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Influence Of Vertical Soil Moisture To Root Growth And Biomass Allocation In *Acacia Senegal* Linn Seedlings From Two Provenances

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Rhizotrons,
Root Allotment,
Water Deficit.

ABSTRACT
Acacia senegal is an important drought-tolerant species, but limited information exists on how vertical soil moisture affects its early growth from different provenances. Uneven moisture distribution in drylands may influence root development and biomass allocation, affecting seedling establishment. Therefore, this study aims to investigate the influence of vertical soil moisture gradients on root growth and biomass allocation in *Acacia senegal* seedlings from two provenances, to determine best provenance and moisture condition for survival. Study was conducted in the greenhouse at Federal University Dutse using 36 glass-fronted rhizotrons (120 x 30 cm) filled with 7 kg sieved sandy-loam topsoil. Acacia seeds from provenances were germinated and transplanted into rhizotrons arranged in Randomized Complete Block Design with watered and 10% drought treatments. Shoot and root lengths, soil moisture at four depths, and biomass at three soil sections were measured for 56 days. Drought significantly reduced shoot length in species, with Nguru provenance showing greater shoot inhibition than Gujba. Root length increased under drought, with Nguru developing significantly longer roots (122.00 cm) than Gujba, indicating a drought-avoidance strategy. Root dry weight was highest in the 0–40 cm depth, and Nguru had greater total root dry weight and higher root:shoot ratios across treatments. Lateral root number varied by depth and provenance, decreasing with soil depth. Water uptake shifted progressively downward as upper layers dried, with root distribution patterns matching water extraction. Nguru (arid) provenance exhibited stronger stomatal closure and deeper rooting, suggesting superior drought adaptation through enhanced water acquisition and reduced transpiration. Nguru provenance showed superior drought adaptation through deeper roots and higher root:shoot ratio, while Gujba maintained greater shoot growth.

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INTRODUCTION

In the Sahelian region of northeastern Nigeria, water is a scarce resource and competition for it is severe" (Nkiaka *et al.,* 2024). One underlying cause of water scarcity is likely to be changing climate patterns that result in a reduction of rainfall (Abubakar and Yamusa 2013). Many plant species in this region experience a prolonged dry season with little rainfall, and the upper soil layer undergoes acute drying" (Obateru *et al.,* 2021). One important and well documented adaptation to such drought is the possession of a deep and thick root system that gives the plant access to water at depth" (Gning *et al.,* 2025). Deeper and larger

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roots are associated with trees in water-limited environments (Ledo *et al*., 2023). Roots show a high degree of morphological plasticity that enables them to cope with water stress (de la Riva *et al.,* 2022). A mechanism for improving water uptake by water-stressed plants may include production of longer, thinner roots that penetrate deeper into the soil (Zhou *et al.,* 2024). Deep rooting systems with a large active surface area improve water uptake. Moisture depletion mainly occurs in the topsoil layer in many soils (Yang *et al.,* 2023). For example, the greatest depletion of water has been observed at depths between two and three meters in a tropical dry forest (Wang *et al*. 2022). One important morphological attribute that affects water uptake and water loss in trees is root-shoot ratio (Jibo 2021; Jibo*;* 2021a Wang *et al.,* 2024). Biomass allocation varies over time, across environments, and among species (Lozano *et al*. 2023; Sun *et al.,* 2022). The understanding of such patterns is important to plant ecology and evolution. The extent to which they vary among species sets limits on biomass production and utilization (Vitali *et al.,* 2022; Pérez-Ramos *et al.,* 2023). Plants growing in arid and semi-arid environments appear to specialize in taking up water (Jibo et al 2021b) from specific soil layers at certain times of the year (Schwendenmann *et al.,* 2022).

Biomass distribution into main and lateral roots in different soil depths (Jibo *et al* 2021) varies among different tree species (Reyes *et al.,* 2023; Zhou *et al.,* 2024). However, our knowledge of the physiological basis underlying the variation in biomass allocation is still poor. Information related to root distributions and knowledge of the basic mechanisms of soil water extraction, and the understanding of such patterns, is of essential importance to plant ecology and has many uses in agricultural practice and implementation. To compare the growth of roots under different soil moisture regimes and understand possible soil water extraction patterns, the root system can be investigated in plants grown in soil-filled glass chambers called rhizotrons (Bindhu, 2024; Ceolin *et al.,* 2025). Rhizotron techniques have been employed by various researchers in studies relating to root form and function (Bindhu, 2024; Cotrufo, 2023). *Acacia senegal* (L.) Willd, a leguminous tree species belonging to the Mimosoideae subfamily, is one of the main agroforestry tree species from the Sahelian region of northeastern Nigeria (Elzaki, 2023; Fang *et al.,* 2024), and is important for the rural economy. The tree is the main source of gum Arabic and a well-established traditional agroforestry component widely distributed naturally in the Sahel. However, the production of this gum is threatened by drought and encroaching desertification (Jibo *et al* 2023, Abdalkreem *et al.,* 2025). The main objective of this investigation is to study the root system characteristics of *A. senegal* growing within a rhizotron in an unimpeded soil medium. Since it was possible to visualize the root growth in the rhizotrons, root traits such as root angle, maximum visible root length at different ages, and number of roots at different depths over time could be measured during growth. The aim of this experiment was to compare the root growth of *A. senegal* from two provenances under two soil water moisture regimes and assess the probable region of soil water uptake by roots of the two *A. senegal* provenances.

MATERIALS AND METHODS

Description of Study Site

The study area is situated at 11°70' N latitude and 9°34' E longitude, with an altitude of 460 meters above sea level. The area, characterized by a tropical climate, receives a mean annual rainfall of 750mm, which provides a favorable condition for agricultural activities (Gidado *et al.,* 2023). The mean annual temperature in the area supports the growth of variety of plants. The area's relatively uniform climate and soil conditions ensured that the results obtained from the study were reliable and applicable to similar agro-ecological zones. The topography of the area is generally flat, with a high land area of approximately 750 meters. The soil type in this region is predominantly sandy-loam, as reported in previous studies (Garba *et al*., 2021). This soil type is characterized by a mix of sand, silt, and clay, providing a suitable medium for plant growth.

Plant Materials

Seedlings of *A. senegal* (synonym *Senegalia senegal* (L.) are grown from seeds obtained from the Rubber Research Institute of Nigeria, Gum Arabic Sub-station, Gashua. The seeds are treated with concentrated sulfuric acid (95%), kept for germination in Petri dishes over moist filter paper. All dishes are kept in laboratory. Filter papers are regularly moistened with distilled water. The seeds germinated at 5 to 8 days and are transplanted to glass rhizotron (120 x 30 cm length x width). The experiment was conducted in a greenhouse for eight weeks in May and July, 2014 at the Department of Biological Sciences, Federal University Dutse, Jigawa State, Nigeria. During the experiment, the average day/night temperature was 27/24° C. Relative humidity ranged from 50 to 70%. The photoperiod was 8 hours of 300 µmol $m^{-2}s^{-1}$ PFD at plant height.

Rhizotron

Thirty-six (36) glass fronted rhizotrons (Figure 1) are made as described in Price *et al*. (2012). Each rhizotron was made from two sheets of 4 mm thick glass (120 x 30 cm length x width). The glass sheets are held 15 mm apart with strips of wood and spacers at the bottom and top. Heavy duty tape was used to seal the base and two sides and the wood removed, while the spacers kept the two sides apart. The space was filled with 7 kg of sieved soil (screened topsoil, sieved to 1 cm) to a volume of 6.3 l, giving a total weight of 13 kg for each rhizotron. The soil used for the

experiment was topsoil (pH 5.5) from an agricultural field at Federal University Dutse. The chambers had a 5 mm diameter drainage hole at the bottom of each side. Rhizotrons are grouped in stacks of six, leaned at an angle of 15° from vertical to encourage the growth of roots down the lower surface. Sheets of expanded polystyrene are placed behind and in front of all stacks of chambers to prevent heat loss and to keep out light.

Experimental Design

Study was conducted in a Randomized Complete Block Design with two factors (provenances and watering regime). There are two treatments that were used: a fully watered (control) and severe drought (10%) and gravimetric water content (GWC).

Sowing and Irrigation

Before sowing, each chamber was saturated with 500 ml water. Pre-germinated seedlings were sown in each chamber on 22 May 2014. Seedlings are grown for 56 days in a greenhouse (minimum temperature 25° C) under daylight conditions. Each rhizotron was connected to a drip irrigation system. Seedlings received their last watering on the evening of Day 53 after sowing and received no more water until they are harvested on Day 56.

Traits Measured

Shoot growth was monitored on a weekly basis as shoot length (length from the soil to the tip of the longest leaf) while the length of the longest visible root in each seedling will be recorded for each seedling. The mass of each rhizotron will be measured using an electronic balance with an accuracy of ± 0.01 g, at a week interval from Day 39 until Day 52 to assess water use in the final three days of the experiment. Soil volumetric water content was measured weekly at 10 cm, 40 cm, 80 cm and 120 cm depth using a calibrated theta probe Delta-T Devices (ML2x) with four rods of 60 mm. Six chambers were not be seeded and used to evaluate background water lost through drainage or evaporation. At Day 49 of plant growth, (Day 28 into the drought treatment)

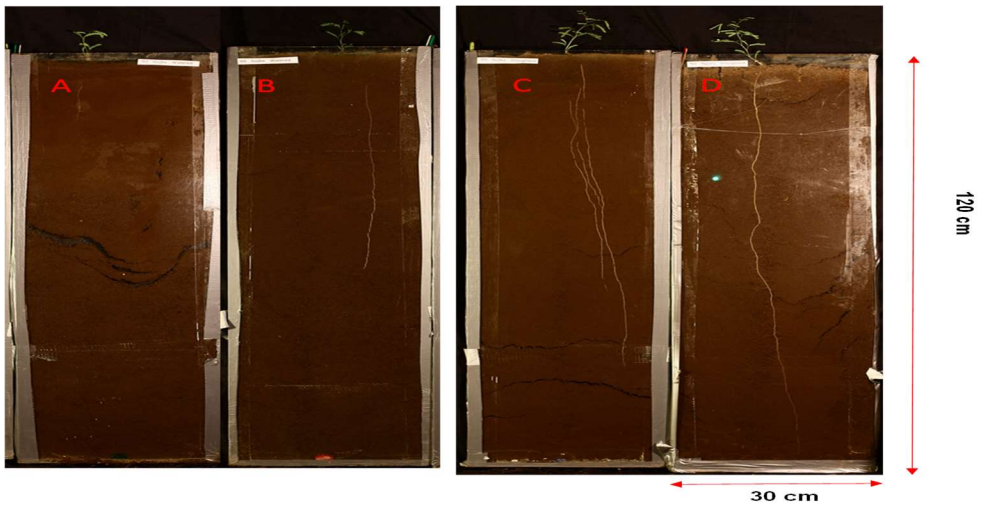


Figure 1: Images of Rhizotron with Different Provenances and Treatments. (A= Gujba provenance + Watered; B= Nguru provenance + watered; C = Gujba provenance + droughted; D= Nguru provenance + droughted) on day 47of plant growth

Harvesting

At the end of the experiment (Day 56), shoots and roots were removed in a single day. Each rhizotron was opened and the entire root system were divided into three sections 0-40 cm (the top), 41-80 cm (middle) and 81-120 cm (bottom). The number of lateral roots at each depth was recorded; the roots are then washed and dried at 80° C for 2 days and dry mass determined. Dry weights of shoots were also be determined.

Data Analysis

Data were analyzed using two-way ANOVA, with significance at $P<0.05$, and Pearson's correlation in MINITAB and SigmaPlot.

RESULTS AND DISCUSSION

Shoot Growth in Rhizotron

Total shoot length (TSL) of all plants in each treatment was presented in Figure 2, during the 56 days of the experiment. Mean TSL of *A. senegal* for the two provenances (Gujba and Nguru), shows a significant difference ($F_{1,\ 16}$= 35.67, $P<0.001$) in the drought treatment (Figure 3). There was also a significant difference in total shoot lengths between the two provenances ($F_{1,\ 6}$=8.49, P=0.010) an interaction between treatment and provenances was also significant ($F_{1,\ 16}$=9.36, P=0.007). The mean shoot lengths in the droughted Nguru seedling was lower (16.80 cm) compared to other treatments (Figure 3).

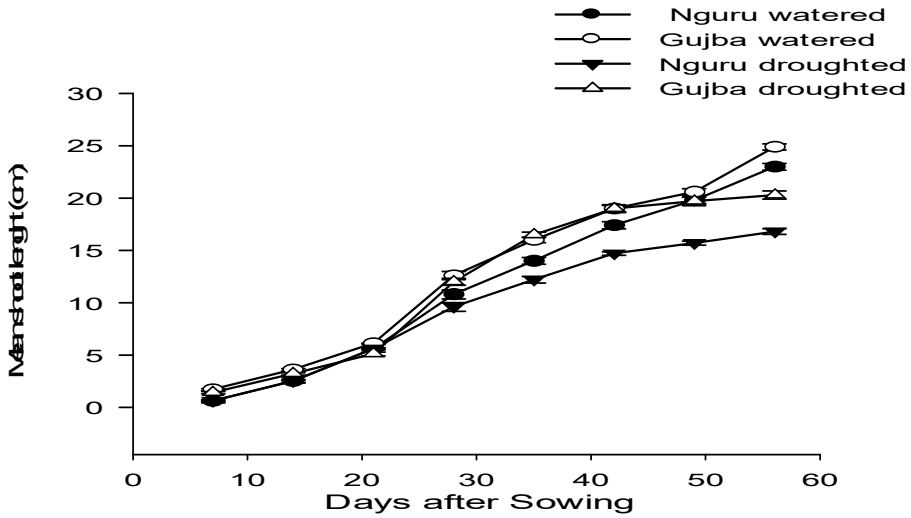


Figure 2: Mean (±SE) total shoot length increased from 7- 52 days after sowing, n=5

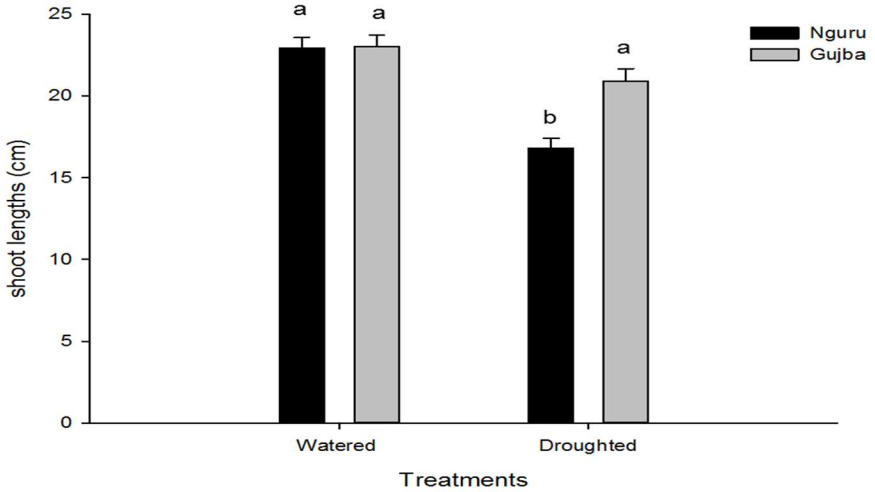


Figure 3: Mean (±SE) total shoot lengths between two provenances of *A. senegal*, n= 5. Mean with different letter is significantly different within treatment after a Tukey test (P<0.05)

Root Growth in Rhizotron

Increase in root length of the longest visible root for all plants in each treatment is shown in Figure 4. Mean root lengths of Nguru and Gujba are significantly different in both treatments. The mean root length in the droughted Nguru seedling was higher (122.00 cm) compared to other treatments (Figure 5) and was affected by the drought treatment. At the end of the experiment, variation in mean root length between the provenances was statistically significant ($F_{1,\ 16}$ = 16.34, P = 0.001), and between treatments ($F_{1,\ 16}$= 29.36, P< 0.001), the interaction between treatment and provenance was not statistically significant ($F_{1,\ 16}$=0.09, P= 0.774).

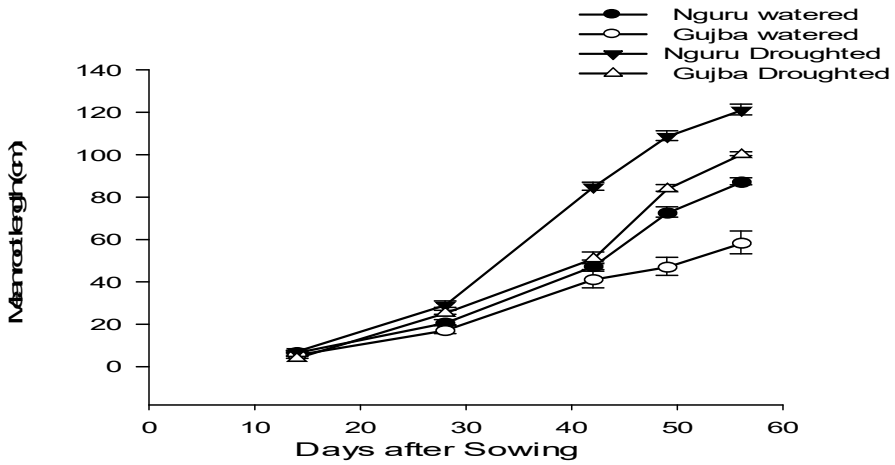


Figure 4: Mean (±SE) maximum visible root length increased from 14 – 52 days after sowing, n=5.

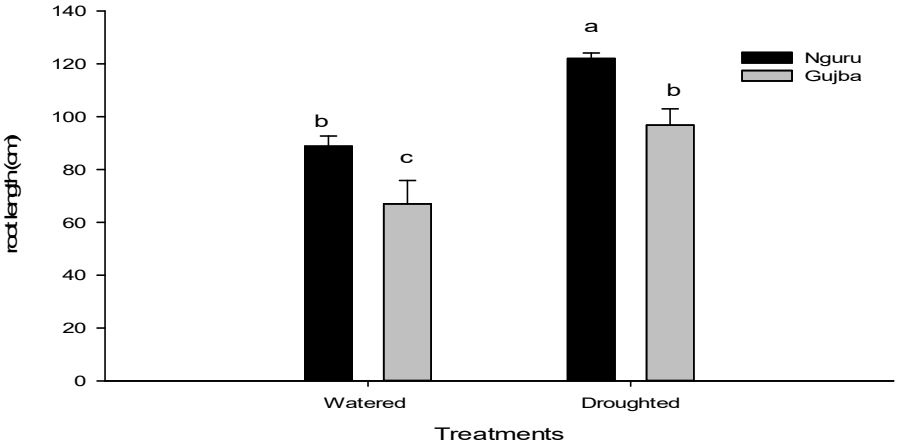


Figure 5: Mean (±SE) root lengths between two provenances of *A. senegal*, n= 5. Mean with different letter are significantly different within treatment after a Tukey test (P< 0.05)

Lateral Roots

The mean number of lateral roots was significantly different between the provenances in all three depths; 0-40 cm ($F_{1, 16}$= 17.04, P=0.001), 41-80 cm ($F_{1, 16}$ =13.844, P=0.002) and 81-120 cm ($F_{1,16}$= 83.903, P<0.001) respectively (Figure 6 A-C). Similarly, there are significant differences among the number of mean lateral roots among the two treatments in two depths 0-40 cm ($F_{1,16}$ = 0.618, P= 0.443) and 81-120 cm ($F_{1,16}$ = 27.129, P<0.001)respectively but not at 41-80cm ($F_{1, 16}$ = 1.725, P=0.208), the interaction between treatment and provenance at depth 0-40 cm was not statistically significant ($F_{1,16}$ = 1.894, P=0.188), however it was significant difference at 41-80 cm ($F_{1, 16}$ = 10.778, P=0.005) and 81-120 cm ($F_{1, 16}$ = 27.129, P<0.001).

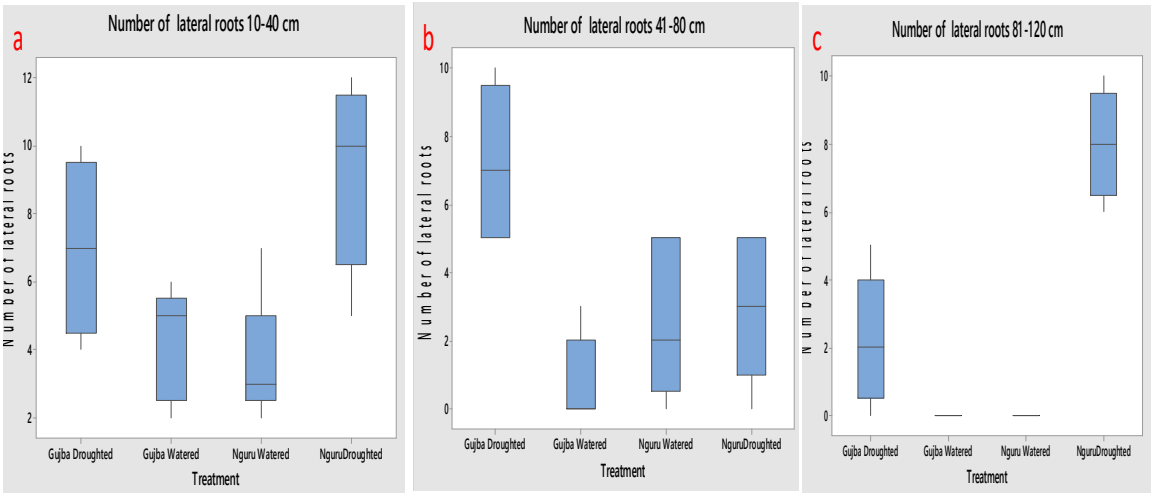


Figure 6 a, b and c: Median number of lateral roots distribution at harvest for all plants at three depth sections (0—40 cm, 41—80 cm and 81—120 cm) in the two provenances

Root Dry Weight Distribution

Root dry weight distribution at harvest of all plant for each three depth sections (0-40 cm, 41-80 cm and 81-120 cm) in the two provenances is shown in (Figure 7. a-c). Root dry weight was highest at in the upper depth (10-40 cm) of the chambers in both provenances and within the treatments. There was more mass in each depth in the drought treatment (Figure 6 a-c) compared to the watered treatment. Nguru had significantly greater total root dry weight and root dry weight at all the depth in all the treatment (Figure 8).

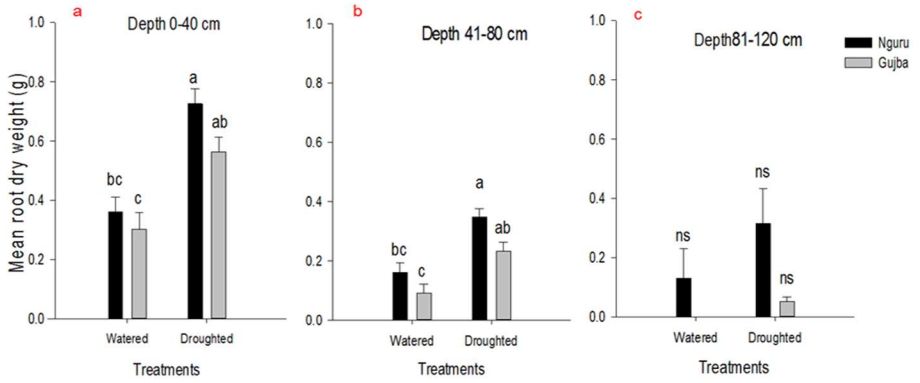


Figure 7 a-c: Mean root dry weight (±SE) in each three depth sections in the two provenances at 56days in rhizotron, n=5. Means with different letters are significantly different within treatment after a Tukey test (P< 0.05).

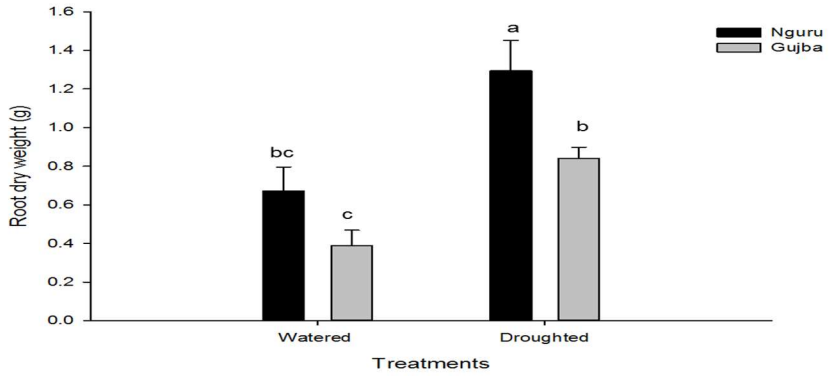


Figure 8: Mean root dry weight (±SE) of *A. senegal* seedlings from two provenances, at 56 days of growth inrhizotrons, n=5. Means with different letters are significantly different within treatments after a Tukey test (P< 0.05)

Root: Shoot ratio

Gujba had a lower mean root: shoot ratio than Nguru in both treatments at the end of the experiment (Figure 9). Root: shoot ratios for the treatment are significantly different ($F_{1,16}$=23.30 P<0.001). Similarly, they are significantly different between provenances ($F_{1,16}$=4.52, P=0.049), however there are no statistically significant interaction between treatment and provenance ($F_{1,16}$=0.004, P= 0.950)

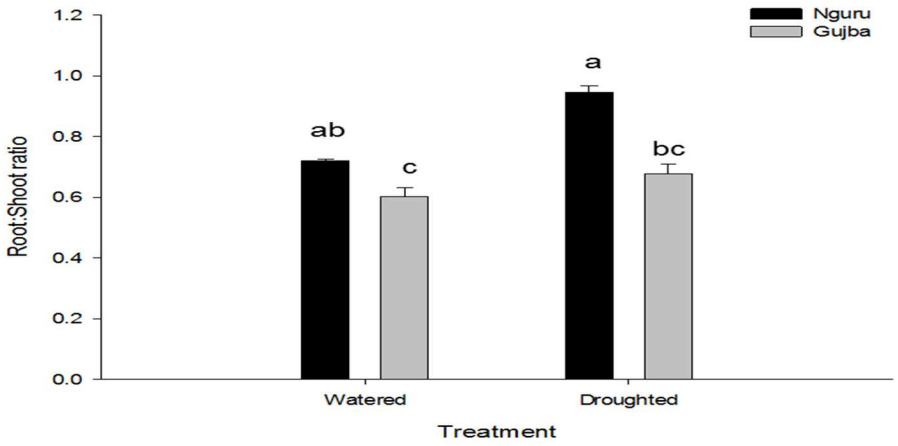


Figure 9: Mean (±SE) root: shoot ratio of seedlings in two provenances of *A. senegal* at 56days in rhizotrons, n=5.Means with different letters are significantly different within treatments after a Tukey test (P< 0.05)

Discussion

Acacia species from the arid and semi-arid areas in NE Nigeria appeared to show different strategies to avoid water stress in this study. Earlier studies have shown that an increase in root length may be associated with soil water deficit (Ahmed *et al.* 2021; Amombo and Wang 2020; Zhou and Zhang 2022). In a comparable study, *Faidherbia albida* (*A. albida*) appeared to tolerate drought stress by producing long taproots, whereas *A. nilotica* developed more branching, presumably allowing exploration of a greater volume of soil (Osman *et al.,* 2022; Gebrehiwot *et al.,* 2020). This is also in agreement with the current findings that Nguru (arid) provenances developed longer roots than Gujba (semi-arid) provenance. This could be a mechanism of tolerating drought stress. An extensive deep-rooting system allows extraction of water from a large volume of soil or from a deep-water table (de Moraes *et al.,* 2021; Gessesse *et al.,* 2023; Kamara *et al.,* 2020). In response to increasing soil water deficit, there was a significant reduction in shoot biomass in this study. Loss of leaf area and inhibition of the expansion of developing foliage are considered an adaptation mechanism to drought. Drought resistant plants are characterized by a reduced transpiration rate to resist embolism in conditions of severe drought. (El Atta *et al.* 2012; Kalapos, 1994). The effect of water deficits would result in change of photosynthetic apparatus and leaf weight. This may be important for maximizing photosynthesis and transpiration efficiency. This suggests that biomass was re-directed to more critical root growth. Increased root: shoot ratio in water stress conditions have been shown in *Acacia tortilis* subsp raddiana (Elfeel and Al-Namo 2011) and *Eucalyptus microtheca* (El-Juhany *et al.* 2008; Elfadl, 2013). *A.senegal* species from the two provenances in the drought treatment had a higher biomassproportion allocated below ground.A similar result has been reported among some other *Acacia* species (Aref *et al,* 2003; Aref and Loutfy 1999). Generally, plants allocate proportionally more biomass to roots in drought conditions (Elfadl 2013). In a drought environmental condition, the balance between the increase in water uptake by deeper roots and the reduction in water loss by stomatal control is critical. It is an important component in adaptation to the environmental conditions (Meinzer *et al.* 2003). Differences in stomatal behaviour between provenances as shown in the case of the *Acacia* trees studied is a strong indication of their differences in rooting patterns and root water uptake. Soil water content appeared to play an important role in controlling stomatal conductance. Decreased soil water content led to a decrease stomatal conductance. This is consistent with other studies involving *Acacia* (Liang and Zhang 1999; Otieno *et al.* 2005). In this study, a more rapid decline in stomatal conductance was observed in the Nguru (arid) provenances than in the Gujba (semi-arid) provenance. Differences in response of stomatal conductance to declining SWC between and within species could therefore serve to demonstrate the differential abilities to access water at different soil layers, root absorption and

strong indication of their differences in rooting patterns and root water uptake.

A. senegal has a tendency to use water preferably in the 0–40 cm topsoil. This could be due to the concentration of soil water content in surface layers since the highest root densities are generally observed in that layer. The soil water content seems to be greatest in the topsoil (0-40 cm) and decrease as soil depth increases. Plants tend to take water first from the upper depths possibly because the internal resistance to transport increases as water is drawn from deeper depths (Meinzer*et al.* 1999; Shaxson and Barber, 2003). Soils dry progressively from the upper to the deeper depths. Root water uptake however, progressively shifted downwards, as water became scarce in the upper soil layers. Species with dimorphic rooting patterns are able to take advantage of short rainfall pulses that occur in the middle of a drought (Jibo, 2021) as well as percolated water resources in the deeper soil layers (Ogle and Reynolds 2004, Jibo 2021). This is consistent with the studies of (February and Higgins 2010) which found a significant negative relationship between soil moisture content and root biomass in semi-arid savannah.

The removal of water from successively deeper section of the soil profile matched root distribution in this study. Water was first extracted in each 40cm depth as root length increased during the weeks. The change in root density may have had a profound influence on the ability of the seedlings to extract soil water and indicates that it is the proliferation of roots into deeper layers that controls water uptake (Snyder and Williams 2003, Jibo *et al* 2021b). There are significant differences among the mean number of lateral roots between the two treatments. Lateral roots are generally more numerous in the two upper layers in seedlings of the Nguru (arid) provenances compared with Gujba (semi-arid) provenances. Lateral roots decrease with increasing soil depth; the number of lateral roots intersects in the 0-40 cm surface soil layer represented 53% of the total throughout the profile. Total root mass and root distributions are markedly different between treatments and provenances, indicating a very high degree of plasticity in root growth in response to even small differences in the rooting environment (Comas *et al.* 2013). The pattern of water extraction from the soil followed patterns of root growth. Water was taken preferentially from the upper layer in the water deficit treatment. The period when water extraction becomes apparent in each section coincided with a major increase in number of roots at that depth.

CONCLUSION

Acacia senegal seedlings from Nguru (arid) and Gujba (semi-arid) provenances exhibited distinct drought response strategies in relation to vertical soil moisture distribution. Nguru seedlings tolerated water stress through deeper rooting, greater root biomass allocation, and stronger stomatal control, producing longer taproots and higher root:shoot ratios under drought. Gujba seedlings maintained greater shoot growth but had shallower root systems. Root dry weight was concentrated in the 0–40 cm topsoil, with water extraction shifting to deeper layers as upper soils dried. These results show that root allotment patterns are strongly influenced by soil moisture gradients, with provenance origin determining drought adaptation mechanisms. Nguru provenance demonstrated superior drought avoidance through enhanced water uptake from deeper soil layers. Prioritize Nguru provenance for afforestation and gum arabic plantation programs in arid zones due to its deeper rooting and efficient water-use strategy under drought. Match provenance selection to site-specific soil moisture profiles, using Gujba provenance for areas with reliable topsoil moisture and Nguru for sites prone to deep soil drying. Incorporate rooting depth and root:shoot ratio as selection criteria in *A. senegal* breeding programs for dryland restoration. Promote nursery practices that encourage early taproot development to improve seedling establishment in water-limited environments. Conduct long-term field trials to validate rhizotron findings and assess survival and gum yield under natural drought

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