CHAPTER FIVE

Study of variations in drought tolerance and water use among seedlings of some selected *Azadirachta indica* seed sources

5.1 Introduction

The ability of plants to function under conditions of low soil moisture depends on their capacity to adjust form and function to offset the damaging impact of negative water potentials in soil and atmosphere (Khalil and Grace, 1992). In dry lands, water availability is the main environmental factor controlling plant growth and survival, depending on the different manifestation of ecological selection pressure towards morphological characters and physiological behavior that enable plant to survive and grow (Kundu *et al*., 2000).

Adaptation is defined as inherent modification in structure or function that increases the probability of an organism surviving and reproducing in particular environment (Karmer, 1983). Adaptive response to water availability include physiological and morphological changes affecting, for instance, plant structure, growth rate, water-use efficiency, tissue osmotic potential and stomata conductance (Li and Wang, 2003).

Plant response to water deficit varies temporally and spatially over long time scale (Li, 1998). Plant capacity to resist drought results from the integration of variety of adaptive characteristics and mechanisms (Jones, 1993; Turner, 1986). Drought avoidance which is generally found in higher plants is achieved primarily through adaptation that retard water losses or increase water absorption (Bewley and Krochko, 1982).

Plants with conservative strategy are adapted to conditions where individuals are isolated with drought periods are supposed to be long. The conservative strategy is usually associated with drought resistances of species and with high water use efficiency and slow intrinsic growth rate (Kramer, 1983; Dickmann *et al.*, 1992; Li, 1999). Recent studies indicate significant genotypic variation in physiological and morphological adaptation to stress, particularly drought, in tree species provenances, families and clones (Abrames, 1988; Cregg, 1993). The effectiveness of any combination of traits in conferring drought resistance will depend on the specific environment (Ranney *et al.*, 1990).

Plant growth analysis has been successfully used in both study of wild plant population dynamics and in studies with more applied orientation, particularly those aimed at increase useful. Due to selective pressure in dry land species to conserve water and thus optimize water use may be an important factor contributing to plant survival in extreme condition (Tuomela, 1997). Leaf water relations provide useful tool to improve the understanding of intra-specific adaptation mechanisms, (Tuomela and Kanninen, 1995).

This study is attempted to improve the understanding of the growth performance of *Azadirachta indica* population on the basis of physiological and morphological behavior and of the climatic conditions in their nurseries habitats.

The main objective of the present study was to identify some morphological growth characteristics of *Azadirachta indica* seedlings from different provenances and examine how they are affected by varying irrigation frequency and investigate how these morphological growth characteristics relate to species performance in term of growth. The study also aimed to investigate inter-specific variations in seedlings characteristics of *Azadirachta indica* among provenances under four irrigation regimes and two soils type.

5.2 Material and methods

5.2.1 Seed sources

Mature yellow and healthy fruits of neem *Azadrichta indica* were collected from four different seed sources in various parts of Sudan. The seed sources used were namely provenances of Elfasher (Northern Darfur state), Eldalang (Southern Kordofan state), Senga (Sinar state) and Gedaref (Gedaref state) western, central and eastern parts of the country. It was collected in 15-20 June 2013. The seed were collected from 24(6x4) of well-formed healthy looking trees in each area and pooled together as one lot.

5.2.2 Collection and processing of neem seeds

Healthy neem fruits mature (yellow) and were collected from selected trees grown in plantations at the specified geographic areas (Table 1). Four sites within each area were selected randomly and a sample of six trees was marked in each site for fruit collection seeds, were collected from the tree crown by shaking with long hooked sticks or by hand. In total one kilogram of fruits were collected from the marked trees. Collected fruits were then transported to the Regional Tree Seed Centre, Elobied-Sudan, where they were cleaned in the processing room and dried under shade in dry weather. Seed processing was carried out in accordance with (ISTA, 1993) and International Neem Network recommendations (Thomsen and Souvannavong, 1994).

Fruits were gently squeezed by the fingers to extract the seeds which were then washed with water several times to remove fruit pulp. Seeds were then dried in thin layers on absorbent sheets in the shade with circulating air.

5.2.3 Experimental design

The study was conducted in the nursery of Natural Resources and Environmental Studies, University of Kordofan in 2013. Seeds from the four origins were directly sown in polythene bags with one seed per bag. The bags were factorial experimental plant medium.

A complete randomize design with three factors i.e., 4 provenances× 4 irrigation levels× 2 soil × 3 replicates× 5 seedlings per replicate resulted in total of 480 experimental units.

The seedlings were raised in the nursery under direct heat and 50 % light from the sun. The seedlings were grown for 45 days, with regular watering before applying irrigation regimes. Four irrigation levels 100%, 75%, 50% and 25% field capacity were applied to the seedlings for 3 months; irrigated every two days by neems seedlings were measured at the end of each week for root collar diameter, length and number of leaves. Treated with soil (clay, sand); irrigation treatment (full field capacity 100%, 75% field capacity, 50% field capacity and 25% field capacity). At the end of the experiment after 3 month destructive sampling for two seedlings per provenances per irrigation level per replicate were randomly selected and harvested, the seedling were measured for root length, shoot length, root fresh weight, shoot fresh weight, shoo/root ratio, number of leaves and leaf area. Roots were uncovered by washing off soil using running tap water. Shoot and root fresh weights were then oven dried at 70-80^{o} C for 48 hours for constant weight for determining the shoot and root dry weight. Shoots and roots were then used to determine the shoot: root ratio. All measurements were carried out using an electronic balance. Root collar diameter was measured by using digital vernier caliper. Leaf area was measured with the LI-COR Model LI-3000.

The data were subjected to analysis of variance (ANOVA) for confirming the differences between the provenances and irrigation level and soil type Duncan Multiple Range Test was used to separate the means.

5.3 Results

Analysis of variances showed highly significant ($p<0.0001$) differences among the provenances in root collar diameter and shoot length significant ($p<0.01$) differences in root fresh weight, significant ($p<0.05$) differences in shoot/root length ratio. The provenances showed no significant differences between them in root length, shoot fresh weight, shoot dry weight, root dry weight, shoot/root dry weight, and leaf area. While the irrigation showed high significant differences in root collar diameter, shoot length, root fresh weight, shoot dry weight, root fresh weight and number of leaves. The analysis revealed only significant differences between them in leaf area. Showed no significant differences in root length, shoot/root length, shoot fresh weight, root dry weight and shoot/root dry weight ratio. Also, the analysis of variances showed high significant differences in root collar diameter, shoot length, shoot/root length, shoot fresh weight, root fresh weight, shoot dry weight, shoot/root dry weight and leaf area, no significant differences in root length and root dry weight Table 5.1.

5.3.1 Root collar diameter

The root collar diameter of the seedlings varied significantly between the provenances. Senga provenance recorded the root collar diameter of (3.1 mm) followed by Elfasher and Eldalang (2.8, 2.7) respectively. Gedaref provenance ranked the last lowest place with a root collar diameter of (2.5 mm) significant differences lowest Senga provenance Table 5.2.

Table (5.3) the root collar diameter of seedlings varied significantly between the provenances affected by irrigation. Elfasher provenance recorded the bigger diameter of seedling (3.2 mm) and Eldalang, Senga and Gedaref in the lowest places (2.7, 2.6 and 2.5) respectively.

In 75% field capacity of irrigation regimes Senga provenance recorded bigger seedling (2.9± 0.6) and Elfasher and Eldalang were mediate (2.9±0.6, 2.7±0.5) respectively. Gedaref provenance ranked in the last lowest place with a root diameter of (2.5±0.6). In 50% field capacity of irrigation regimes Senga and Eldalang provenances recorded bigger diameter of seedlings (2.6±0.4) and Gedaref provenance ranked in the last lowest rank place (2.3±0.5) and Elfasher provenance in mediate (2.6±0.6). Senga provenance recorded bigger diameter of seedling in 25% field capacity (2.4±0.4). Gedaref provenance ranked in the last lowest place (2.3±0.4) and Elfasher, Eldalang provenances in mediate (2.5±0.4, 2.4±0.4) respectively Table 5.4.

Table 1

Table 1

Table2

Table3

Table 4

Table 4

Table (5.5) Senga provenance recorded bigger root diameter of seedling in sandy soil by different soils (3.3± 0.5) and were Elfasher, Eldalang mediate (2.8±0.7, 2.6±0.5) respectively. Gedaref provenance ranked in the last lowest place with a root diameter of (2.2±0.4). In clay soil by different soils Senga provenance recorded bigger diameter of seedlings (2.9±0.5) and Gedaref provenance ranked in the last lowest place (2.6±0.6) and Elfasher and Eldalang provenances in mediate (2.8±0.7, 2.7±0.6) respectively.

5.3.2 Shoot length

The length of the seedlings varied significantly between the provenances. Elfasher provenance recorded the longest seedling (23.5 cm) and was significantly longer from Senga, Eldalang and Gedaref provenances with a length of (21.3, 21.1, 20.2 cm) respectively. They came in second place with no significantly differences between them Table 5.2.

Table (5.3) the shoot length of seedlings varied significantly between the provenances by the effect of irrigation. Elfasher provenance recorded the highest seedling (23.1 cm) and Eldalang, Senga and Gedaref came in the second lowest places (21.4, 21.1 and 20.5 cm) respectively.

Elfasher provenance recorded bigger seedling (22.6± 2.1) and Eldalang, Senga and Gedaref provenances ranked in the last lowest place with a root diameter of (19.1±2.7, 23.4±2.6, 17±2.3) respectively. Elfasher provenance recorded bigger seedling in sandy soil by different soils (24.5± 3.6) and Eldalang, Senga and Gedaref provenances ranked in the last lowest place with a root diameter of (19.4±2.6, 23±4, 20.3±0.5) respectively Table 5.5.

Table 5

5.3.3 Root length

The provenances showed no significant variations in root length characteristic Table 5.2.

Table (5.3) the root length of seedlings varied significantly between the provenances because of the effect of irrigation. Elfasher provenance recorded the longest root seedling (19.1 cm) and Gedaref came in the second place (18.4 cm) while Senga and Eldalang provenances ranked in the last lowest places with a root length of (18.2, 16.9 cm) respectively.

Gedaref provenance recorded bigger root length of seedling in 100% field capacity of irrigation regimes (20.7 ± 4.9) and Elfasher and Eldalang were mediate (19.5 ± 4.8, 17.9 ± 4.6) respectively. Senga provenance ranked in the last lowest place with a root length of (16.7 ± 2.6) Table 5.4.

5.3.4 Shoot/root length ratio

The provenances showed significant variations in shoot/root length ratio. Elfaher provenance had significantly longer value (4.1) compared to the rest of the provenances. However, Senga, Eldalang and Gedaref provenances ranked in the second place with significant differences between them recorded values of (1.3, 1.2, 1.2) respectively. Which was showed in Table 5.2.

In table (5.5) Elfasher provenance recorded high value of shoot/root length raito of seedling in sandy soil (1.3± 0.3) and were Eldalang, Senga and Gedaref provenances ranked in the last lowest place with a shoot/root length ratio of (1.1±0.2, 1.1±0.3, 0.9±0.3) respectively. Elfasher and Eldalang provenances recorded high value seedling in clay soil by different soils (1.5±0.4, 1.3±0.3) respectively and Senga and Gedaref provenances ranked in the last lowest place with a shoot/root length ratio of (1.6±0.6, 1.9±1.1) respectively.

5.3.5 Shoot fresh weight

The provenances showed no significant variations in shoot length characteristic Table 5.2.

Table (5.3) the shoot fresh weight of seedlings varied significantly between the provenances affected by irrigation. Elfasher provenance recorded the highest shoot fresh weight of seedling (1.6 g) and Senga and Eldalang provenances ranked in the second places (1.4 g) respectively and Gedaref ranked in the last lowest place with a shoot fresh weight of (1.3 g).

5.3.6 Root fresh weight

Elfasher and Senga provenances recorded significantly bigger root fresh weight compared to the remainder of the provenances. However, root fresh weight values ranged from (1.1, 1.2 g). Eldalang recorded no significant difference and ranked in the second place. The least root fresh weight value was recorded by Gedaref provenance which showed no significant difference and occupied the second place as shown in Table 5.2.

Table (5.3) the root fresh weight of seedlings varied significantly between the provenances affected by irrigation. Elfasher provenance recorded the highest root fresh weight of seedling (1.23 g) and Eldalang provenances ranked in the second place (1.05g), which Senga and Gedaref came in the last lowest place with a root fresh weight of (0.97, 0.94 g) respectively.

Elfasher provenance recorded high value of root fresh weight of seedling in 75% field capacity by irrigation regimes (1.2±0.4) while Senga, Eldalang were mediate (1.2±0.5, 1.1±0.6) respectively. Gedaref provenance ranked in the last lowest place with a root length of (0.8±0.7) Table 5.4.

Elfasher provenance recorded high value of root fresh weight of seedling in sandy soil (1.5±0.5) and Senga provenance ranked in the second place (1.1±0.4). Gedaref provenance ranked in the last lowest place with a shoot fresh weight of (0.8±0.4). Elfasher provenance recorded high value of root fresh weight of seedling in clay soil (1.5±0.4) and Senga provenance ranked in the second place (1.2±0.5). Gedaref provenance ranked in the last lowest place with a shoot fresh weight of (0.9±0.8) Table 5.5.

5.3.7 Shoot dry weight

The provenances showed no significant variations in shoot dry weight characteristic Table 5.2.

5.3.8 Root dry weight

The provenances showed significant variations in root dry weight characteristic Table (5.2). Elfasher provenance recorded significantly bigger root dry weight (0.44) compared to the remainder of the provenances. The least root dry weight value was recorded by Senga and Gedaref Table 5.3.

Provenances recorded high value root dry weight of seedling (0.5±0.2, 0.5±0.1, 0.3±0.2) respectively. Eldalang provenance ranked in the last lowest place with a root fresh weight of (0.4±0.1). Elfasher, Senga and Gedaref provenances recorded high value of root dry weight of seedling in clay soil by different soils (0.5±0.1, 0.4±0.2, 0.3±0.3) respectively. Eldalang provenance ranked in the last lowest place with a root dry weight of (0.4±0.2).

5.3.9 Shoot/root dry weight ratio

The provenances showed no significant variations in shoot/root dry weight ratio characteristic Table 5.2.

Elfasher and Gedaref provenances recorded high value of shoot/root dry weight ratio of seedling (2.4±1.2, 2.3±1) respectively. Eldalang provenance ranked in the second place with a shoot/root dry weight ratio of (1.7±0.8). Senga provenance ranked in the last lowest place with a shoot/root dry weight ratio of (1.9±1.3) Table 5.5.

5.3.10 Number of leaves

Elfasher, Eldalang and Senga provenances showed significant variations in number of leaf characteristic and recorded 8 leaves. The least number of leaves was recorded by Gedaref 7 leaves Table 5.2.

Table (5.3) the number of leaves of seedlings varied significantly between the provenances affected by irrigation. Elfasher provenance recorded the highest number of leaf of seedling (8.5) and Eldalang provenances ranked in the second place (7.8) and Senga in third place (7.1). Gedaref in the last lowest place with a number of leaves (6.4).

Elfasher, Eldalang provenances recorded high value number of leaves of seedling in 100% field capacity by irrigation regimes (9.2±3.3, 8.9±2.9). Senga and Gedaref provenances ranked in the last lowest place with a number of leaves of (8.2±1.9, 7.9±3.2) respectively. In 75% field capacity by irrigation regimes Elfasher, Eldalang and Senga provenances recorded high value of number of leaves of seedling (6.8±1.7, 7.8±2.6, 8±2.5) respectively. Gedaref provenance in the last lowest ranked place with a number of leaves (7.3±2.4). Elfasher, Eldalang and Senga provenances recorded high value of number of leaf of seedling in 50% field capacity by irrigation regimes (6.8±1.7, 7.3±2, 7.5±1.9) respectively. Gedaref provenance in the last lowest ranked place with a number of leaves (6.7±1.9) Table 5.4.

Table (5.5) Elfasher provenance recorded high value of number of leaves of seedling (2.8±0.6). Eldalang and Senga provenances recorded second value of number of leaves of seedling in sandy soil by different soil (2.6±0.5, 3.3±0.3) respectively. Gedaref provenance in the last lowest ranked places with a sandy soil (2.6±0.6). Eldalang and Senga provenances recorded high value of number of leaf of seedling in clay soil by different soil (2.7±2.6, 2.9±2.5) respectively. Elfasher and Gedaref provenances in the last lowest ranked places with a clay soil (2.8±0.6, 2.6±0.6).

5.3.11 Leaf area

The leaf area of the seedlings varied significantly between the provenances. Senga provenance recorded the largest seedling leaf size (21.2 cm^2) and was significantly largest from Elfasher, Eldalang provenances with an area of (18, 15.5 cm^2) respectively. Gedaref provenance recorded in the last place (14.4 cm^2) Table 5.2.

Table (5.3) showed that the leaf area of seedlings varied significantly between the provenances affected by irrigation. Elfasher provenance recorded the largest leaf area of seedling (22.4 cm^2) and Eldalang provenance ranked in the second place (17.1 cm^2) .Senga and Gedaref in the last lowest ranked places with leaf area of (15.2, 14.4 cm^2) respectively.

Table (5.4) Elfasher and Senga provenances recorded large leaf area of seedling in 75% field capacity by irrigation regimes (19.7±6.7, 18.8±8.4). Eldalang and Gedaref provenances ranked in the last lowest place (15.6±7.4, 14.1±6.7) respectively.

Eldalang provenance recorded high value of leaf area of seedling (19.2±5.3). Elfasher, Eldalang and Gedaref provenances ranked in the last lowest place (13.4±4.1, 11.7±2.4, 8.6±1.7) respectively. Eldalang provenance recorded high value of leaf area of seedling (23.9±2.9). Elfasher, Eldalang and Gedaref provenances ranked in the last lowest place (22.7±2.9, 20.6±7, 20.2±6.7) respectively.

5.4 Discussion

A reduction in all parameters measured in response to water uses, as observed in this study, demonstrated the ability of species to tolerate and acclimate to a wide range of water levels by morphogenetic and plastic response. The water use treatment resulted in significant decreases in all measured parameters. Observed phenotypic variation is generally assumed to reflect the inherent genotypic variation among and within the provenances grown under uniform environmental conditions (Dangasuk *et al*., 1997). Such variations in morphological and physiological characteristics of the seedlings in the nursery have been shown to be useful when assessing seedlings growth potential under field conditions (Patiwal *et al*., 1999). The effectiveness of any combination of traits in conferring drought resistance will depend on the specific environment, (Ranney *et al*., 1990).

Senga provenance the biggest seedlings RCD, indicating that Senga provenance is enjoying the best growth requirements (water, soil) while on the other hand

Gedaref is somewhat suffering from harsh or short growth conditions that lead the plant to minimize seedling size towards the economy of water and/or materials. The other two provenances have moderate growth conditions. However, Elfasher have the greatest root collar diameter than the other provenances as an effect of irrigation. Senga provenance seedlings had greater collar diameter at 75, 50, 25% field capacity. All measured growth parameters declined gradually in all provenances and decrease in growth in response to less water regime and this indicated that the water has major effects on plant growth (Wilson *et al*., 1998).

Shoot length is indirectly related in addition to other growth requirements to light intensity (West *et al.*, 1996; Cutini and Nocentini, 2000) found similar results in other plant species. This may explain why Elfasher scored longest seedlings where the sun intensity in the environment is high so the seedling grew longer searching for light. The recorded high pattern of provenance variation indicates genetic differences among these provenances. Similar results were observed in many studies (Mohamood *et al*., 2003; Maelim, 2003; Arnold and Cuevas, 2003; Marunda, 1993). This type of variation in adaptation traits is necessary for selection of the populations to be included in a conservation plan (Gradual, *et al*., 1997).

Elfasher had greater root length compared to the other provenances at the four irrigation regimes. Gedaref, provenance seedlings had greater root length for 100% field capacity indicating that the provenance is well adapted to more humid environments prevailing the area.

Elfasher provenance had greater shoot/root length ratio than the other provenances. This high value in both sandy and clay soil indicate some sort of adaptation to water availability as genetic differentiation. Genetic differentiation of drought tolerance has been found within tree species with extensive geographic or habitat range (Bongarten *et al*., 1986; Abrams *et al*., 1990; Parker and Pallardy, 1991; Kundu, 1997). A lower shoot/root length ratio may indicate drought tolerance in a provenance. Since Elfasher provenance had the longest seedlings or shoot lengths than it is expected to have also greeter root length to avail enough water for this shoot system and this is why it has the greeter root/shoot root length ratio.

As a result of the high shoot/root ratio, Elfasher provenance had the greatest shoot fresh weight compared to the other provenances at the four irrigation regime.

Elfasher and Senga provenances had a greater root fresh weight than the other provenances as the result of irrigation and soil type. Fresh weight variable seems to be an indicator to efficient use of resources within the environment of origin. These two provenances were shown to be more adapted compared with the other two provenances tested in this experiment.

The root dry weight characteristic indicates the amount of carbohydrate production and consequently, the photosynthetic efficiency of the plant. Since Senga and Elfasher provenances showed significantly greater RDW value, it might be inferred that both provenances were environmentally adapted to the type of environmental conditions prevailing in their origin and where they were tested.

Elfasher and Gedaref provenances produced significantly bigger shoot/root ratio on the sandy soil compared to the other four provenances. Adaptive response to water availability include physiological and morphological changes affecting, for instance, plant structure, growth rate, water-use efficiency, tissue osmotic potential and stomata conductance (Li and Wang, 2003). Reduction in leaf area, increased root density and depth are important adaptive features as is also suggested by Ekenayake *et al*. (1985), and Pulkkinen and Tigerstedt (1992).

Elfasher, Eldalang and Senga had the greater number of leaf; this indicates that Elfasher, Eldalang and Senga provenances were enjoying the best growth requirements (water, soil) while Gedaref was somewhat suffering from harsh or short growth condition that lead the plant to minimize seedling size towards the economy of water and/or materials. Elfasher has great number of leaf than the other provenances at the four irrigation regimes. Elfasher, Eldalang provenances recorded high value number of leaf of seedling in 100% field capacity by irrigation regime. In 75% field capacity by irrigation regime Elfasher, Eldalang and Senga provenances greater, also, in Elfasher, Eldalang and Senga provenances recorded high value number of leaf of seedling in 50% field capacity by irrigation regimes.

Elfasher and Senga provenances have greater leaf area of seedling in 75% field capacity by irrigation regime. Eldalang provenance has greater value of leaf area of seedling in sandy and clay soils.