 and 2). The Diamenar provenance Dia27B16 had the highest stomatal density but was only significantly greater than Ngane Ng21B1 (Figure 3. 2a). Ngane displayed significantly larger stomata than all other provenances and significantly lower stomatal density than the Diamenar provenance (Dia27B14 and Diam27B16) (Figure 3. 1 and 2).

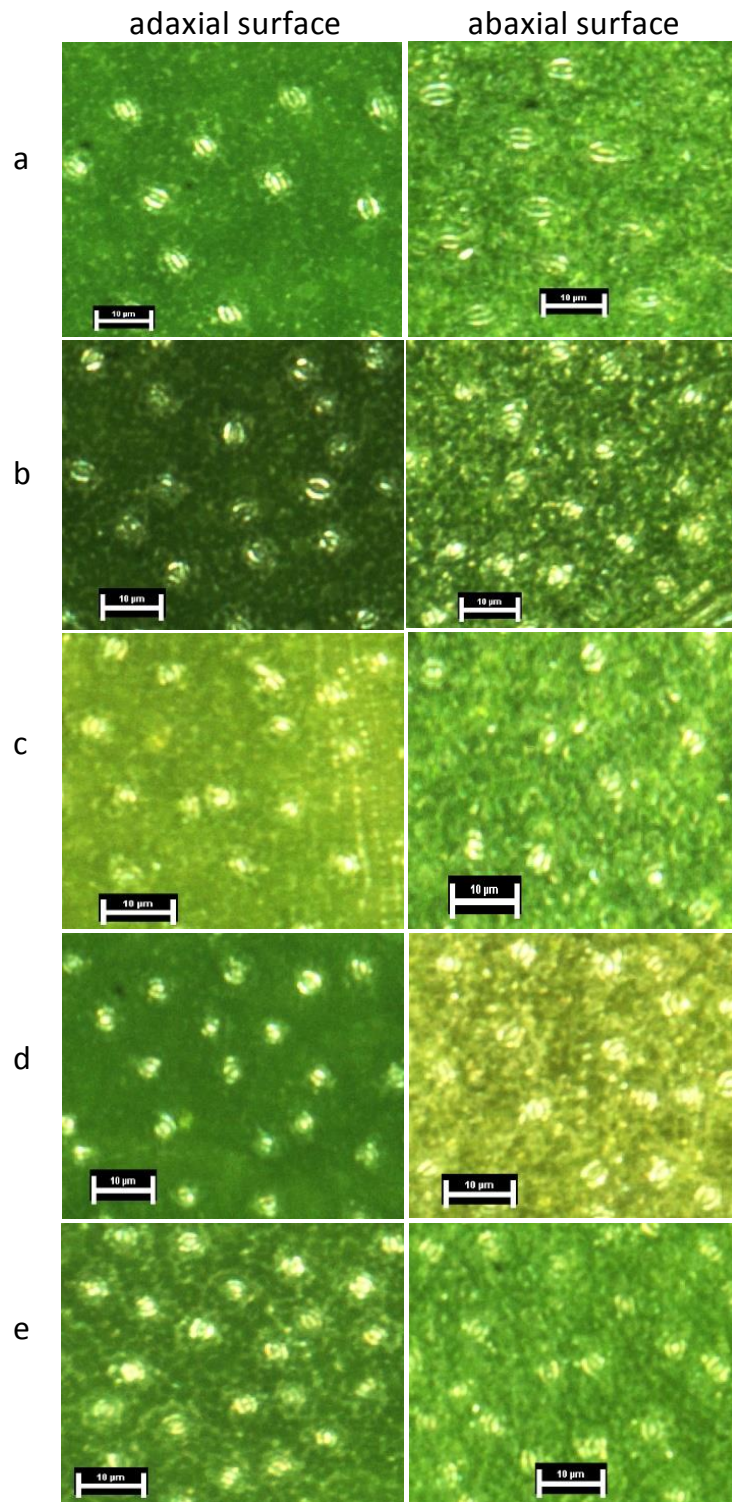


Figure 3.1: Stomata on the abaxial and adaxial leaf surfaces of 12-week-old *Senegalia senegal* seedlings. Ng21B1 (a), K4B19 (b), K17B19 (c), Dia27B14 (d), Dia27B16 (e).

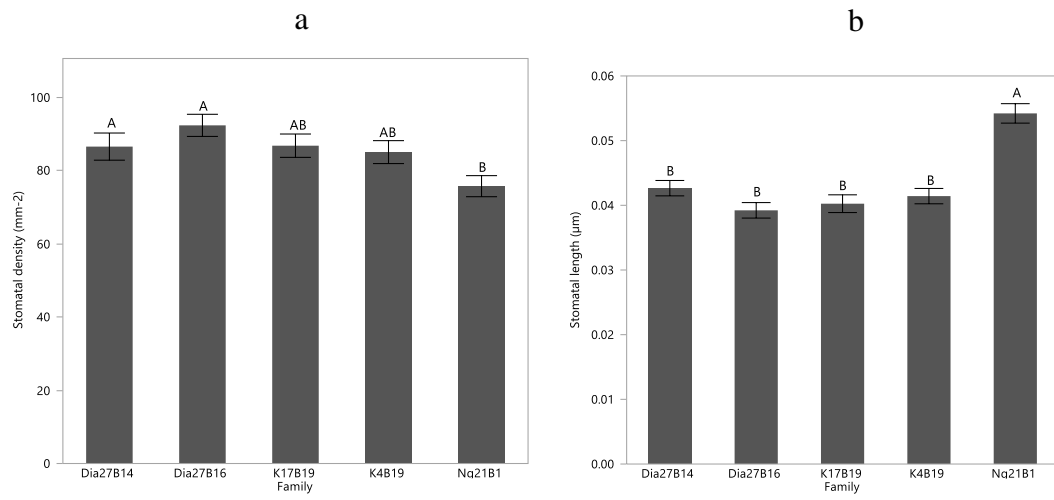


Figure 3.2: Variation of stomatal density (a) and size (b) among *Senegalia senegal* seed sources

3.3.2. *Leaf gas exchanges and chlorophyll content*

Prior to drought stress, a seed source x fertilizer, and seed source x salt interaction was significant for A, G_s , E, and WUE (Figure 3. 3a and b). With the exception of Dia27B16, fertilizer significantly increased A. G_s and E were also generally increased with fertilizer, but only in a few seed sources where they increased significantly (Figure 3. 3a). Since both A and E increased with fertilizer, the WUE was generally unaffected by fertilizer. However, K17B19 displayed higher WUE. The salt treatment significantly decrease G_s in all seed sources and E was lower in all seed sources, but only significantly decreased in Dia27B16, K4B19 and Ng21B1. A was lower in the salt treatments in all seed sources, but only significantly decreased in K4B19. WUE was increased in all seed sources, but only significantly in Dia27B16 and Ng21B1 (Figure 3. 3b).

No interaction effect was found to be significant for total chlorophyll content, which was similar among seed sources 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 ($P < 0.0001$) 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.

At the end of the 3 weeks of drought there was a significant salt x seed source interaction for A, G_s , E, and WUE. Gas exchange rates were lower after the drought stress and the salt treatment generally still decreased G_s and E, and increased WUE. However, the magnitude of these effects varied with seed source (Figure 3. 4).

3.3.3. *Biomass and relative growth rates*

There was a significant seed source x salt x fertilizer interaction for all biomass parameters (Table 3. 2). K17b19 was the only seed source 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 3. 2). No other family had growth changed by the salt treatment. Growth was generally increased (more than 100%) in all seed sources in response to added fertilizer (Table 3. 2). Salt generally had a negative impact on this fertilizer growth enhancement.

The main effects of seed source and salt treatments had a significant influence on specific leaf area (SLA) and root/shoot ratio. Ng21B1 had the lowest SLA and root/shoot ratio while the other seed sources did not differ across source (Figure 3. 5 and 6). 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. 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 (Figure 3. 7a). In contrast, fertilizer only increased RGH prior to drought (Figure 3. 7b). A significant difference among seed sources was found on RGH, whereas RGD was similar between seed sources. Prior to the drought, Ng21B1 had significantly greater RGH than all other seed sources. During drought, RGH was greatly reduced and K17B19 displayed the highest RGH, but it was only significantly higher than Dia27B16.

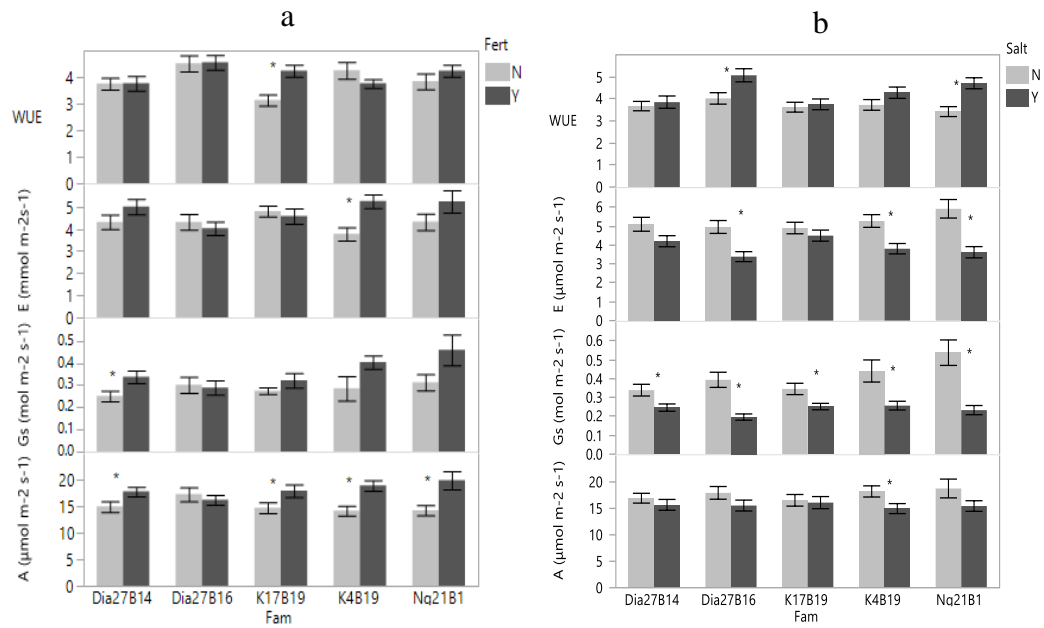


Figure 3.3: The effect of fertilizer (a) and salt (b) treatments on net photosynthetic rate (A), stomatal conductance (Gs), transpiration (E), and intrinsic water use efficiency (WUE) of *Senegalia senegal* seed sources.

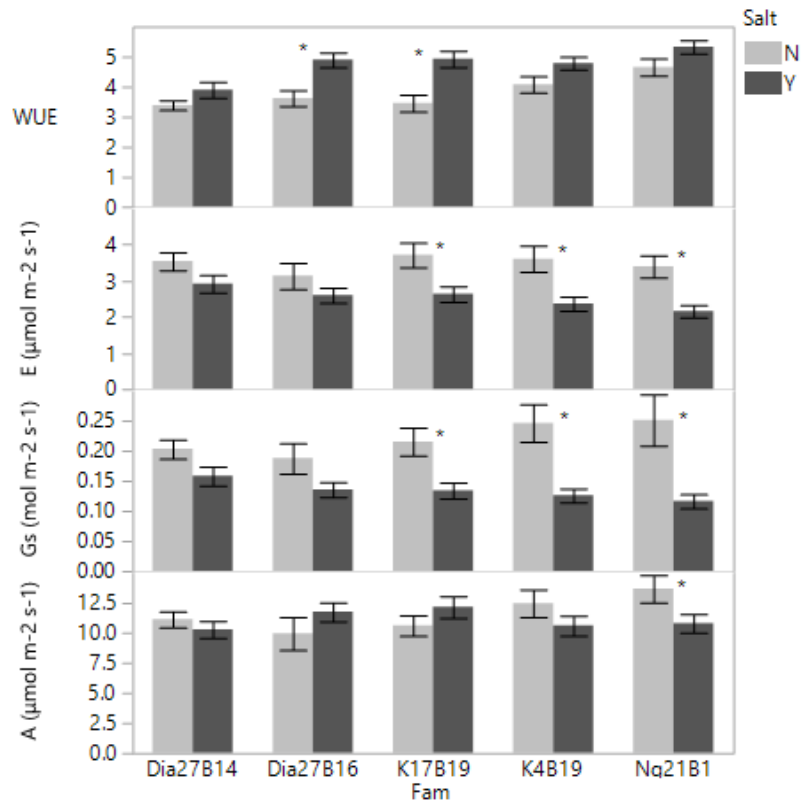


Figure 3.4: Effect of salt on net photosynthetic rate (A), stomatal conductance (G_s), transpiration (E), and intrinsic water use efficiency (WUE) of *Senegalia senegal* seed sources under drought stress.

Table 3.2: The effect of salt and fertilizer treatments on growth parameters of *Senegalia senegal* seed sources.

Variable	Treatment	Seed sources				
		Dia27B14	Dia27B16	K4B19	K17B19	Ng21B1
Leaf (g)	Control	1.4±0.47Aa	1.7±0.28Aab	2.3±0.61ABab	1.5±0.17Aa	3.7±0.40Bb
	Salt	1.4±0.24Aa	1.6±0.12Aa	1.7±0.18Aa	2.1±0.16Aab	1.6±0.17Aa
	Fert	4.2±0.11Ab	4.9±0.45Ac	4.2±0.33Abc	4.8±0.41Ac	5.3±0.30Ab
	Salt*fert	2.9±0.09BCab	2.3±0.21Aab	3.7±0.36ABbc	3.3±0.30ABCbc	4.2±0.32Cb
Stem (g)	Control	1.3±0.40Aa	1.9±0.34Aa	1.7±0.47Aa	1.2±0.16Aa	2.7±0.23Aab
	Salt	1.3±0.23Aa	2.0±0.11Ba	1.8±0.14ABa	2.1±0.15Bab	1.5±0.17ABa
	Fert	3.9±0.24Ab	5.3±0.65Ac	4.8±0.62Ab	5.0±0.52Ac	5.2±0.25Ac
	Salt*fert	3.0±0.17ABab	2.5±0.21Aab	4.0±0.35Bb	3.3±0.31ABbc	4.2±0.35Bbc
Root (g)	Control	2.0±0.35ABab	2.7±0.17ABab	2.9±0.45ABa	1.7±0.25Aa	3.3±0.29Bab
	Salt	1.5±0.30Aa	2.4±0.22ABab	2.6±0.38Ba	2.5±0.25ABab	1.7±0.07ABa
	Fert	3.9±0.36Ac	5.4±0.40Ac	5.2±0.34Ac	5.1±0.68Ac	5.2±0.20Ac
	Salt*fert	1.8±0.19Aab	1.7±0.15Aa	3.3±0.14Bab	2.0±0.22Aab	3.6±0.41Babc
Leaf area (cm ²)	Control	242.2±111.3Aa	279.9±57.1Aa	366.9±106.9Aa	295.1±52.9Aa	551.5±61.6Aab
	Salt	263.2±58.7Aa	237.7±22.4Aa	380.6±90.6Aa	346.0±72.1Aab	226.2±50.5Aa
	Fert	891.6±80.6Ac	909.9±113.8Ac	686.8±111.3Aab	818.0±134.8Ac	756.1±106.1Ab
	Salt*fert	435.1±74.0Aab	323.9±38.4Aab	560.8±118.0Aab	522.5±49.2Aabc	546.6±92.7Aab
Total dry matter (g)	Control	4.6 ±1.16Aa	6.2 ±0.75ABa	6.8±1.41 ABab	4.4±0.49 Aa	9.7±0.76Bb
	Salt	4.2±0.71Aa	6.0±0.22 ABa	6.2±0.63 ABa	6.7±0.53Bab	4.8±0.38 Aa
	Fert	12.0±0.57 Ab	15.6 ±1.39Ab	14.1±0.90Acd	14.9±1.51 Ac	15.7±0.43 Ac
	Salt*fert	7.8±0.30ABab	6.5±0.45Aa	11.0±0.77 BCbc	8.6±0.80 ABb	12.0±0.99 Cbc

Values for each variable within the same treatment marked with different capital letters are significantly different (P<0.05 Tukey test) between seed sources. Values for each variable with different small letters are significantly different (P<0.05 Tukey test) for treatments within the same seed source. Values represent the mean of 5 replicates ± standard error

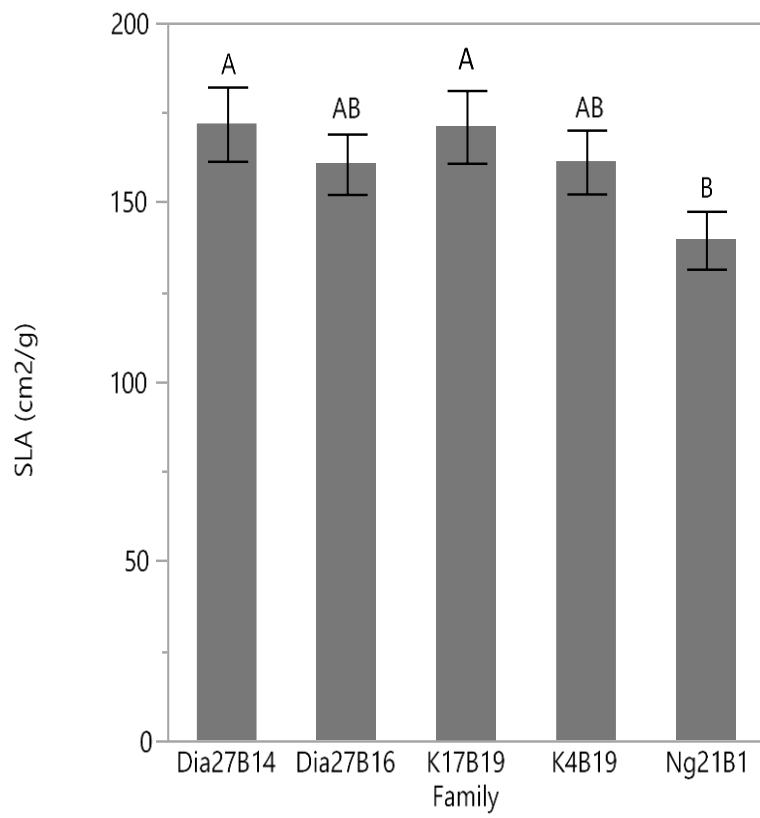


Figure 3.5: Variation of specific leaf area (SLA) of *Senegalia senegal* seed sources.

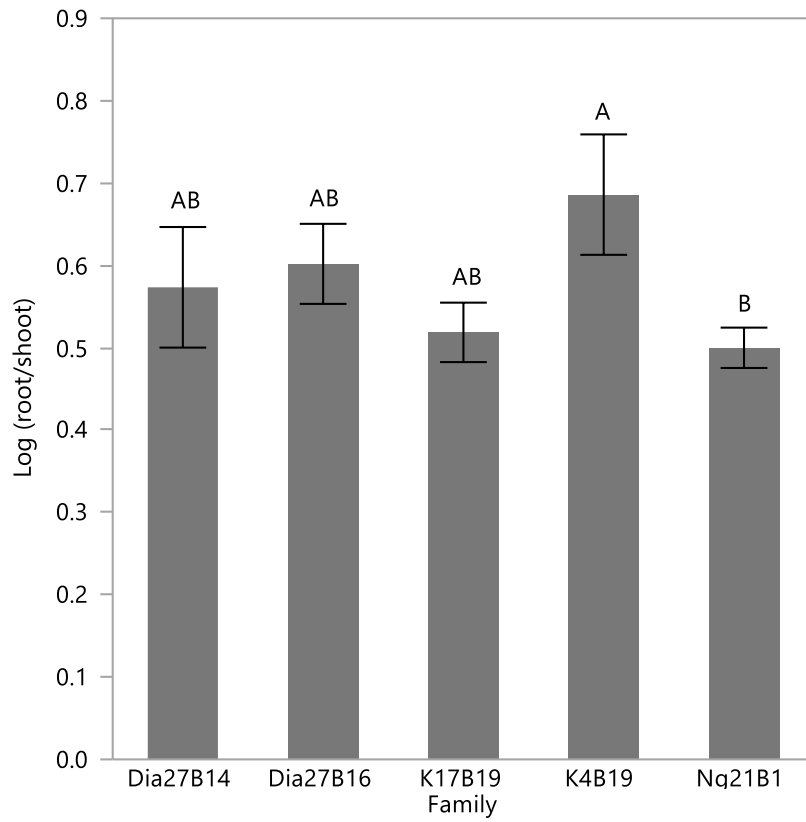


Figure 3.6: Variation of root/shoot ratio among *Senegalia senegal* seed sources seedlings.

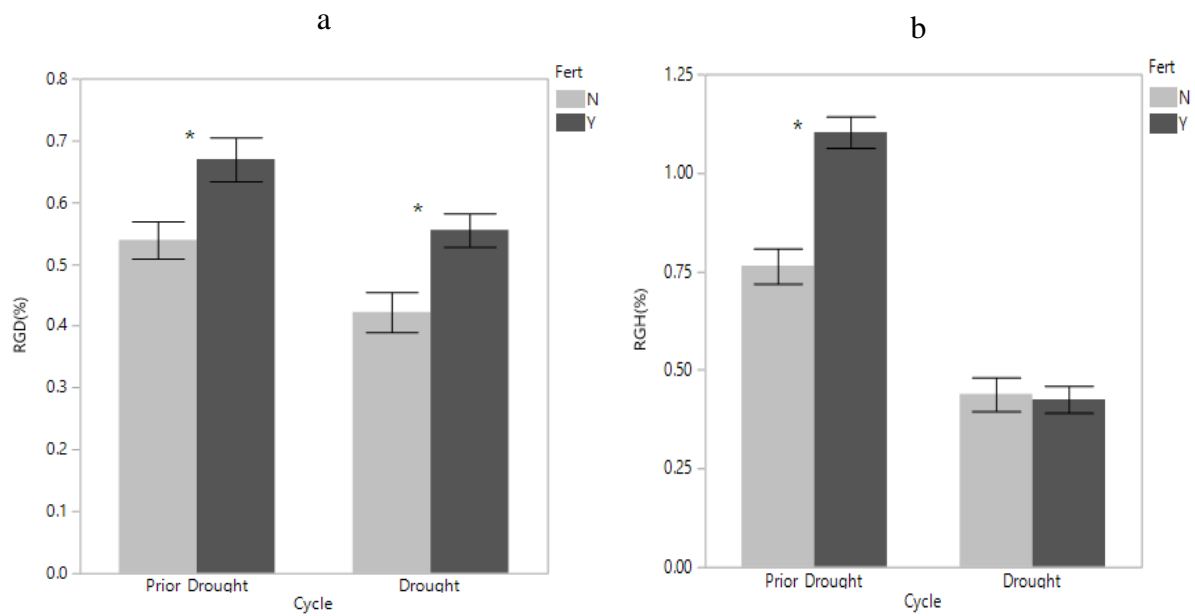


Figure 3.7: The effect of fertilizer on relative growth rate of diameter (RGD) (a) and growth rate of height (RGH) (b) of *Senegalia senegal* seedlings when watered (prior drought) and during drought stress (drought). Asterisks mark on the bar indicates significant effect of fertilizer at $P < 0.05$.

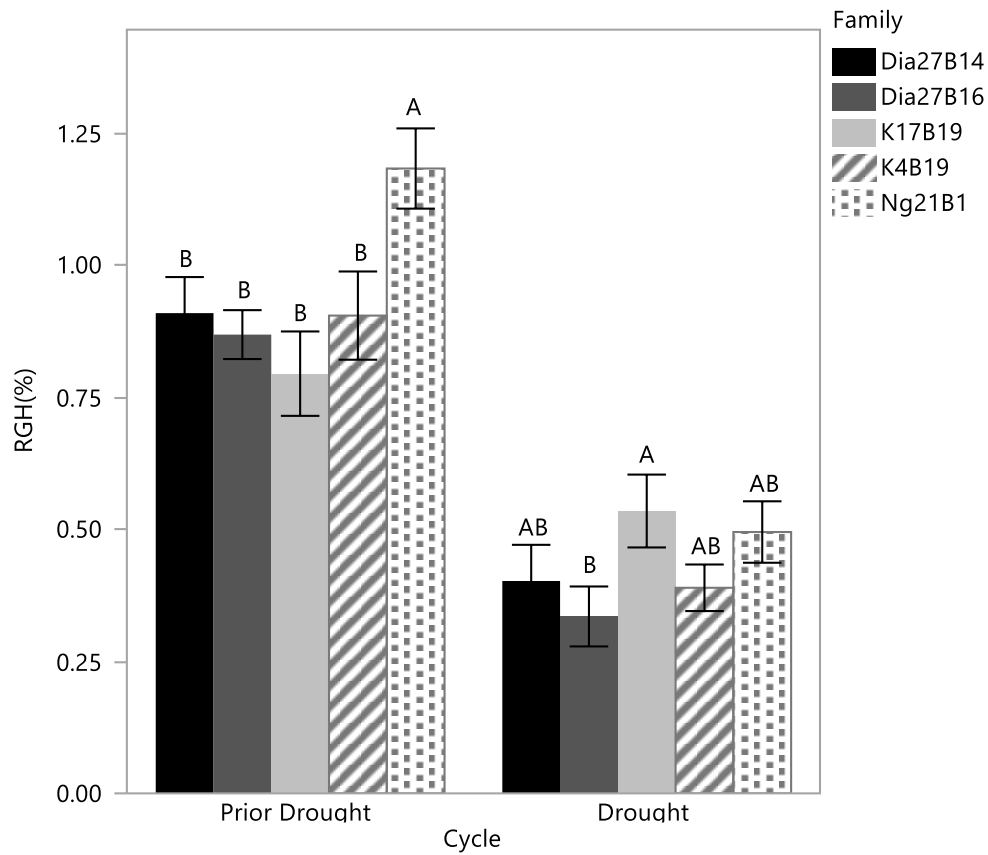


Figure 3.8: Variation of relative growth rate of height (RGH) between *Senegalia senegal* seedlings when watered (prior to drought) and during drought stress.

3.4. Discussion

This study was the first attempt to look for growth performance and physiological adaptation under salt, drought, and fertility conditions among different *Senegalia senegal* seed sources collected in Senegal. Results of this study confirmed our previous finding (Chapter 2) that photosynthetic capacities of *Senegalia senegal* seedlings are similar among seed sources in non-limiting water conditions. However, significant differences in stomatal density and size, SLA, root/shoot ratio, and RGH may be indicative of different responses to environmental stress among tested seed sources. The significant salinity by seed sources interaction on dry matter accumulation found in this study implies that some *Senegalia senegal* seed sources perform better on salt affected lands, than on non-salty lands (Flowers and Yeo, 1986). Ng1B1 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 (Hardikar and Pandey, 2007). However, they used a mixture of seeds collected from one coastal area and did not examine seed sources, so it is possible they utilized a susceptible source such as our Ng21B1.

The significant decrease of total biomass observed in Ng21B1 reveals its higher sensitivity to salt stress than the other genotypes tested in this study. Despite originating from more saline areas, Ng21B1 tended to be more sensitive to salinity, a finding than what was reported in an earlier study by Briggs (1978) who revealed that topodemes from areas subjected to salinity are more salt tolerant than those from non-salted areas. In contrast, according to Muturi (1993) salt tolerance in *Senegalia tortilis* might be attributed to genetic factors that may be site independent. *Senegalia senegal* is known as a salt tolerant species (Fall, 2016; Pandey, 2007; Sambou, 2010) 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 (Cha-um *et al.*, 2013; Fung *et al.*, 1998; Greenway and Munns, 1980; Munns, 2005; Ghoulam *et al.*, 2002). Surprisingly, K17B19 grew better in saline conditions, increasing total biomass by 51%, than in non-saline conditions, even though the increase was not significant. Munns (2005) argued that increasing growth of plants under saline soils is due to specific genes that control cell growth and leaf function. K17B19, K4B19, Dia27B14, and Dia27B16 may have inherent ability to control of water loss and improve ionic balance to their favor, therefore maintaining growth and biomass under salinity (Yadav *et al.*, 2011). 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 (Fall, 2016; Taiz *et al.*, 2015).

Unexpectedly, stomatal density and total chlorophyll content were significantly increased by salinity. Past studies found salinity to negatively affect stomatal density and chlorophyll content (Barbieri *et al.*, 2012; Cha-um *et al.* 2013; Larcher 1995; Reginato *et al.* 2013; Yang and Wang, 2000; Zhang *et al.*, 2006). The presence of salt increased WUE (Figures 3. 3b and 3. 4) and decreased SLA. 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 and SLA reported by Rao *et al.*, (1995), who suggested lower SLA as a selection criterion for enhanced WUE. Our study revealed that *Senegalia senegal* response to salt depended on genotypes and whether or not drought was induced. The increased WUE in Dia27B16 and Ng21B1 in response

to salt when water was not a limiting factor may be attributed to significant decrease of transpiration relative to assimilation rate through stomata closure (Figure 3. 3b). The same response was observed on K17B19 under drought condition. The increased WUE likely is due to high photosynthesis, or low transpiration, or both (Ehleringer *et al.* 1993; Li, 1999). However, under drought stress the higher WUE in response to salt without a significant change in stomata conductance observed in Dia27B16 might be influenced by other traits such as mesophyll conductance. Besides stomatal control, inherent differences in mesophyll CO₂ fixation capacity may influence the rate of photosynthesis and accordingly WUE (Flexas *et al.*, 2013; Pallardy, *et al.*, 2008). It is reported that enhancing mesophyll conductance/stomata conductance ratio can improve WUE (Flexas *et al.*, 2013; Flexas *et al.*, 2015). Moreover, notable effects of salinity and drought stress on mesophyll conductance have also been reported in *Vitis vinifera* (Ferrio *et al.*, 2012). Stomatal conductance did not differ between seed sources for the drought experiment only, but was decreased in saline conditions by 37% during drought and 39% by prior drought averaged across all seed sources. Still, influence of stomatal closure on A inhibition and regulation of stomatal conductance are strongly determined by both mesophyll and stomatal resistance to CO₂ diffusion (Nilsen, 1996).

As expected, the fertilizer significantly increased total biomass and biomass partition of all seed sources. A positive effect of N-P-K supply on *Senegalia senegal* growth has been revealed in an earlier study (Daldoum, 2016). The significant increase in total biomass was likely due in part to the significant increase in net photosynthetic rate under fertilizer conditions in most seed sources (Figure 3. 3a). However, the increased biomass partitioning was higher in leaves and stems than in roots, inducing a lower root/shoot ratio (Figure 3. 7b). It is reported that in young seedlings nutrient addition favored shoot growth more than root growth (Fernandez, 2006;

Stovall *et al.*, 2012; Pallardy *et al.*, 2008). 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 Dia27B16, were similar to what was found in their parent trees grown in the field which displayed lower height for Dia27B16 (396 cm) compared. In Other seed sources, height varied from 510 cm for K4B19 to 590 cm for K17B19 (Chapter 3). We assume that height and RGH are highly heritable traits among *Senegalia senegal* genotypes. According to Dvorak *et al.* (1998) heritability of growth traits for some forest trees do not change significantly between early and mature growth stages. With the exception of Ng21B1, 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.

3.5. Conclusion

This study testing the physiological and morphological adaptation of *Senegalia senegal* seedling under salinity, fertility, and drought 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 and photosynthetic capacities. Large stomata size with low density was observed in Ng21B1, which tended to be more sensitive to salinity despite lower specific leaf area. Diamenar and Kidira provenances in general and particularly the K17B19 seed source might be suitable for afforestation of salt affected lands. All genotypes have notably increased biomass

under the fertilizer effect. However, 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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4. Chapter 4: Identifying predictors of gum arabic yield in a *Senegalia senegal* (L.) Britton progeny trial in Senegal

Abstract

Gum arabic yield is highly variable due to genetic potential and physiological conditions of the tree and the environmental conditions. However, growth characteristics and morphological parameters are believed to affect gum arabic yield. We aimed in this study to assess growth traits and WUE, and potential relationships with gum yield variability among field *Senegalia senegal* provenances. A *Senegalia senegal* progeny trial, in Dahra, Senegal was used in this study. Gum yield was assessed by tree over four successive years. Height, diameter, crown width, leaf area index and $\delta^{13}\text{C}$ analysis were determined at the last harvested annual gum yield. Results showed a high variability of gum yield among provenances and families. Daiba was a consistent low gum producer while Diamenar, Kidira, and Ngane had consistently similar gum yield. Furthermore, Diamenar and Ngane showed similar annual gum yield per hectare despite a lower tree survival rate of Ngane than Diamenar. Gum yield was found to be significantly correlated with height ($r^2=0.16$), crown width ($r^2=0.11$), stem volume index ($r^2=0.23$), and crown area index ($r^2=0.19$). Growth traits, especially height and stem volume index were larger on Ngane provenance, which also displayed significantly higher WUE and lower LAI than the other provenances. WUE was positively associated with gum yield, but the coefficient of correlation was only 2%. Differences found in growth and gum yield may be attributed to genotype-specific variation. However, a significant correlation between soil pH and tree survival rate was found ($P=0.0051$; $r^2=0.36$). This study confirmed a possible improvement of the gum arabic sector through genotype based selection. Ngane seems to be more profitable to grow in Dahra than the other tested provenances.

But, we should test in the future the effect of soil pH on tree survival among provenances, and investigate the relationship between site quality improvement, tree survival rate and gum yield.

Key words: *Senegalia senegal*, provenances, gum yield, WUE, $\delta^{13}\text{C}$ analysis

4.1.Introduction

Gum arabic is among the most valuable non-timber forest product (NTFP), and it represents a substantial component of household income for many local communities in Africa (Barbier, 1992; Chikamai and Odera, 2002; Gaudiose and D'Haese, 2012; Wekesa *et al.*, 2010). Increasing demand for gum arabic creates interest in domesticating *Senegalia senegal* to promote potential economic return. However, gum production fluctuates and is affected by both abiotic and plant physiological factors, which make estimation of gum yield uncertain. Many authors have reported factors influencing gum exudation. Studies have shown that variation in gum exudation appears to be related to the topographical location, the rainfall received in the year before tapping, the temperature at tapping and gum collection, the soil temperature, and the time and intensity of tapping (Ballal *et al.*, 2005; Dione and Vassal, 1993; Unanaonwi and Bada, 2013; Wekesa *et al.*, 2010). Even though progress has been made in identifying how abiotic factors such as rainfall and temperature affect gum yield, our ability to prescribe management options for increasing gum production is limited by an incomplete understanding. Factors controlling gum yield have been investigated for centuries. Gum exudation was first attributed to fungal infection (Fagg and Allison, 2004). Later, it was found that water deficit was a major factor that triggered gum exudation (Fagg and Allison, 2004; Fagg and Allison, 2004). Some reports revealed that initial gum harvest on *Senegalia* trees can begin on four or five-year-old trees, with the ages of seven to twelve being the most productive time (CNI, 2008; Iqbal, 1993). Another researcher stated that age and size of the tree are of no major significance (Larson and Bromley, 1991). More recently, it was revealed that provenances which grow faster with a high photosynthetic rate result in good gum production (Raddad *et al.*, 2006). Additionally, Ullmann (1983) and Mouret (1987) correlated high gum yield to unfavorable growing conditions. Clearly

the relationships between *Senegalia senegal* gum production and tree form and physiological status remains unclear. However, because of severe and frequent drought occurrences in dry and semi-dry areas where *Senegalia senegal* grows, optimizing gum arabic production requires consideration of indicators of adaptability such as survival, growth, and water use efficiency (WUE).

WUE determines the relationship between plant production and water consumption. It refers to the ratio of photosynthetic production to the rate of transpiration and determines growth performance for drought adapted species. Correlation between WUE and stable carbon isotope discrimination provides a simplified measurement of WUE. Carbon isotope analysis has become a valuable tool in plant ecophysiology to help understand the link between photosynthesis and water use of plant species (Farquhar *et al.*, 1989). It may be used as a criterion for ecotype selection for improved WUE in trees (Farquhar *et al.*, 1989) and possibly in gum yield from *Senegalia senegal*.

Recently, it was revealed that high gum yield is associated with low water use efficiency (Gray *et al.*, 2012; Raddad and Luukkanen, 2006). Higher WUE is usually positively correlated with drought resistance (Bacelar *et al.*, 2007; Rivero *et al.*, 2007). However, research on the association between WUE and gum Arabic yield is very limited. It may therefore be useful to examine the relationship between gum yield and WUE in *Senegalia* species. The main objective of this study is to investigate tree variables related to gum yield. Specifically, we are examining WUE as measured by stable isotope composition in foliage and tree growth traits (height, diameter, crown width, stem volume index, and crown area index) on gum arabic yield among *Senegalia senegal* genotypes.

4.2. Materials and methods

4.2.1. Site description and trial setup

The field experiment was conducted in Dahra (northern Senegal; 15° 35 N, 15° 43 W, 43.2 m altitude) at the Centre de Recherches Zootechniques of Institut Senegalais de Recherche Agricole (ISRA). The climate in Dahra is type sub-tropical, hot semi-arid, with mean annual rainfall over the last 10 years (2006 -2015) of 398.35 mm. The monthly average temperature varies between 19°C in January to 41°C in May. Soil is sandy dune, type arenosol covered with 96% sand/grass and 4% trees (Brandt *et al.*, 2014; Gottsche *et al.*, 2016). A *Senegalia senegal* progeny trial set up in 1994 was used in this study, with details and history of the trial being described by Diallo *et al.* (2016). The trial consists of a randomized complete block design with 30 blocks as replications. Each block contains 60 trees planted at a 5-meter spacing, derived from open-pollinated seeds collected from 4 natural populations or provenances (Daiba, Diamenar, Kidira, and Ngane). Each tree in each block is considered as a family, and the progeny test counted 15 families per provenance. The trial has been used to test for genetic assessment among populations (Diallo *et al.*, 2015; Diallo *et al.*, 2016), but this current study will be the first experiment involving carbon isotope composition analysis and gum arabic yield over four successive years. To minimize effects of site heterogeneity, only the twenty contiguous blocks are used in this analysis instead of all thirty in the entire trial.

4.2.2. Tapping treatment and Gum harvest

A tapping treatment was performed on mature *Senegalia senegal* trees in order to induce gum exudation from stems and branches. The standard method used in Senegal was adopted in this study, consisting of removing a portion (4 cm wide and 30 cm to 1 m long) of the bark of stems and branches with a special tool called a “Daba” (Harmand *et al.*, 2012). Tapping was

initiated in October when trees began dropping their foliage as recommended by Adam *et al.* (2013) and Dione and Vassal (1998). Four gum collections were manually completed per tree over the season. Harvested gum from each picking was dried for a couple days and then weighted.

4.2.3. Growth characteristics, survival rate and leaf area index measurement

Tree height, diameter, crown width, and leaf area index were measured for each tree in October 2015. At the same time foliage was collected for ^{13}C analyses. Stems were measured with a laser caliper at 0.5 m from the ground since *Senegalia senegal* presents usually several stems with lateral branches from near the base. The crown width was the average of the crown diameter in two perpendicular directions. A tree stem volume index (diameter squared x height) and crown area index (height x crown width) were estimated. Survival for each source was determined by dividing the number of living trees over the number of trees planted after establishment.

Leaf area index (LAI), the amount of one-sided leaf area per unit area of ground, was determined by tree with the LAI-2200 plant canopy analyzer (PCA, Li-Cor, Inc., Lincoln, NE). Readings were taken under uniform light conditions, using one sensor mode with a 45-degree view cap. We conducted 5 readings under each tree canopy coupled with one reading outside the tree canopy.

Table 4.1: Mean annual rainfall, temperature minimal ($T_{\min}^{\circ}\text{C}$) and temperature maximal ($T_{\max}^{\circ}\text{C}$) at gum arabic collection (picking) in Dahra, Senegal

Year	Rainfall (mm)	First picking		Second picking		Third picking		Fourth picking	
		T_{\max}	T_{\min}	T_{\max}	T_{\min}	T_{\max}	T_{\min}	T_{\max}	T_{\min}
2012	417.3	35.8	14.9	36.2	20.6	37.3	17.6	41.6	20.7
2013	318.7	34.9	15.5	31.3	14.23	35.5	19.3	39.8	19.3
2014	301.5	31.4	18.2	33	18.75	36.1	22.9	40.7	25.6
2015	315.5	35.4	16	39.6	21.8	34.3	17.9	40.7	23.5

4.2.4. Carbon isotope composition ($\delta^{13}\text{C}$) analysis

Carbon isotope analysis was conducted in the laboratory of the Department Forest Resources and Environmental Conservation, Virginia Tech, Virginia, USA. From each *Senegalia senegal* tree, green mature leaves were collected from the four corners of each tree to form a composite foliage sample. Leaf collection was completed in late October, a critical time to assess WUE in *Senegalia senegal* species. A total of 448 leaf samples were collected and quickly air dried and then transported to Virginia Tech and stored in an oven at 65°C. Dried leaf samples were ground with a ball mill, placed in clean tin capsules and weighed on an electronic balance (Mettler Toledo XP6, Columbus, OH, USA), and loaded into an automatic sampler. An elemental analyzer continuous flow stable isotope ratio mass spectrometer (IsoPrime100, Isoprime Ltd., UK) was used to determine the carbon isotope ratio $^{13}\text{C}/^{12}\text{C}$. The $\delta^{13}\text{C}$ values were expressed relative to the Pee Dee Belemnite international standards (Craig, 1953; Farquhar *et al.*, 1989).

4.2.5. Soil pH and electrical conductivity

Electrical conductivity and soil pH were assessed in each block and under each measured tree. We sampled 5 points by block. Each sample consisted of collecting soil to a 10 cm depth from 6 randomly selected sub-samples to form a single composite sample. The same protocol was adopted for each tree by collecting 5 randomly selected sub-samples under the canopy to form one mixed composite sample. Analyses were conducted in the laboratory of Institut Senegalais de Recherche Agricole (ISRA), Dakar Senegal. EC and pH analysis were conducted in a 1:1 soil/distilled water mixture for each sample. The electrical conductivity was measured

with a multi parameter analyzer (Consort C535, Dorval, Quebec, Canada) and the pH with a microprocessor pH meter (HI 223, Hanna instruments, Woonsocket, RI, USA).

4.2.6. Experimental design and data analysis

The experimental layout was a completely randomized block design with 20 replications. Data were subjected to an unbalanced analysis of variance due to the different survival rate among seed sources and blocks. We used Box-cox Y transformation to improve normality and constant variance when needed. For growth analysis, three outliers for height and one for crown width were identified and omitted from the dataset. Three outliers were also identified and removed for the electrical conductivity analysis. The diameter represented either tree diameter with one stem or total summed diameter for trees with two or multiple stems, measured at 0.5 m from the ground. Differences in least squares means were assessed at $P < 0.05$ using Tukey test. Simple linear regression analysis was performed between variables in general and particularly between gum yield and each of the other measured variables. Gum yield average was computed using the four annual gum yields. A zero was recorded for each tree which did not produce gum. Pearson correlation analysis was performed to determine the relationship between growth characteristics and gum arabic yield.

4.3. Results

4.3.1. Tree survival rate, Growth characteristics, LAI, Soil pH, and electrical conductivity

Analysis of variance of tree survival rates, height, stem diameter and crown width revealed significant differences among provenances and families. Except K25, in which no tree

in the 20 blocks was sampled in 2015, the survival rate varied between 10% for K7, K23, Da4, and Ng11 to 85% for Da8. Deviation from the mean value varied from -36% to 48% (Figure 4. 1a). Crown width and diameter were not statistically different among families. Height ranged significantly from 547cm for Ng25 to 372 cm for Da16. Within provenances, the average tree survival rate varied between 28% to 48% for Kidira and Diamenar, respectively, corresponding to -10% to 10% deviation from mean (Figure 4. 1b). Ngane height averaged 504 cm, which was significantly higher than Daiba (427 cm), Diamenar (437 cm), and Kidira (453 cm), which were not statistically different. Diameter was only significantly different between Daiba and Diamenar, which averaged, respectively, 20.6 cm and 24.2 cm (Figure 4. 2b). Also, Daiba displayed a significantly narrower crown width (5.76 m) in comparison to Kidira (6.20 m). The crown width was not statistically different between Diamenar (5.76 m) and Ngane (5.74 m) and between them and the other provenances (Figure 4. 3b). Ngane had lower LAI than Daiba and Diamenar which were not significantly different (Figure 4. 3a). LAI varied significantly among families from 2.31 for Da4 to 0.62 for Ng15. Stem volume index and crown area index remained lower for Daiba and higher for Ngane (Figure 4. 4a and b). Between families, crown area index varied significantly between 0.34 m² for Ng18 and 0.16 m² for K16, the same as stem volume index which also varied from 0.21 m² for Ng25 and Ng18 to 0.05 m² for K16.

No significant difference was found for soil electrical conductivity and soil pH under tree canopies by family or by provenance. However, there was a significant positive correlation ($P = 0.0051$, $R^2 = 0.36$) between average block tree survival and average block soil pH (Figure 4. 5).

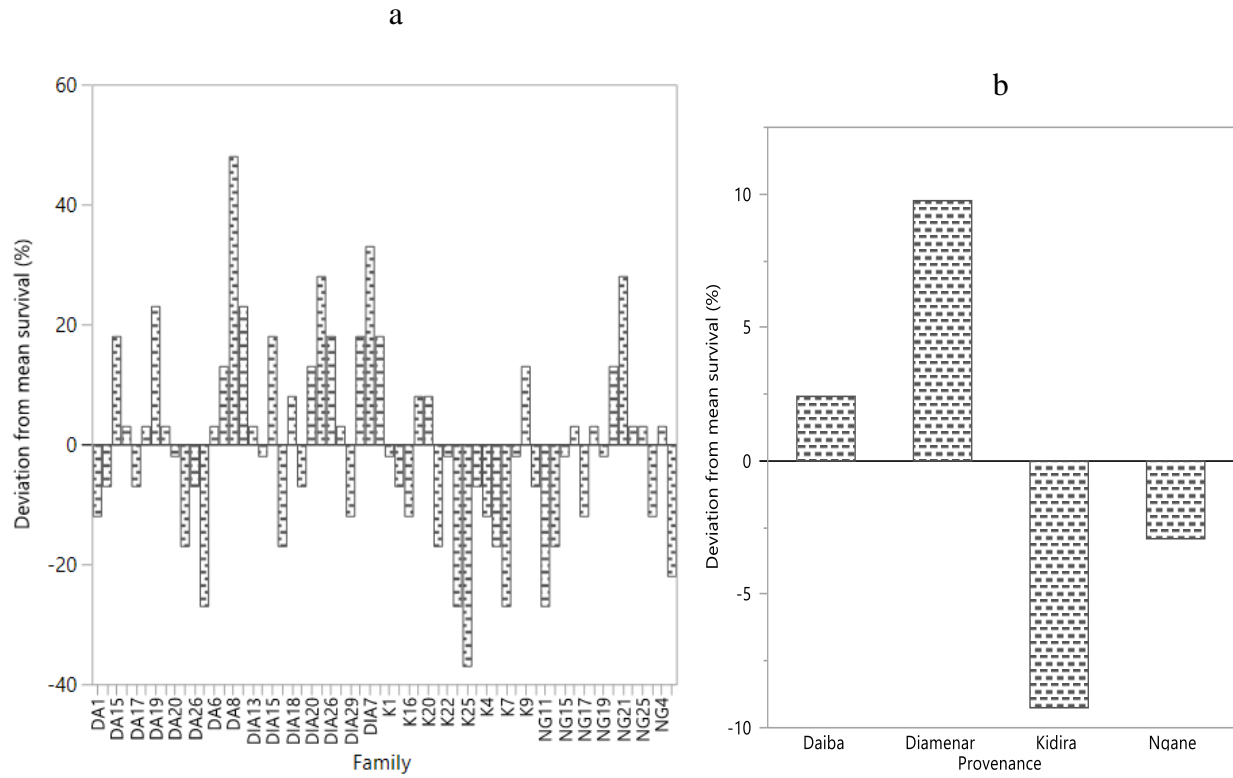


Figure 4.1: Tree survival by family (a) and provenance (b). Mean value in percent for each family and provenance is a deviation from the overall mean.

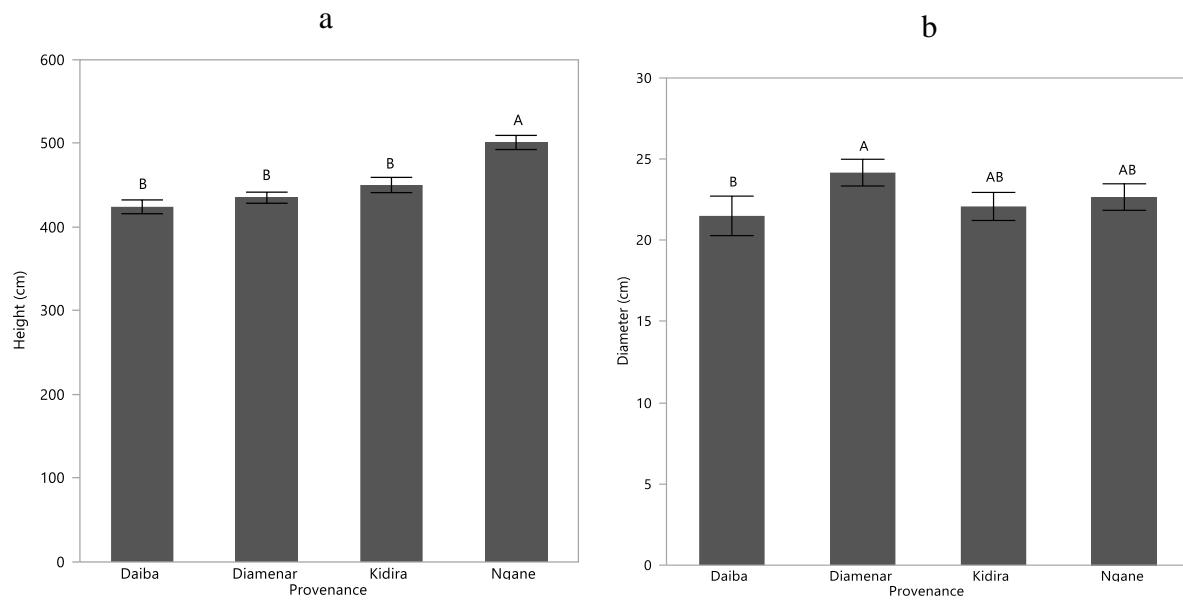


Figure 4.2: Height (a) and diameter (b) variation among 21-year-old *Senegalia senegal* provenances. Different letters indicate significant differences between provenances.

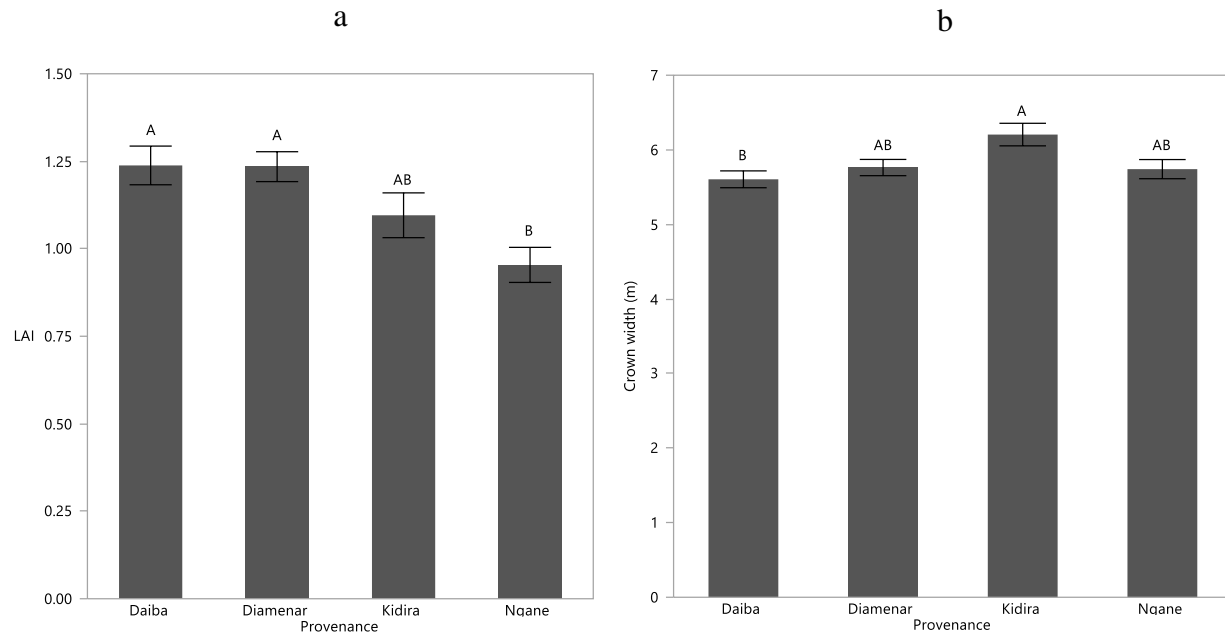


Figure 4.3: Leaf area index (a) and crown width (b) variation among *Senegalia senegal* provenances. Different letters indicate significant differences between provenances.

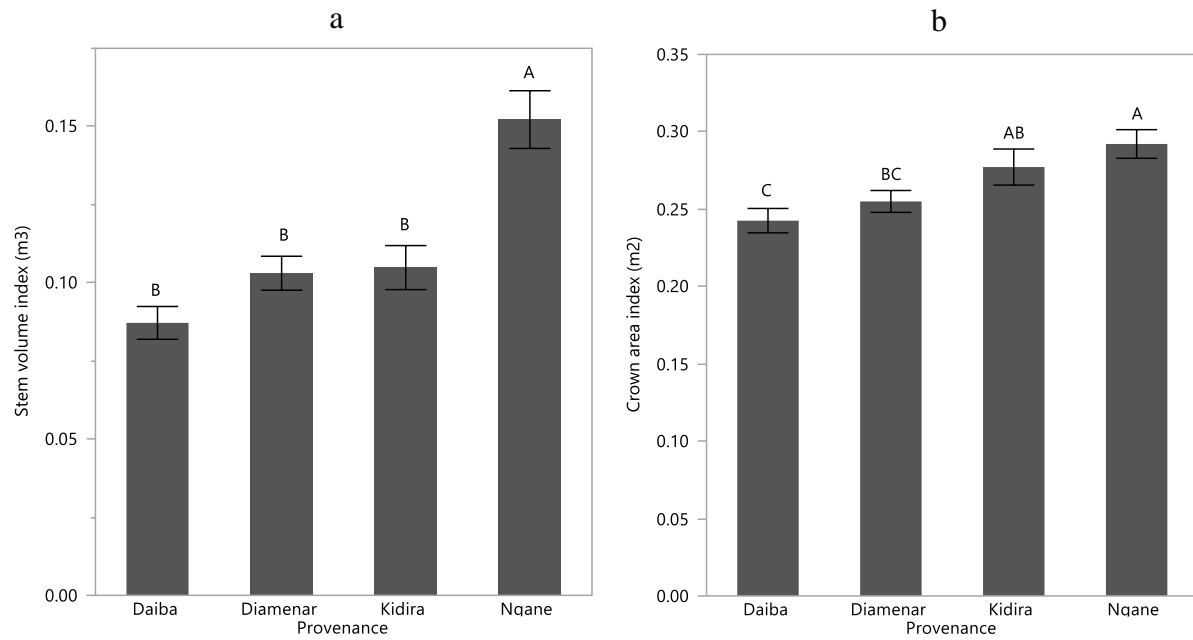


Figure 4.4: Variation of stem volume index (a) and crown area index (b) among *Senegalia senegal* provenances. Different letters indicate significant differences between provenances.

4.3.2. Gum yield

Gum yield was significantly affected by provenance and families, and that finding was consistent between years (Figure 4. 6a). Daiba was consistently the lowest gum producer (Figure 4. 6 a and b). Between families, the average gum yield across 4 years varied significantly from 645.6 g for Dia27 to 207.5g for K16. Gum yield per hectare between provenances was found to be significant, with Diamenar and Ngane having higher yields (Figure 4. 7). There was a significant positive correlation with crown width and height explaining 12% and 16% of the variation in gum yield, respectively (Figure 4. 8a and b). Similarly, gum yield was positively correlated with stem volume index and crown area index which explained 23% and 19% of gum variation, respectively (Figure 4. 8c and d).

4.3.3. Carbon isotope composition ($\delta^{13}C$)

Significant difference in carbon isotope composition was found among families. Ng17 displayed the highest value (-26.52 ‰), whereas the lowest value was found in Dia2 (-28.69 ‰). Within provenances, Ngane had significantly higher $\delta^{13}C$ than the other provenances, which were not statistically different (Figure 4. 9). $\delta^{13}C$ was found to be significantly and negatively correlated with diameter and LAI, explaining 7% and 12 % of $\delta^{13}C$ variation, respectively (Figure 4.10a and b). ANOVA also revealed that $\delta^{13}C$ is significantly correlated with tree volume index ($P<0.0001$) and crown area index ($P=0.0350$) (Figure 4. 10a and b), explaining 12% and 19% of the variation, respectively.

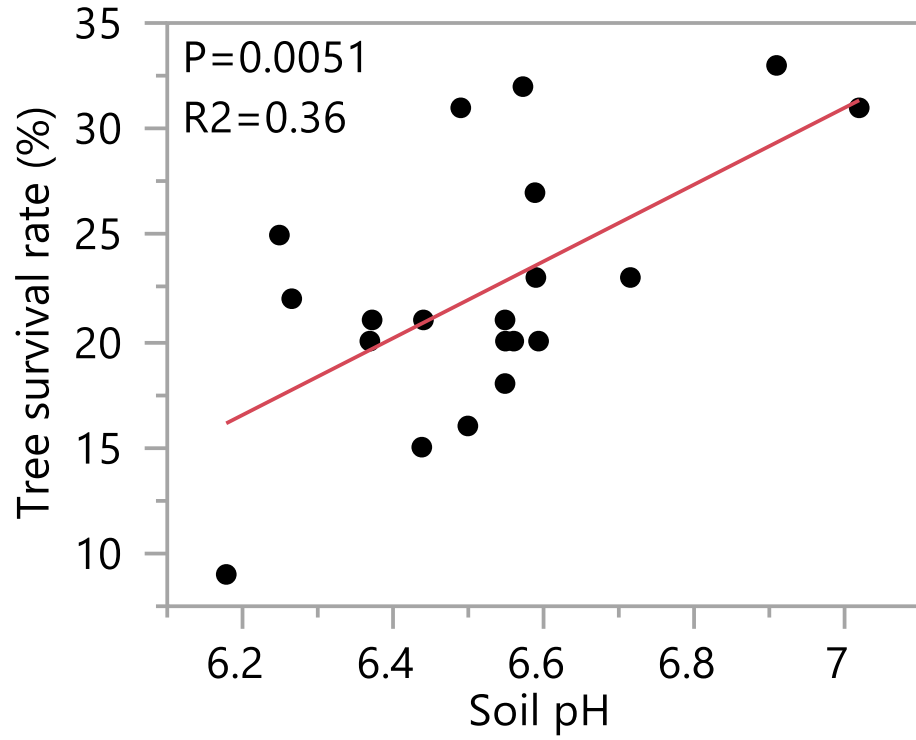


Figure 4.5: Soil pH versus tree survival (all provenances and families).

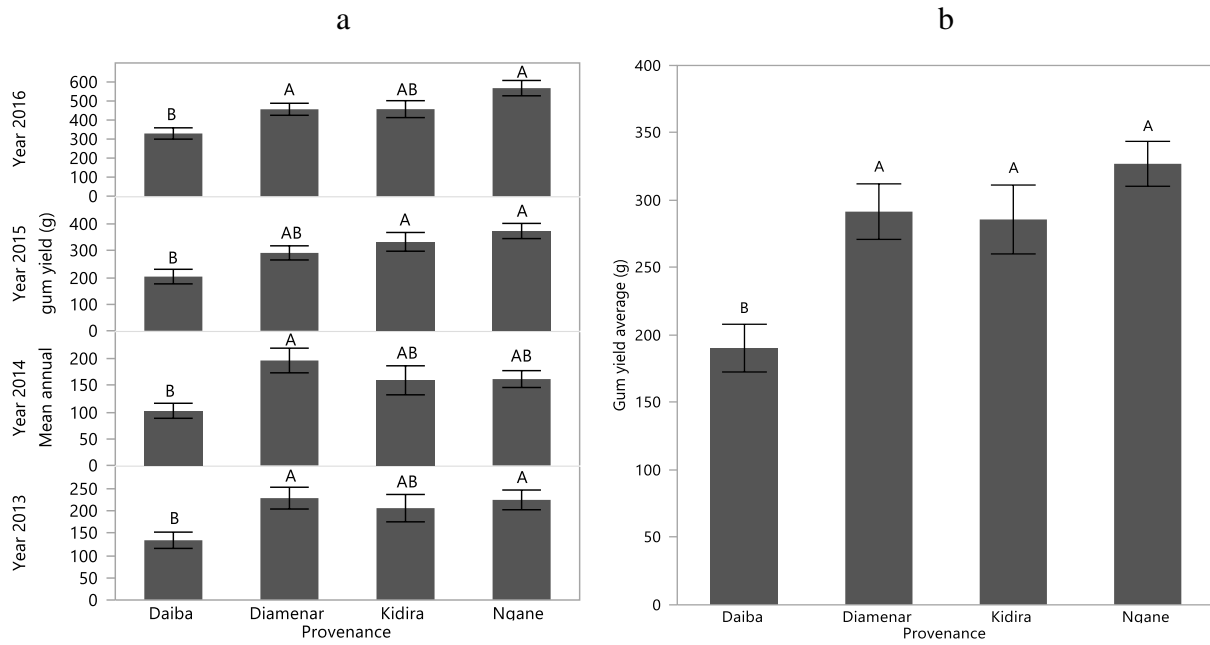


Figure 4.6: Annual gum yield production as affected by *Senegalia senegal* provenances (a) and gum yield average across 4 years per provenance (b). Different letters indicate significant differences between provenances.

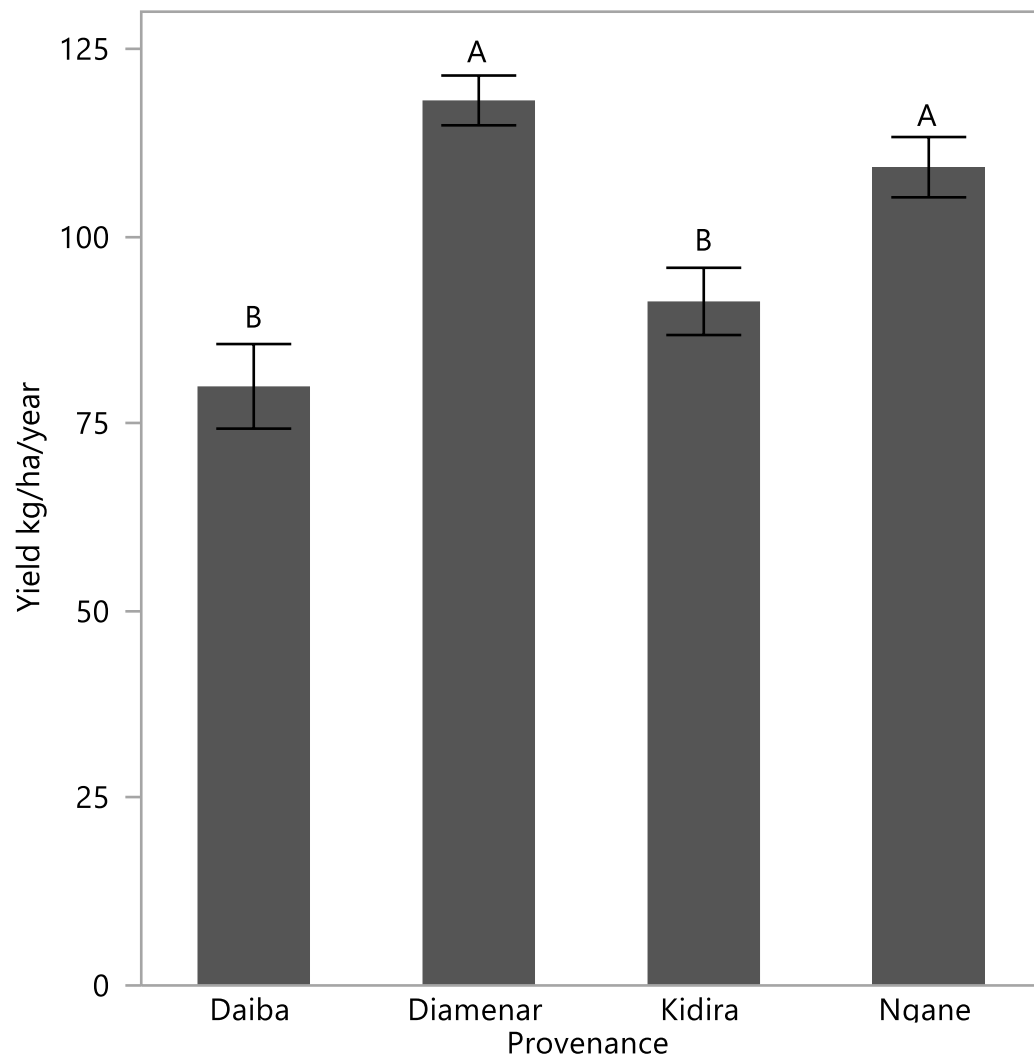


Figure 4.7: Total annual gum yield (kg) per hectare per provenance. Different letters indicate significant differences between provenances.

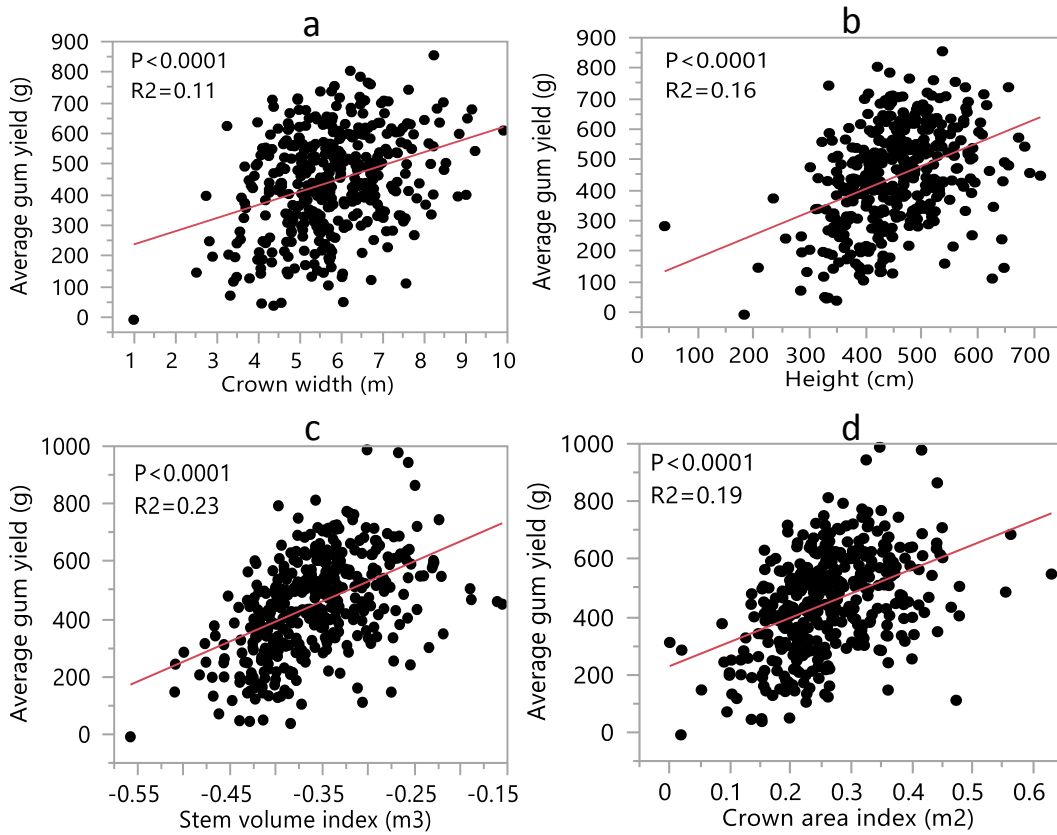


Figure 4.8: Relationship between average gum yield (g) and crown width (a), and height (b), and stem volume index (c) and crown area index (d). Stem volume index and crown area index data were Cox-box transformed to meet assumption for linear regression.

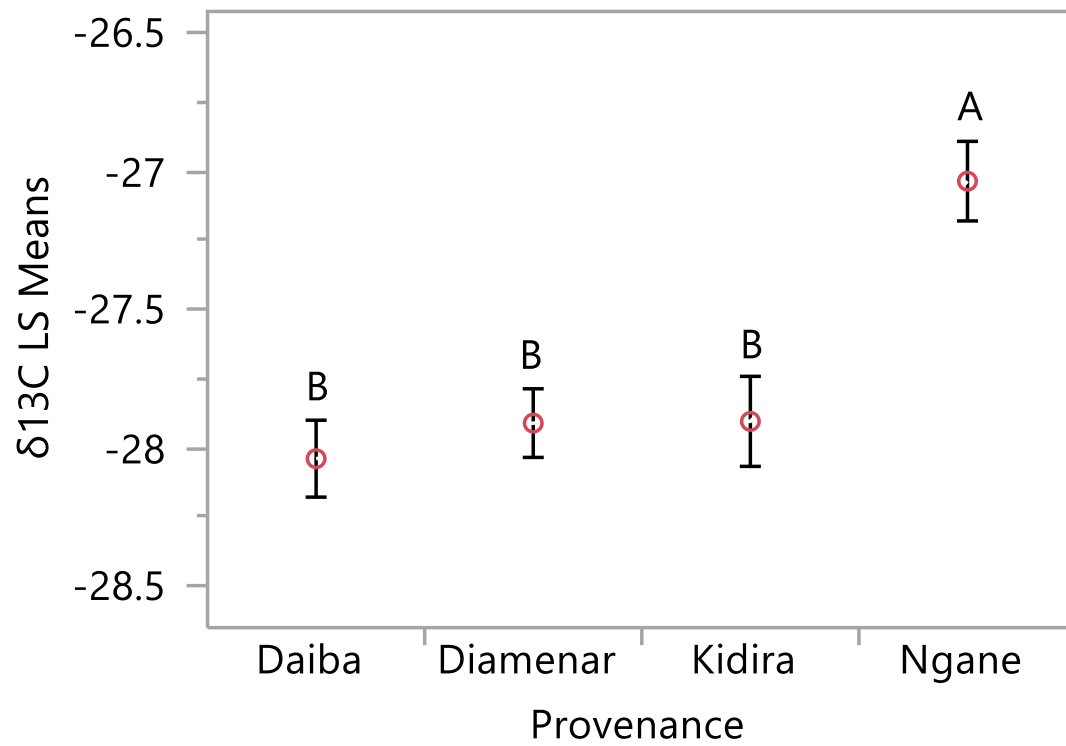


Figure 4.9: Variation of $\delta^{13}\text{C}$ among *Senegalia senegal* provenances. Different letters indicate significant differences between provenances.

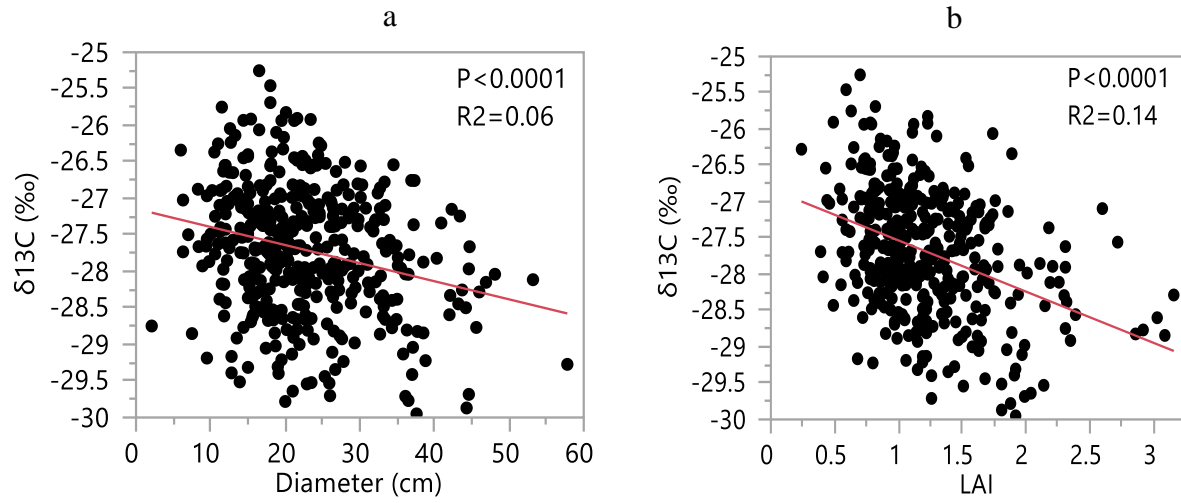


Figure 4.10: Relationship between $\delta^{13}\text{C}$ and diameter (a) and $\delta^{13}\text{C}$ and leaf area index (LAI) (b) of 21-year-old *Senegalia senegal* in Dahra Senegal.

4.4. Discussion

To meet the objectives of assessing gum yield variability among and within *Senegalia senegal* populations, population of uniform age and common growth conditions were used. This approach is important for an understanding of inter and intra genetic variabilities, which is critical in tree selection (Bowman, 1972). The significant differences observed between provenances and families on gum yield suggest a possible identification and selection of superior genotypes. There is a high variability in gum production among the sampled families. Families such as Ng21, Dia27, and DA15 exhibited a high gum yield over 4 years while Da18, Da20, K7 were low gum producers. The same trend was also noticed at the provenance level. Diamenar, Kidira, and Ngane displayed higher gum production than Daiba. Gum yield variability between *Senegalia senegal* provenances were already observed in earlier studies (Josiah *et al.*, 2008; Mulumba *et al.*, 2012; Omondi *et al.*, 2010). Despite variability in gum yield over the four years sampled (Figure 4. 6a), gum yield per provenance remained relatively consistent. Annual variation in gum yield is mostly attributed to the amount of precipitation and temperature variations (Bateson *et al.*, 1988; Bayene, 1993; Pearce *et al.*, 1990). In contrast to the study by Ballal *et al.* (2005), a relationship between gum yield and rainfall was not observed in this study and it is possible that it may be biased by the age of the stand. Actually, gum arabic yield in a given year is influenced by the age of the stand. According to Pearce (1988) and FAO (1995), 15 years is the maximum productive age of gum arabic from *Senegalia senegal*. Moreover, Abd EL Rahman (2001) showed that gum yield increases up to the age 15 years and then begins to decline.

Beside gum yield variability among provenances, we observed significant difference in growth variables and survival rate, which also are indicators of performance and adaptability for provenances evaluation (Raebild, 2002). Raddad *et al.* (2006) suggested the use of both growth traits and gum production as criteria for provenance selection. Our results revealed Ngane as taller than the other provenances used in this study. We also found a higher relative height growth rate exhibited by seedlings of this provenance under well-watered conditions (chapter 2). The significant relationship between gum yield and height (Figure 4. 8b) found in this study corroborates Abd El Rahmane (2001), who revealed similar relationship for *Senegalia senegal* from natural stands. Also, positive correlations found between gum yield and crown width, stem volume index and crown area index (Figure 4. 8a, c, and d) indicate their importance in tree selection.

Assuming that abiotic factors such as rainfall, temperature, tapping date and method are identical for all the trees, significant differences observed on measured traits would be attributed mostly to the genetic potential. This study confirmed many findings in the genotype based selection to improve gum supply (Fakuta *et al.*, 2009; Fakuta *et al.*, 2015; Gray *et al.*, 2013; Raddad and Luukkanen, 2006; Raebild *et al.*, 2003).

Based on our findings, Daiba does not seem to be profitable to grow in Dahra and likely in areas with similar pedo-climatic characteristics. Ngane, Kidira and Diamenar have similar and better average gum yield performance by tree (Figure 4. 6a and b). However, despite lower survival rate than the average mean (Figure 4. 1a and b), Ngane was able to maintain as high annual gum yield per hectare as Diamenar (Figure 4.7) which displayed the significantly highest survival rate. It appears clear for Diamenar and Ngane provenances that a trade-off between survival rate and gum yield may limit gum productivity. This finding implies a possible increase

of gum yield in a stand, by improvement of tree survival rate. Thus, improving long-term survival of *Senegalia senegal* is an area of research which should be investigated in order to maximize gum yield potential. Many reports estimate gum production per hectare of a stand by considering optimal survived trees (ITC, 1983; Speece, 1997). Such cases are usually far from the reality because of low tree survival due to abiotic and biotic factors. Perhaps this could be improved through better management options such as weeding control and protecting seedlings from browsing animals.

The positive correlation between soil pH and tree survival rate found in this study (Figure 4. 5) demonstrates the influence of soil conditions on tree survival. Best survival occurred at soil pH close to 7.0. This finding brings into question Von Maydell (1986) who reported that *Senegalia senegal* is adapted to a wide range of soil pH. Thus, further studies testing provenance responses to different soil pH levels would be of great importance.

The negative correlation between gum yield and WUE reported recently by Gray *et al.* (2013) and Raddad and Lukkanen (2006) was not confirmed in this study. In contrast, a significant positive relationship between $\delta^{13}\text{C}$ and gum yield was found (data not shown), but the coefficient of correlation was very small (only 2%). However, WUE is critically important to investigate in tree selection for species such as *Senegalia senegal* which grows in dryland ecosystems with frequent and severe drought events. Substantial variations in isotopic composition values were found among provenances. This result is supported by finding in other earlier studies reporting differences of $\delta^{13}\text{C}$ between species in general and especially among *Senegalia senegal* provenances (Cregg *et al.*, 2000; Dawson *et al.*, 2002; Farquhar *et al.*, 1989; Garten and Taylor, 1992; Gebrekirstos *et al.*, 2011; Gray *et al.*, 2012; Raddad and Lukkanen, 2006; Zhang and Cregg, 1996).

A higher WUE (as expressed by higher carbon isotope composition) was observed in Ngane (Figure 4.9), meaning that Ngane may have a faster net photosynthetic rate, a lower transpiration or both (Ehleringer *et al.*, 1993). This may explain why Ngane grows faster than the other provenances. This finding corroborates studies in ponderosa pine, black spruce and peanuts which indicated that sources that grow faster displayed higher WUE (Cregg *et al.* 2000; Hubick *et al.*, 1988; Johnsen and Major, 1999). Interpretation of WUE based on foliar $\delta^{13}\text{C}$ may change according to soil water availability and sampling date (Blum, 2005; Blum, 2009; Gebrekistos *et al.*, 2011). However, Gebrekistos *et al.* (2011) revealed a contrasting water use strategy in *Senegalia senegal* between moisture periods and drought periods. We did not explore $\delta^{13}\text{C}$ during the growing season, but based on earlier findings on *Senegalia senegal*, plants displaying higher or lower WUE under drought, may exhibit lower or higher WUE, respectively throughout the growing season (Gebrekistos *et al.*, 2011; Gray *et al.* 2013). Based on this assumption, it could be that Ngane would exhibit higher water use strategies (lower WUE) during the growing seasons as well. According to Blum (2005) genotypic variation in WUE under drought is more affected by variation in water use behavior. This is illustrated by the better growth traits (especially height) observed in Ngane, which clearly seems to be more drought tolerant than Daiba, Diamenar, and Kidira.

The LAI was assessed in late October, which corresponds to the beginning of the dry season in Senegal. At that period, deciduous trees such as *Senegalia senegal* already start shedding leaves to minimize water loss in response to water stress. Nonetheless, our results indicated a significant difference of LAI (interpreted in this study as foliage density) between Ngane and Daiba and Diamenar (Figure 4. 3a). The different observed patterns of LAI between provenances may reflect different sensibilities to environmental stress such as water limitation. The lower

LAI, which could be related to earlier leaf shedding, may have contributed to increase WUE in Ngane. This result confirms Jones *et al.* (1985) who showed that plants with lower LAI tended to increase their WUE. A more recent study completed in California ecosystems also supports that conclusion (Malone *et al.*, 2016). Also, results of chapter 2, where a negative association between intrinsic WUE and SLA were found, strengthens the evidence of the negative association between WUE and LAI. This is illustrated by the negative association between $\delta^{13}\text{C}$ and LAI (Figure 4.10b). Furthermore, whole tree transpiration is generally known to increase with LAI (Breda and Granier, 1996; Cregg *et al.*, 2000) and therefore intrinsic WUE and total leaf area are important parameters to consider when examining plant adaptations to water stress.

4.5. Conclusion

Overall, this study emphasizes the importance of genotypes-based selection for the gum arabic sector improvement. Significant differences on tree survival rate, growth characteristics, WUE, and gum yield among *Senegalia senegal* provenances and families were found. Our results indicated a relatively high influence of stem biomass index and crown area index on gum yield, but a clear relationship between WUE and gum yield was not evident in this study. However, WUE varied significantly between families and provenances and revealed potential differences in their water use behavior. Ngane seemed to be more drought tolerant because of higher carbon isotope composition assessed during a period of drought stress. Although improving drought tolerance is a good option to improve tree survival in dryland ecosystems, a key result from this study is that soil pH affects tree survival rate and accordingly gum yield. Ngane, Diamenar, and Kidira produced consistently more gum than Daiba. Thus, identification and selection of performant sources in terms of gum productivity and drought tolerance might be

a starting point for improvement of the gum arabic sector in Senegal. Investigation of silvicultural management practices aimed at improving seedling survival and growth of the trees is also important for optimizing gum production of a stand.

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5. Chapter 5: Conclusions and perspectives

Past research has emphasized the effect of multiple factors both abiotic, biotic, and genetic on gum arabic yield and productivity. Results of this study confirm that stress caused by multiple factors effect gum arabic yield. Over four successive years of gum yield assessment by tree, a significant provenance effect was found. Ngane, Kidira, and Diamenar maintained higher gum yield in contrast to Daiba, which remained consistently a low gum producer. This finding highlights the potential of tree improvement and genetic selection for increasing production of gum arabic. Stem volume index, crown area index, and height were positively correlated with gum yield. This implies the importance of growth and biomass effects on gum yield. Also, our results revealed a significant effect of fertilizer on all biomass components of *Senegalia senegal* seedlings. This finding should be confirmed on mature trees in the field as well as determining the effect of fertilization on gum yield. The growth and fertilizer results suggests that proper silvicultural management can also increase gum yield.

Under field conditions, it is challenging to study the effect of one factor while many other factors are varying. For this reasons, we combined both field and greenhouse studies to better assess genotype by environment interactions. Our study showed throughout that Ngane has superior growth characteristics compared with the other provenances. Ngane accumulated more above ground biomass as mature trees and seedlings, both under limited and non-limited conditions. Ngane, being able to produce more above ground biomass than the other provenances despite the same water limited conditions might be considered as more drought tolerant. This assertion is further confirmed by the higher WUE exhibited by Ngane through $\delta^{13}\text{C}$ analysis. However, the interpretation of WUE analyzed through $\delta^{13}\text{C}$ under limited water condition is still under debate. Therefore, traits such as osmotic adjustment, turgor potential and modulus of

elasticity might provide additional insights on water relation proprieties among provenances. The contrasting finding between our study and those from Gray *et al.* (2013) and Raddad and Luukkanen, (2006) on the relationship between WUE and gum yield has to be clarified.

Therefore, we suggest more studies on gum yield assesement and WUE.

Before embarking on domestication or breeding programs of *Senegalia senegal*, forest geneticists need to understand the physiological variability within this species. Besides intraspecific variabilities found on gum yield, drought tolerance, and biomass, our results also showed different responses to salinity stress among provenances. Ngane appeared to be more sensitive to salt stress despite originating from more saline area than the other provenances tested, which did not seem to be affected significantly in terms of biomass accumulated. This finding is very important for reclamation of salt affected land in Senegal.

One of the main goals of this study is to promote the sustainable management of gum arabic in order to address economic and environmental concerns. Planting genetically improved seedlings which are more tolerant to drought and possess better growth characteristics and gum arabic yield would be biologically a good choice to overcome scarcity and optimize profit.

However, optimizing site conditions for better survival and growth may result in higher gum yield and thus a good socio-economic impact. Therefore, a management plan appears crucial for guiding the financial viability of *Senegalia senegal* plantations in Senegal.

Literature cited

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