Effect of seed collection site on the tolerance of Melia azedarach L. seedlings to drought stress

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Abstract:

As temperatures warm and precipitation patterns shift as a result of climate change, interest in the identification of tree genotypes that will thrive under more arid conditions has grown. *Melia azedarach* L. is a deciduous tree of the Meliaceae family that is introduced to Iraq and planted as an ornamental tree in parks and streets. To study the mechanism of drought resistance in *Melia azedarach* and evaluate the drought resistance capacity of each tree and site, we selected fifteen tree from three sites (Domis, Sarsink, and Brifka) as the research subjects and set four levels of drought stress treatment (CT: 80% of field capacity, mild drought: 60% of field capacity, moderate drought: 40% of field capacity, and severe drought: 20% of field capacity). In this study, the seedling survival, height and the ground diameter, the leaf number, dry biomass of shoot, root length, root width, root number, dry weight of root system, leaf area, stomatal conductance size and number, and the content of chlorophyll a, b, and ab decreased with the increasing drought stress levels spatially in the seedlings growing under moderate (40%) and severe (20%) drought. The seedlings were damaged to varying degrees. The minimum percentage of seedling survived in severe drought was in the Brifka site in ratio (24.44%), while the seedlings in the Sarsink locality more tolerance to severe drought stress where the success rate of the seedlings exceeded 50%. Variations in seedling characters among different sites can be used as an indicator for selection trees of this species and used for tree breeding and development programs under drought stress condition.

Keywords: *Melia azedarach* L.; sites; seedling survival; morphology; physiology characters; drought stress

Introduction:

Climate change, characterized by rising temperatures, changing precipitation patterns, and an increasing frequency of extreme weather events, has emerged as a primary stressor in forest ecosystems [19,20]. Recent studies indicate that prolonged droughts, such as those observed in the different countries of the world, contribute to widespread tree mortality, with species such as *Pinus ponderosa and Quercus sp. ilex* experiencing significant population decline [43,64]. Similarly, altered temperature regimes enable the expansion of invasive species and pests, exacerbating forest decline [54]. Climate change is challenging forestry management and practices. Among other things, tree species with the ability to cope with more extreme climate conditions have to be identified. However, while environmental factors may severely limit tree growth or even cause tree death, assessing a tree species' potential for surviving future aggravated environmental conditions is rather demanding [15].

ISSN 2072-3857

Droughts pose significant challenges to agriculture, water resources, ecosystems, and societies worldwide [40]. This comprehensive A basic definition of drought tolerance according to [36] is the ability to survive, and sometimes grow, during periods of water shortage. Survival and growth are often correlated, with trees exhibiting a history of below average growth or abrupt decreases in growth having higher mortality [27]. Forests are one of the most critical components of the Earth's biosphere are essential for global ecological, economic, and social stability; however, they are increasingly threatened by global environmental changes. Forest plantations play an important role in maintaining a supply of high-quality timber from managed forest. With an expected increase in the prevalence of drought in some forested areas, climate change increases concerns about future seedling growth. Planting seedlings with improved resistance to drought may offer a solution to increase survival and improve the resilience of forest plantations because seedlings are particularly vulnerable to drought during many years after plantation, given that they lack a well-developed root system. However, before implementing this strategy at the operational scale, the performance of the genetic material improved for current climatic conditions needs to be tested under conditions that will emulate future more adverse conditions [56]. Studying the genetics of adaptation to new environments in ecologically and industrially important tree species is currently a major research line in the fields of plant science and genetic improvement for tolerance to abiotic stress. Specifically, exploring the genomic basis of local adaptation is imperative for assessing the conditions under which trees will successfully adapt in situ to global climate change [11].

Tree breeding for drought tolerance uses both traditional methods like pedigree selection and modern molecular techniques like marker-assisted selection to create plants better able to survive and produce under water-scarce conditions. Key strategies focus on improving root systems for deeper water access, enhancing physiological mechanisms like stomatal closure and osmotic adjustment, and selecting for specific traits that improve water use efficiency. Success relies on a comprehensive approach that combines genetic knowledge, physiological understanding, and advanced breeding tools. Breeding climate-resilient forest trees with improved levels of abiotic and biotic stress resistance as a response to climate change presents both opportunities and challenges. Drought resistant forest trees are essential for the establishment of protection plantations in arid and semi-arid regions. Provenance studies established in the mid-20th century to identify seed zones for replanting or highly productive genotypes have been re-purposed to investigate potential responses to climate change [41,42,51]. The response of trees to drought conditions can be complex. Drought stress directly or indirectly influences seedling growth by inhibiting cell growth, stomatal conductance and metabolic activity due to reduced photosynthesis and carbohydrate availability [13]. The results of [33] show that the drought response of Norway spruce seedlings includes a number of physiological and biochemical changes that enhance the seedlings' ability to survive and thrive during drought periods. In addition to the expected reduction of growth rate due to drought-induced stress, trees growing under severe drought can undergo modifcations of their xylem structure [5].

Chinaberry tree (*Melia azedarach* L.) It is an Asiatic multipurpose tree, of worldwide cultivation, mainly for its ornamental beauty and landscape value. Is a fast-growing, It is deciduous and perennial tree of the Meliaceae family that grows naturally in China, India, and Iran; however, it has also spread into other countries [35]. Introduced into Kurdistan region and planted as an ornamental tree at the nurseries, parks and

ISSN 2072-3857

streets [1]. It is very adaptable tree that tolerates a wide range of climates and soils. In arid regions, and semiarid areas, afforestation occurs in warm temperatures with low relative humidity and high transpiration. It adapts well to hot climates, poor soils, and dry seasonal conditions [16]. The main utility of *M. azedarach* is with long lasting wood, used as a component of agro forestry systems with inter cropping annual species [48]. *M. azedarach* is a multi-purpose tree species. It is a fast-growing and high-quality timber tree; it is also a good nectar plant and a vital plant pesticide [25]. The wood is used to manufacture agricultural implements, furniture, plywood, boxes, poles, fuel, tool handles, pulp, and particleboard industries [16]. It is also used in cabinet making as well as in construction [37]. The leaves can be used as green manure as well as supply a wide range of medicinal applications and insecticide. It is a good source of natural compounds such as azadirachtin - which is found only in seeds- with potent insecticide and antimicrobial action [50,68]. Drought tolerant varieties are with high demand which seems to be a great challenging task to plant breeders however difficulties are combined by the difficulty of forest growth on the genetic and physiological bases. Therefore, this study, will conduct a common garden experiment with Chinaberry tree (*Melia azedarach* L.) seedlings from different locations and trees, treated under control and drought lath house conditions as a proxy to investigate the effect of breeding on forests´ resilience and adaptability to drought. Is a step towards selecting good genotypes for agroforestry plantations, which can be multiplied to produce quality planting material that will be further available to forester on selection.

Materials and Methods: -

- Site Determination and Location:

Healthy and mature fruits at the time of natural seed dissemination of this species were collected from the South to North from three sites. Five opened pollinate trees per location (vigor trees of taller straighter bole, regular crown, fine attractive branching habit, and delayed stem-core decay) which has adapted naturally to ecological condition, were selected in November 2024. Representing the various physiographic regions of Dohuk province at the least 80 Km. apart from the sites located between the periods of precipitation. The Domis site it is located between (400-500ml), Sarsink site it is set between (700-800ml), and Brifka site it is placed between (900-1000ml) according to moisture sources and spatio-temporal variation of isotopic signatures in Iraqi precipitation [2] as is shown in map figure (1). Fruits from each locality and trees with relatively average growth were transplanted to research area in the laboratory and lath house of forest department of College of Agricultural Engineering Sciences/ Duhok University during growing seasons (2024-2025), located in the district of Sumail (42°, 52′, 02″ E, 36°, 51′, 38″ N, and altitude 456m. over sea level and average annual rainfall 400 – 450 mm) in the province of Dohuk governorate / Kurdistan region Iraq. (Some tree characters and sites and physiographic data for the selected sites are shown in table (1), the Temperature and humidity data for the period 2014–2024 were analyzed to observe long-term climate trends over eleven years).

Seeds after fruit flesh and endocarp are removed manually without any intermission after fruit gathering cleaning, and put in moisture-proof containers, and stored in a cooling chamber at a low temperature (2-4C°) until experimented. Seeds planted in polyethylene sacks filled with sand soil (three seeds in each polyethylene sack and six polyethylene sacks for each treatment unit with three replicates) planted between February and March 2025 inside a lath house, where the percentage of light was about 50%. After

ISSN 2072-3857

seeds sowing, allowed to germinate and grow.

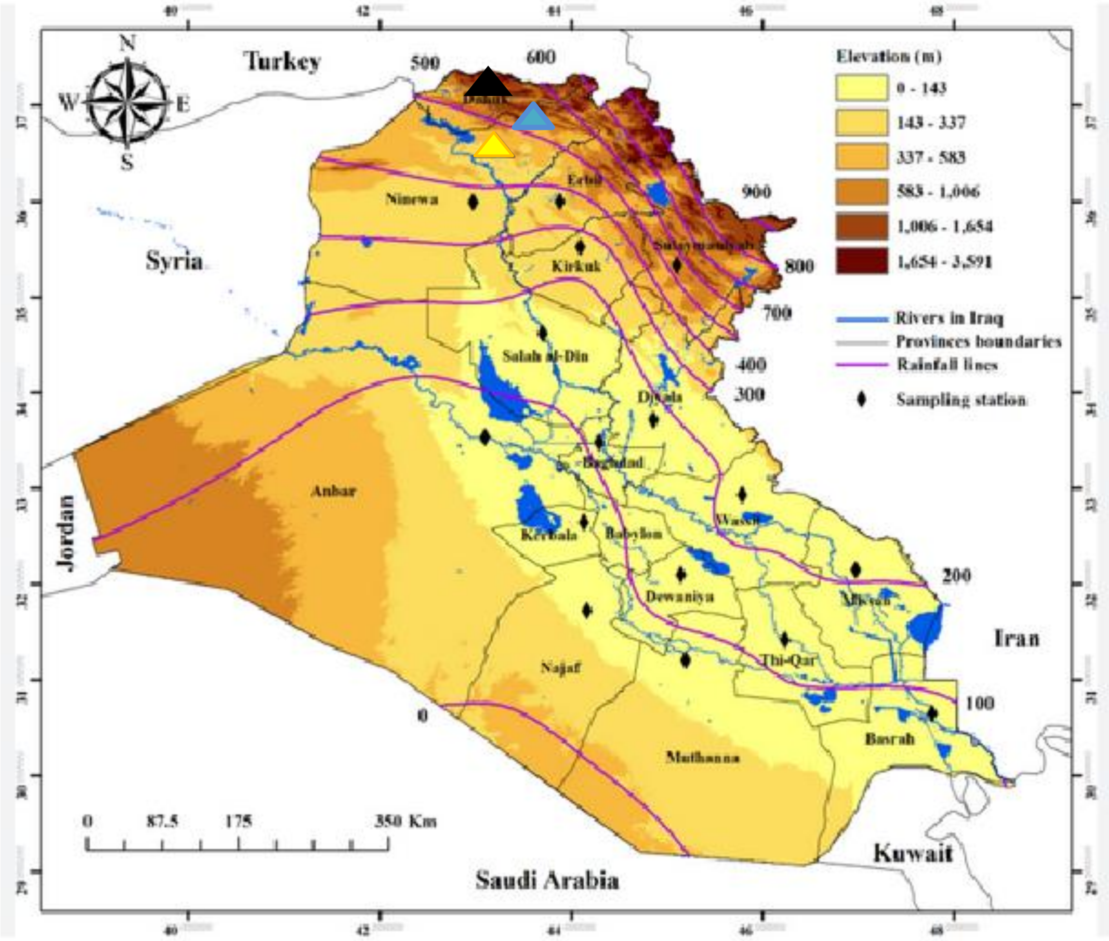

Figure (1) illustrates the moisture sources and the spatio-temporal variation of isotopic signatures in Iraqi precipitation [2]. Yellow denotes the Domis area, blue represents the Sarsink area, and black indicates the Brifka area.

Table 1. Selected tree characteristics, site attributes, and physiographic data for the studied locations.

Location		Domis	Sarsink	Brifka
Height (m)		4.7-6.5	6.5-8	5-9
First branch (m)		1.7-2.49	1.67-2.8	1.35-2.32
Diameter at D.B.H (cm)		15-26.5	21.03-23.2	16-25.9
Crown		5.75-8.1	5.1-8	6.45-7.8
Weight of 1000 seed(g)		524.97-645.5	565.79-715.98	516.9-1090.47
Precipitation mm		319.2- 953	681.5-1646.5	548.4-1292.6
Relative humidity %		36.16- 47.01	40.24-51.24	47.83-57.63
Air temperature C⁰	Min	-7.01-(-1.01)	-15.7-(-5.21)	-24.18-(-12.32)
	Max	46.54-48.47	41.63-43.7	34.78-36.71
Latitude(N°)		36°.7933348	37°.03915	37°.22036

(E°) Longitude	42°.9126319	43°.34040	43°.21770
(M) Altitude	443	1036	912

- **Treatment Seedlings with Water: -**

Seedlings Initially, germinated (i.e. after hypocotyls emerged from the soil) assigned to the drought treatment received water every day until post-emergence, after which watering will be stopped. After that in this experiment used the artificial water control method for potted (15*30) plants, and four drought stress gradients were set in this experiment: Control treatment (soil water content was 80% of field capacity), mild drought stress (soil water content was 60% of field capacity), moderate drought stress (soil water content was 40% of field capacity), and severe drought stress (soil water content was 20% of field capacity). The soil field capacity of the potting substrate was 19.66%, based on which the water weight under four drought stress treatments was calculated accordingly. Each container was weighed individually starting on June 10, 2025. Until October 19, the water content in each container remained almost constant within the specified limits. After that, an electronic scale was used every two days at 10:00 AM to weigh each container to replenish lost water and maintain the water content within the predetermined range field capacity.

- **Growth and Maintenance of Seedlings: -**

The seedlings were cared for in the greenhouse under standardized conditions, including regular watering and weeding as needed, until the end of the experiment. At the beginning of November, four seedlings were randomly selected from each treatment for each tree at each site, and the following seedling characteristics were evaluated:

1. **Survival percentage of seedlings: -**

The percentage of the germination and living plants will account at the end of the experiment for all treatments, and then data will transfer to the Angular or Arcsine Transformation.

2. **Morphological characteristics of seedling (Phenotypic evaluation)**

such as Stem length (cm), Stem diameter (mm), Number of leave per Transplant, Largest leaflets and leaf area (cm^2). Root Length (cm), Root Diameter (mm), and dry Weight of Root System (g). The largest leaf of the transplant will be select and calculat via (ImageJ 1.52a) program according to [52].

3. **Chlorophyll Content in Seedlings Leaf**

Chlorophyll a (Ch a), Chlorophyll b (Ch b), Total Chlorophyll ab (Ch ab): The chlorophyll quantity was measured via using a spectrophotometer tool, through taking leaves from each transplant in fresh weight of 0.5 g after removing the middle vein of the leaf. The leaves were later cut into small pieces and put in flasks of 50ml capacity which include 30 ml of the absolute ethanol alcohol and then they were kept in darkness for 24 h. The operation of extraction was repeated more than three times to guarantee the chlorophyll extraction completely. after that; the final volume reached 90ml and the volume completed to 100ml [30]. The absorption of the solution was measured in

ISSN 2072-3857

two wavelengths (665 nm and 649 nm) by using Spectrophotometer.

4. Stomatal Length, Stomatal Width, and Stomatal:

According to the [32] stomata measurements, the abaxial leaf surface of mature leaves about 0.1 cm^2 was coated with transparent nail polish, and then the lower epidermis was peeled off and put on a glass microscope slide for stomata analyses. 15 stomatal apparatus measured for each leaf, indicted the stomatal apparatus length, width, number of Stomata per mm^2 were measured by using the Olympus microscope connected to DinoXcope digital camera.

-Data Statistics and Analysis

Data analysis was performed by SAS software. Data analysis between the different sites, and water treatment were tested by one-way ANOVA, and the significance of differences was obtained by Duncan's test. The graphs in the text were created using origin pro.

Result and discussion:

- **Effect of drought stress on variability of seedling characters**

The result of statistical analysis including (ranges, coefficient of variability and means ± standard deviation) presented in Table 2, indicates there were important and wide range of variation in all traits of seedlings of *M. azedarach*. High variability within seedlings characters, it is a clear index of the direct influence of different levels of drought-induced stress over the two consecutive growing seasons treatments on seedling survival and characteristics in this investigation. We observed reductions of values for all traits related to growth (morphologically, physiologically, and biomass index) under drought stress conditions as compared to normal conditions. The effect of drought-induced stress on most traits between the two drought stress treatments was also significant (moderate drought vs severe drought, $P<0.01$ for all trait). The results indicate that multi-trait genetic selection for drought stress response at a small age of seedlings could represent a promising approach to increase resilience to drought. The variation between seed and seedlings characters among sites in this study it is a good opportunity and importance for selection and breeding among Chinaberry tree seedling populations. This genetic variability and field evaluation may give greater insight into the success of improvement of this species to drought resistance in the futures.

According to [56] suggested the planting seedlings with improved resistance to drought may offer a solution to increase survival and improve the resilience of forest plantations because seedlings are particularly vulnerable to drought during many years after plantation, given that they lack a well-developed root system. The conditions set up by [62] in order to be able to predict survival and growth of the seedling in the field. The high significant differences among families for all of the growth parameters surveyed, in addition to the significant interaction effect of the population source by family have implied that certain families from different population will produce seedlings of superior growth combination characteristics.

ISSN 2072-3857

Table 2: Show the variation in seed and seedlings of *Melia azedarach* L. characters among treated and untreated seeds with colchicine.

Seed and Seedling Characters	Mean	Maximum	Minimum	Standard Deviation (SD.V.)	Coefficient of Variation (C.V.)
Seed Germination (%)	81.508	100.000	16.667	24.322	29.840
Seedling Survival (%)	43.518	100.000	0.000	29.129	66.934
Stem Length (cm)	28.102	49.667	3.111	8.142	28.972
Stem Diameter (mm)	2.590	3.943	1.510	0.511	19.715
Number of Leaves / Transplant	11.045	21.333	5.333	3.214	29.094
Shoot Green Weight (gm)	2.360	5.165	0.378	1.124	47.617
Largest Leaf Area (cm^2)	41.169	88.940	5.130	17.368	42.188
Root length (cm)	31.868	51.667	16.000	6.889	21.618
Root diameter (mm)	4.285	7.277	1.520	1.133	26.442
Number of secondary roots	5.496	13.333	1.667	2.558	46.548
Root System Green Weight (g)	4.747	11.303	0.690	2.334	49.173
Root System Dry Weight (g)	1.687	4.580	0.130	0.972	57.587
Chlorophyll (a) Content (mg/g)	8.225	19.648	3.476	2.762	33.573
Chlorophyll (b) Content (mg/g)	5.351	10.280	1.264	1.613	30.151
Total Chlorophyll Content (mg/g)	13.576	24.155	6.214	3.448	25.399
Stomata Length (µm)	20.087	37.785	11.873	4.055	20.185
Stomata Width (µm)	13.522	24.733	7.503	2.578	19.063
Number of stomata / mm^2	295.007	515.460	85.910	88.210	29.901

- Effect of drought stress of seedling survival (%)

The overall mean of seed germination among the sites was 81.582 ± 24.322, with minimum and maximum values of 16.66 and 100.0 % respectively. Also, the Coefficient of Variation of the entire sample (29.840%) was not low (Table 2). In the other side, Results showed that survival percentage of *Melia azedarach* L**.** seedlings was high significantly differed (P > 0.01) under different level of drought. the total mean of seedling survival observed to be 43.52± 29.13 % with the range values varied from 0.00 and 100.0 %, high variability was found in percentage seedling survival between the sites (C.V.%= 66.93%). An average mortality of 56.58% is considered to be high for M. *azedarach* species in this experiment, these indicated that the seedlings was greatly affected and inhibited by artificially drought treatment (Table 2). The analysis of variance (Table 3) validated there were high significant effects at 0.01(probability level) of different drought stress treatment on seedling survival rate among the sites. The results showed the seedling survival ration declined under mild drought, moderate drought, and severe drought, comparison to control drought. Drought severely impacts forest seedlings, causing high mortality, especially in the first year, due to shallow roots and competition, but effects vary by species and light conditions. Species with "conservative" traits (like high leaf mass) or deciduous habits often fare better, while high-light conditions increase growth but also drought-induced mortality, and overall survival depends on species-specific adaptations, functional traits, and microclimate. Therefore, sites of higher survival percent should be selected to ensure a greater number of desired seedlings for the purpose of regeneration.

Table (3) shown that the *Melia azedarach* Seedling survival was decreased in different level of drought in the different sites. The lowest of survival percentage of seedling was noted in moderate drought, and severe drought. The decreases of exposure time of water were negatively affected on the percentage of surviving seedlings compared to the control's drought. The great ratio of surviving of seedling was observed in the Sarsink location by the value (60.28%) which differ significantly with other sites, while the lower rate of seedling survived was found in the Brifka site in percentage value (30.28%). Young seedlings may be more susceptible to drought than older seedlings, which have had more time to develop larger root systems, buffering them against drought. Many studies have shown that mortality is very high for young seedlings and declines with age [9,12,22]. [17] found the effects of drought on mortality and wilting behavior varied greatly among species, so that relative survival in the dry treatment ranged from 0% to about 100% of that in the irrigated treatment. Drought stress was the main factor in mortality, causing about 90% (median) of the total mortality observed in the dry treatment.

Table 3. The survival ratio of seedling of *Melia azedarach* among different sites under different drought stress.

Characters	Seedling Survival (%)				Sites	Relative Declined (1%)	Relative Declined (2%)	Relative Declined (3%)
Drought treatment	0	1	2	3				
Domis	60.00 ab	33.33 cde	34.44 cde	32.22 de	40.00 b	44.45	42.60	46.30
Sarsink	70.00 a	65.56 a	48.89 bc	56.67 ab	60.28 a	60.34	30.16	19.04
Brifka	45.56 bcd	21.11 e	30.00 de	24.44 e	30.28 c	53.67	34.15	46.36

Note: Different lowercase letters in the same line indicate a significant difference between different treatments of the same variety ($p<0.01$). 0: control treatment, 1: mild drought; 2: moderate drought; 3: severe drought.

- **Effect of drought stress on shoot growth system:**

Drought stress significantly reduces shoot growth by inhibiting cell expansion (due to water loss and reduced turgor), limiting photosynthesis and carbohydrate supply, and triggering hormonal changes that close stomata, ultimately decreasing plant height, biomass, leaf area, and overall productivity. Shoot characteristics include seedling height, ground-level diameter, shoot weight, and number of leaves per seedling. These features are normally used for seedling grading, and evaluation in the field. Much work should be done to define *Melia azedarach* seedlings of best performance. With the deepening of the drought stress and the extension of the stress time, the seedling growth traits of the three sites declined to varying degrees (Table 2). The average length of shoot was 28.10 cm with the maximum 49.67 cm and minimum 3.11cm. The shoot diameter is highly variable. The average value is found to be (2.59mm) with range (1.51-3.94 mm). The mean number of leaves per seedlings comes to be 11.04, with the range of (5.33 – 21.33). Shoot dry weight is one of the important parameters used for

ISSN 2072-3857

assessing seedling quality and performance in the field. Our data clearly indicate significance of these characters in evaluating seedling growth parameters. The overall mean of shoot weight (0.853 gm) is found to be variable throughout its natural range of distribution (1.99 to 0.10 gm) as indicated by C. V= (51.25%).

Results of analysis of variance in Table 4, showed that increments of stem height, stem diameter, leaf number, shoot dry weight were significantly ($P < 0.01$) affected by different drought intervals and sites, where the highest significant value of stem height increment (36.66cm), stem diameter increment (3.10 mm), leaf number increment (14.29), great weight of dry shoot obtained (1.26gm) were obtained from the seedlings watered under control (80% of field capacity), while the value of these growth parameters decreased by increasing drought stress time as the lowest increments of them were 19.51cm decreased by (46.78%), 1.81mm reduced by (41.61%), 8.58 leaves declined by (39.96%), and 0.57gm lessened by (54.76%|) respectively in the seedlings in different level of drought a specially in moderate and severe drought. Drought can have a devastating impact on plant growth [57]. Moreover, [28] concluded that seedling growth inhibits by lowering carbon domestication and allocation as well as slowing cell expansion. In the other side, [65] found that the he reduction in plant height is mainly due to reduced cell expansion, increased leaf abscission, and impaired mitosis under drought conditions. Morphological changes in plants under drought stress include a decrease in the size, area, and number of leaves. These results confirmed that *Melia azedarach* L. seedlings grew very well when they were irrigated well. However, drought stress had negative impact on the growth of the studied species. In the severe drought (20% field capacity) treatment the data showed the taller shoot (24.29cm) and extreme number of leaves (9.81 leaf) produced in seedling of the Sarsink site, while the Domis locality created wider shoot diameter (2.51mm) and heavy shoot dry weight (0.63gm). Water stress may have a negative impact on growth due to alterations in a number of physiological processes. Due to the reduction in turgor pressure, the formation of cells is thought to be one of the physiological processes that is most vulnerable to dryness [44].

Similarly to [25] the results showed that the growth in the seedling height and the ground diameter, the leaf relative water content, transpiration rate, net photosynthetic rate, stomatal conductance, and the content of chlorophyll decreased with the increasing stress levels. Also, [56] they expected, the drought-induced stress treatments led to a decrease in biomass index, radial and apical growth of seedlings. They observed that the effect was more significant for the severe drought stress treatment on seedling growth (control vs severe drought $P<0.001$) than for the moderate drought stress treatment (control vs moderate drought, $P<0.01$). [46] is found that water stress significantly reduced height and base diameter, total chlorophyll per leaf, biomass allocation of *Platanus orientalis* L. seedlings. Addition, Hamad (2023) in his on *Paulownia tomentosa* seedlings the results showed that survival percentage was not significantly differed ($P > 0.05$) under different irrigation periods, while as the increments of stem height, stem diameter and leaf number were significantly ($P < 0.05$) affected by different irrigation intervals. [6] showed that stem height and stem diameter of *Cunninghamia lanceolata* significantly reduced when the seedlings grown under low water content condition compared with well-watered condition.

ISSN 2072-3857

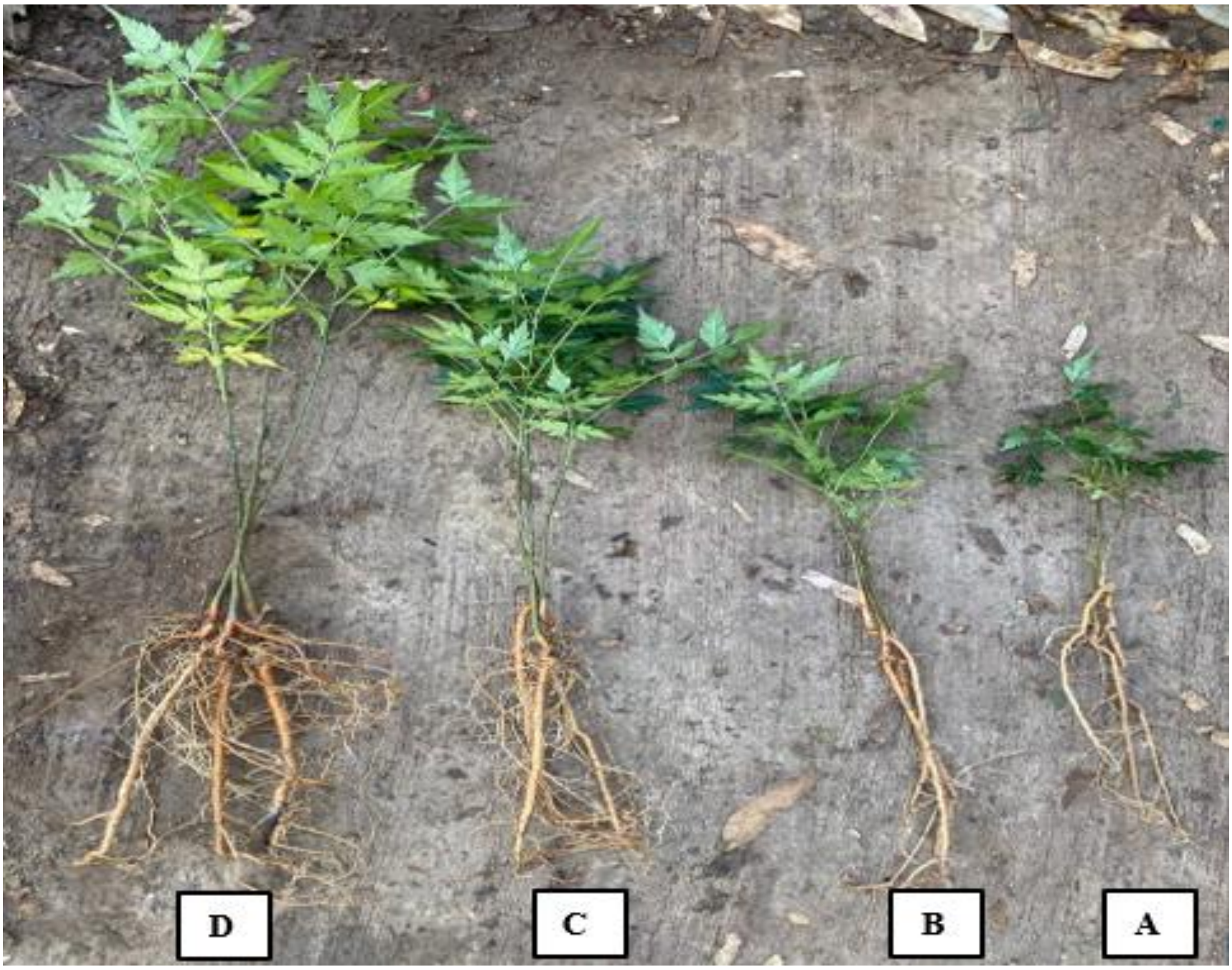

Figure 2: Effects of different drought stress levels on seedling morphology, showing variations in length, shape, and size. Treatments include well-watered control (D), mild drought stress (C), moderate drought stress (B), and severe drought stress (A).

ISSN 2072-3857

Table 4. The shoot characters of seedling of *Melia azedarach* among different sites under different drought stress.

Seedling Character	Shoot Length (cm)				Sites	Shoot Diameter (mm)				Sites
Sites	0	1	2	3		0	1	2	3	
Domis	36.66 a	26.87 bc	26.82 bc	23.34 cde	28.42 a	3.10 a	2.70 bc	2.51 cd	2.51 cd	2.70 a
Sarsink	35.74 a	29.62 b	25.23 bcd	24.29 cd	28.72 a	2.85 ab	2.54 bcd	2.28 d	2.32 d	2.48 b
Brifka	35.12 a	19.51 e	20.53 de	22.24 cde	24.35 b	3.10 a	1.81 e	1.93 e	2.40 cd	2.31 c
Seedling Character	**Number of Leaves / Transplant**				**Sites**	**Shoot Dry Weight (gm)**				**Sites**
Sites	**0**	**1**	**2**	**3**		**0**	**1**	**2**	**3**	
Domis	12.96 ab	10.38 cd	10.57 cd	8.68 d	10.64 ab	1.25 a	0.96 b	0.90 bc	0.63 d	0.937 a
Sarsink	14.29 a	11.68 bc	10.33 cd	9.81 cd	11.53 a	1.12 ab	0.88 bc	0.68 cd	0.61 d	0.821 ab
Brifka	12.49 ab	8.77 d	8.95 d	9.22 d	9.86 b	1.26 a	0.57 d	0.59 d	0.49 d	0.729 b

-Effect of drought stress on root system:

Drought stress significantly alters seedling root systems, generally reducing overall growth (length, surface area, volume) while increasing the root-to-shoot ratio as roots thicken and grow deeper to find water, enhancing water absorption, though severe stress damages root structures and inhibits growth. The values of root length vary from 16.0 – 51.67 cm, with the overall mean of 31.86 cm (Table 2). This high variability and differences in root length play an important role in phenotypic plasticity of the species. Variations in root length can be used as an indicator for planting this species and tree development under drought stress condition [46]. While, the root diameter of Melia azedarach seedling is found to be as important as shoot diameter if not more in determining growth of the plant. The mean value of root diameter is observed to be 4.28 mm. The range varies from 1.52 to 7.27 mm. Observed the number of secondary roots is found to be highly variable throughout the range of distribution in each of the three sites under different drought stress (C.V. = 46.54%). The values vary from 1.66 to 13.33 with an overall mean of 5.49 roots. Also, high variability (C.V.= 57.58%), was indicated by the descriptive statistics was recorded for underground biomass (root dry weight). The average root dry weight reaches as much as 1.68gm with range of 0.13 – 4.58gm. Root weight is often used as a subjective measure of seedling quality [62].

Results of drought treatments in Table (5) exposed that all underground biomass parameters (root length, root diameter, number of roots, and root dry weight) of seedlings of the three sites were significantly (5% and 1% of the probability) impacted by drought treatments. This root characters were highly decreased when treated with mild drought, moderate drought, and severe drought compere to control treatment. The longer root (33.90cm) and higher root diameter (3.10mm) when seedling watered well was found in the Domis site and in contrary, the shorter root (23.83cm) and thinner root diameter (2.32mm) were observed in the severe drought treatment in the seedlings belong to the Brifka and Sarsink locality respectively. In the other sides, the seedlings were irrigated well (control treatment) produced seedling with high numbers of secondary roots (14.29 root) and heavy root weight (1.26gm) in the Sarsink and Brifka site respectively. Seedling with a smaller number of roots (8.77 root) and Lightweight, dry root they formed when seedlings treated with mild and severe drought in the Brifka location. Improved dry mass allocation to roots may be resulted from a drought-induced reduction in the sink strength of the aboveground plant tissues, making more assimilates available for root growth [53]. An increase in root length was greater in water-stressed seedlings as compare to well-watered seedlings, demonstrating that decreasing water availability had less effect on root growth than on leaf growth [26]. On the contrary, [25] reported that the morphological characters in plants changes under drought stress include a decrease in the size, area, and number of leaves and an increase in the length of roots and shoots. [45] he found while under drought condition with 30% of (WHC) a significant increase was found for both root length and root to shoot ratio compare to other treatments. An increase in root length was greater in water-stressed seedlings as compare to well-watered seedlings, demonstrating that decreasing water availability had less effect on root growth than on leaf growth [26].

Table 5. The root characters of seedling of *Melia azedarach* among different sites under different drought stress.

Character	Root Length (cm)				Sites	Root Diameter (mm)				Sites
Drought level	0	1	2	3		0	1	2	3	
Domis	33.90 a	33.54 ab	34.57 a	29.69 bc	32.92 a	5.83 a	4.03 cd	4.04 cd	3.77 cde	4.41 a
Sarsink	33.71 ab	33.04 ab	32.47 ab	29.70 bc	32.23 a	5.10 ab	4.09 c	3.42 de	3.49 cde	4.02 b
Brifka	34.46 a	24.62 d	26.13 cd	23.83 d	27.26 b	5.66 ab	3.34 e	3.40 de	3.55 cde	3.98 b
Character	Number of Secondary Root/Transplant				Sites	Root Dry Weight (gm)				Sites
Drought level	0	1	2	3		0	1	2	3	
Domis	8.03 a	5.36 bc	5.09 bcd	3.92 cd	5.60 a	2.67 a	1.57 b	1.63 b	1.26 bc	1.78 a
Sarsink	8.03 a	5.67 b	4.46 bcd	4.06 cd	5.55 a	2.31 a	1.62 b	1.29 bc	1.04 bc	1.56 a
Brifka	8.34 a	3.56 d	3.50 d	3.75 d	4.78 b	2.70 a	1.29 bc	1.27 bc	0.93 c	1.55 a

- **Effect of drought stress on chlorophyll content:**

Under greenhouse conditions, noticed the leaves of the seedlings induced to different drought stress contained significantly fewer concentrations of chlorophylls and become yellowing leaves than the seedlings irrigated well. The changes in

ISSN 2072-3857

the chlorophyll content were distinct in each of the three locations. As the stress degree and duration increased, the chlorophyll content of the site's studies dropped or increased initially and subsequently decreased. Data of the descriptive statistics given in (Tables 2) indicates a high amount of variability exhibited by chlorophyll content within the sites and different drought time. The analysis of variance and Test of means of sites using Duncan's test (Table-6) reveals significant differences mostly between control treatment and severe drought treatment in the variable sites. Chlorophyll is the most important group of photosynthetic pigments responsible for light absorption and is found in the thylakoids of the chloroplasts. Both the composition and concentration of chlorophyll [chlorophyll-a, chlorophyll-b, the chlorophyll a/b ratio, and total chlorophyll (a + b)] have a direct influence on photosynthesis and dry weight [32]. Drought stress imposed during vegetative growth significantly reduces chlorophyll content (chlorophyll a, b, and total) in plants by damaging chloroplasts, inhibiting synthesis, and increasing degradation, leading to yellowing leaves (chlorosis) and limiting photosynthesis, though tolerant varieties show less severe reductions than sensitive ones. This decrease directly impairs the plant's ability to capture light energy, affecting growth, yield, and overall plant health.

The maximum amount of chlorophyll a, b, and totally chlorophyll (ab) was observed no doubt in the seedlings when were well watered (control and mild) belongs to the Brifka site by value (10.23, 5.90 and 16.13 mg/g) respectively. And as usual the minimum value of chlorophyll (a), (b) and (ab) contain were recorded in the seedlings growing under severe drought stress by the amount value 5.06, 3.25, and 8.31 mg/g by a declined in rate 50.53%, 44.91%, and 48.48% respectively. The results in (table 6) indicted the Sarsink site among sites under severe drought treatment produced seedling had a higher amount of chlorophyll (a), (b) and total chlorophyll concentrations in the amount value (7.93. 5.40, and 13.33mg/g) respectively, it is apparent that the chlorophyll content of the Brifka position was the lowest in each period and under each treatment. The chlorophyll content is another critical component affected by drought stress and is plant species dependent. Therefore, the characterization of plant species that are resistant to chlorophyll degradation under drought stress is the target of plant breeders to improve abiotic resistance [29].

[55] described that the chlorophyll plays an indispensable role in the absorption, transmission, and transformation of light energy in photosynthesis. Plants accumulate a considerable quantity of reactive oxygen species in their bodies during drought stress, leading to changes in membrane structure and consequently altering chlorophyll concentration. They mentioned that decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species. [7] revealed that the drought stress causes photosynthetic inhibition, which happens as a result of stomatal closure, chlorophyll degradation, and damage to the photochemical equipment, leading to a reduction in the intercellular CO_2 concentration (Ci). According to [14,21,25] the value of chlorophyll A under sufficient water stress was higher than that under moderate and severe drought stress. Moreover, [38] detected that the under-drought stress, the thylakoid membranes in the chloroplast are damaged, adversely affecting the synthesis of chlorophyll. Drought stress caused a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content in all sunflower varieties investigated [34]. A study

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conducted with Norway spruce (*P. abies* L. Karst) seedlings under water stress showed a slight reduction in photosynthetic activity as a result of degradation in chlorophyll-a and chlorophyll-b content in the needles of water-stressed spruce seedlings [49]. In oil palm (*Elaeis guineensis*, tenera), the ratio of chlorophyll a/b and total chlorophyll (a + b) was significantly decreased under water stress [4].

Table 6. The chlorophyll content of the seedling leaves of *Melia azedarach* among different sites under different drought stress.

Character	Chlorophyll (A)				Sites	Chlorophyll (B)				Sites
Drought level	0	1	2	3		0	1	2	3	
Domis	9.74 a	7.89 bcd	7.05 cd	6.55 de	7.81 a	5.27 a	5.63 a	5.10 ab	4.88 ab	5.22 ab
Sarsink	8.71 abc	8.86 ab	7.70 bcd	7.93 bcd	8.39 a	5.61 a	5.65 a	5.68 a	5.40	5.58 a
Brifka	10.23 a	9.04 ab	6.66 de	5.06 e	7.74 a	5.90 a	5.64 a	4.06 bc	3.25 c	4.71 b

Character	Chlorophyll (AB)				Sites
Drought level	0	1	2	3	
Domis	15.01 ab	13.52 bcd	12.15 cde	11.43 de	13.03 ab
Sarsink	14.32 abc	14.52 ab	13.38 bcd	13.33 bcd	13.88 a
Brifka	16.13 a	14.68 ab	10.71 e	8.31 f	12.46 b

- Effect of drought stress on leaf area, stomata size and density:

A. Leaf area:

Drought is one of the most severe environmental stresses affecting plant productivity. Different levels of drought stress significantly reduce leaf area by inhibiting cell expansion, increasing leaf shedding (senescence), and causing wilting, as plants try to conserve water; mild stress might just slow growth, while severe stress dramatically cuts leaf size and number, impacting photosynthesis [66].

The changes were observed in the seedlings during their life under greenhouse by the harmful effects of drought stress are a gradual decline in the leaves' numbers and area, leaf wilting, cell elongation in the leaves, and enhanced senescence of leaves which ultimately cause a reduction in the total plant height. The maximum and minimum value of leaf area was observed to be 88.94 and 5.13 cm^2 respectively, with an overall mean of 41.17± 17.37cm^2. The descriptive statistics given in (Tables 2) indicates a high amount of variability (C.V= 42.188%) exhibited by leaf area within the sites under different period of drought tension. Significant difference (at P <0.01) were found in leaf area trait between sites and different drought treatments. Larger leaf was observed in the seedlings growing under control treatment (80% field capacity), in contrary the smallest leaf was in the severe drought stress as shown in table (7) and figure (3). Brifka areas most affected by the newly created drought among other sites, which produced bigger leaf when it is seedlings irrigated well or under control treatment by the area in value (56.18 cm^2) then decreased significantly in mild drought, moderate drought, and severe drought stress to the value 24.98, 34.70, and 25.70 cm^2 by decking in the ratio 55.53%, 38.23%, and 54.25% respectively. [63] found the heavy drought decreased leaf length, leaf width, leaf area, leaf shape index, fresh weight, saturated fresh weight, dry weight and specific leaf area significantly, while also increasing leaf density significantly. Also, [46] found the leaf area and total chlorophyll content decreased significantly because of drought treatments when the highest levels were obtained in the control (D0).

While the Domis locality of its seedlings was less affected by the drought treatment, it produced the largest leaf area when its seedlings grew under harsh drought conditions. This means that *M. azedarach* retains high water absorption ability and decreased leaf area to reduce water transpiration to guarantee the necessary water content, which is a key factor for the survival and growth of plants. [23] described the since leaf morphology is important in plant growth and development, it significantly affects plant yield. The symptoms observed in the leaves in response to drought are important in the anatomical adaptation of the cultivars and are used in cultivar selection. [47] concluded that the first response of plants to drought is the change in the osmotic pressure of the cell. As the turgor pressure decreases, the cell water potential decreases, and cell expansion is limited, this is reflected in the morphological characteristics of the plant, and the first affected organ is the leaf, which is responsible for photosynthesis. Several previous studies reported that the reduction in the leaf area, change in the turgor, and canopy temperature, under drought, affect plant growth through a decline in the rate of photosynthesis [58].

Figure3: Differences in leaf size and leaf area among three sites: (a) Domis, (b) Sarsink, and (c) Brifka, under drought stress conditions.

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B. Stomata size and density:

Drought stress significantly alters stomata in forest trees, generally leading to smaller stomata and decreased stomatal density to conserve water, though responses vary by species and drought severity; plants adapt by reducing pore size for faster closure and decreasing numbers to maximize limited water use efficiency, impacting gas exchange and survival. Some studies show drought decreases stomatal size and increase density, while other research indicates drought can reduce overall stomatal number in severe cases, highlighting complex, species-specific responses. [39] investigated that Stomata, small pores primarily located on the abaxial surface of leaves of many plant species, play a fundamental role in plant gas exchange, balancing CO_2 uptake and water loss to optimize photosynthesis and water use efficiency (WUE). As shown in Table (2), there were high variation in stomata length, width, and stomata numbers in the abaxial surface of the leaves, results of great significant effect of different drought stress and sites on *M. azedarach* seedlings. Observed the stomata were bigger and greater number or density in control treatment, than those seedlings growing under drought stress which have smaller stomata and lesser number of stomata in it is leaves. Drought induces stomatal closure to avoid water loss through transpiration to stabilize the water status of the shoot [13].

Table (2) illustration the averages and range of the stomata length, width and density of plants were 20.08 (11.87 - 37.78), 13.52 (7.50 – 24.73µm), 295.01 (88.21-515.46 stomata/ mm^2) respectively. The analysis of variance and Duncan's test of means in table (7), investigated also there were high significant effect ($P< 0.01$) of sites and irrigation time and amount on stomata size and density in leaf area. With the deepening of the drought stress, the transpiration rate, net photosynthetic rate, and stomatal conductance of the eight provenances showed a general trend of decline, and the drop in the net photosynthetic rate was mainly due to stomatal and non-stomatal factors [25].

The data of this experiment indicated that the high variation in stomata dimension and numbers were between control treatment (80% of field capacity) and severe drought stress (20% of field capacity) between different sites. In control treatment observed that the longer stomata were in Domis site by the value (23.33µm), the wider stomata and higher number of stomata in unit area in the Brifka locality with value (15.40 µm), and (388.31 stomata/mm^2) respectively. In the other side, in severe drought stress the smallest stomata was created by the leaves of seedlings of the Brifka site to be more tolerance the drought by the length (16.79 µm) and diameter (10.35 µm), while the minimum numbers of smallest stomata size in density (222.79 cells/mm^2) were in leave of seedlings from the Doims sites. The maximum reduction in the number of stomata resulted in greater water retention in the plant due to less transpiration. The opening and number of stomata decreased with the increase in the drought. These characteristics of the stomata such as size, density, and conductivity are very important to reduce the loss of water and the flow of CO_2 in the cells of plant leaves that take part in photosynthesis [60,67]. Whereas, [66] informed that the plants have extensive mechanisms for optimizing gas exchange and preservation of water, which is highlighted by the extraordinary plasticity of stomata in response to drought. This illustrates their capacity to grow even in difficult circumstances. [31] stated that there are two processes plants can resist drought condition. Whole-plant responses to water stress range from stomata closure to increased root/shoot ratio, leaf area reduction. Such that mechanisms either increase water availability or reduce water loss, in that way increasing plant water-use efficiency but decreasing biomass productivity. Drought generally triggers stomatal closure, but also leads to smaller stomatal pores (reduced pore length/width), which directly reduces water loss through transpiration. Drought stress influences stomata in forest trees by often increasing stomatal density and sometimes decreasing stomatal size, especially in developing leaves, to conserve water by reducing surface area for transpiration, though responses vary with drought severity (moderate vs. severe), species, and leaf age, sometimes leading to initial density increases followed by later decreases [8]. [3,23] reported that the drought is the condition of prolonged dryness, which adversely affects the anatomical, morpho-physiological and other fundamental processes in the plant leading to limited plant growth and productivity. It also affects the location and density of the stomata on the leaf surface. [61] they concluded that the cell walls and cuticle thickness of the lamina epidermal cells of the genotype, which is moderately drought resistant, also increased. In moderately drought-tolerant sugarcane, with drought, stomata size increased in leaves, while a decrease was observed in the highly drought-tolerant genotype. The cell walls and cuticle thickness of the lamina epidermal cells of the genotype, which is moderately drought resistant, also increased. In moderately drought-tolerant sugarcane, with drought, stomata size increased in leaves, while a decrease was observed in the highly drought-tolerant genotype [59]. Drought stress triggers significant leaf changes, including smaller size, thicker cuticles, reduced stomata/veins, and rolled leaves (bulliform cells) to conserve water, impacting photosynthesis and yield [10].

Table 7. The leaf area and stomata characters of the seedling leaves of *Melia azedarach* among different sites under different drought stress.

Character	Leaf Area (cm^3)				Sites	Stomata Length (µm)				Sites
Drought level	0	1	2	3		0	1	2	3	
Domis	56.06 a	35.79 bcd	45.63 ab	41.14 bc	44.65 a	23.33 a	20.47 bcd	19.66 cd	18.92 cde	20.59 a
Sarsink	55.20 a	36.17 bcd	32.84 cd	27.68 d	37.97 b	21.44 abc	20.03 cd	19.92 cd	18.03 de	19.85 a
Brifka	56.18 a	24.98 d	34.70 bcd	25.70 d	35.39 b	22.93 ab	14.97 f	16.52 ef	16.79 ef	17.80 b
Character	**Stomata Width (µm)**				**Sites**	**Stomata Density (Stomata No./mm^2)**				**Sites**
Drought level	**0**	**1**	**2**	**3**		**0**	**1**	**2**	**3**	
Domis	15.27 ab	14.14 ab	13.63 abcd	12.19 de	13.81 a	343.64 ab	284.08 cd	258.88 cde	222.79 e	277.35 a
Sarsink	13.53 bcd	13.93 abcd	13.49 bcd	13.16 cd	13.52 a	361.39 a	300.11 bc	279.49 cde	230.24 de	292.81 a
Brifka	15.40 a	10.81 ef	10.96 ef	10.35 f	11.88 b	388.31 a	269.18 cde	249.14 cde	234.82 de	285.36 a

ISSN 2072-3857

- **Conclusion:**

Chinaberry tree (Melia azedarach L.) It is an Asiatic multipurpose tree, of worldwide cultivation, mainly for its ornamental beauty and landscape value. Is a fast-growing, it is deciduous and perennial tree of the Meliaceae. Introduced into Kurdistan region and planted as an ornamental tree at the nurseries, parks and streets. An evaluation of drought resistance was performed for three sites of *Melia azedarach* from the South to North of Duhok province. The data from present study showed that an increase drought stress was significantly influenced morphological and physiological parameter of *M. azedarach* L. Water stress condition is a conventional method to develop performance of this species seedling in water stress areas. The results showed ddifferent sites respond to drought resistance. The morphological and physiological mechanism of the drought stress response in the seedlings of three different sites was preliminarily identified from the growth traits and physiological responses in this study, and the drought resistance ability of the three locations was ranked. In general, the seedling survival percentage, shoot and root length, shoot and root diameter, number of leaf and roots, biomass weight of shoot and root system, leaf area, chlorophyll content, stomata size and density of seedlings of different sites growing under the moderate and severity of the drought stress where it decreased by varying percentages compared to the seedlings charades when irrigated well.

ISSN 2072-3857

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