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Research Article
Physiological Responses of Moringa (*Moringa stenopetala* L.) Seedlings to Drought Stress under Greenhouse Conditions, Southern Ethiopia

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Abstract
Background and Objective: *Moringa stenopetala* L. is a plant called miracle plant due to its adaptability and versatility in use. However, in spite of its marvelous importance, the plant has got little attention for research based on stress physiology. Therefore, in this study, pot experiment was conducted to investigate the chlorophylls concentration, Leaf gas exchanges, stomata traits and leaf water status responses of *Moringa stenopetala* L. accessions seedlings to drought stress under greenhouse condition. **Materials and Methods:** The experiment was consisted of 3 accessions (Konso, Arba Minch Zuria and Humbo) and 4 drought stresses (daily, 5 days interval, 10 days interval and 15 days interval watering) assigned in completely randomized design with 3 replications. **Results:** The accession "Arbaminch Zuria" was higher in all parameters except stomata number. Regarding to water stress, the experiment shown that, increased water stress reduced all parameters except stomata number. Additionally, photosynthetic rate, transpiration rate, stomatal conductance and leaf relative water content were significantly influenced due to the interaction effect of accession and drought stress. Accession "Arbaminch Zuria" grown under daily watering was higher in all traits those indicated significant due to interaction. **Conclusion:** Therefore, Arbaminch Zuria can be taken as a better accession both under stressed and unstressed conditions for production of moringa seedling. Furthermore, study is still needed on more indigenous and exotic accession under different drought stress for longer periods of time as a perennial crop.

Key words: Photosynthesis, moringa, chlorophyll, drought stress, seedlings

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Under water deficit conditions, Moringa could be affected and reduced its potential growth, development and functions by a number of biotic and abiotic factors during seedling stage. While among numerous factors, water does an essential component for the plants life owes to the crucial role that water plays in physiological processes[1]. The properties of water make it essential for many functions in plants such as: a constituent of most parts of plants, a solvent providing a good medium for biochemical reactions[2]. However, even though moringa is drought tolerant plant, during prolonged drought stress, there is a reduction in water content, diminished leaf water potential and turgor loss, closure of stomata, inhibit photosynthesis, lead to dehydration, impair metabolism processes, decrease/and inhibit cell expansion, enlargement, growth and development of plants especially during early stage[3-5]. But, Amoatey *et al*.[6] reported that moringa is the plant with a wide variability. Moreover, different accessions collected from different area could probably have variations in genetic potential and/or the inherent variations across the collection environments to water deficit imposed by drought stress[7-9]. As a result, proposing research to comparing accessions under water deficit has advocated, being familiar with the extent of the water deficit tolerance and evaluate accessions through their physiological traits. Therefore, giving attention to reveals focusing on the selection of drought tolerant accessions to water deficit are playing crucial role to explore breeding objectives, particularly, considering shortage of water. Physiological traits such as photosynthetic pigments (a, b and total chlorophylls), photosynthetic rate, transpiration, stomatal conductance and stomata traits like aperture, leaf water related like relative water content percentage and water use efficiency are of the most important physiological traits. Photosynthetic pigments are important to plants mainly for harvesting light[10]. Gas exchange of the plants (photosynthetic rate, transpiration rate and stomatal conductance) is the principal plant process responsible for plant biomass production and for plant adaptation to adverse environment[11]. While stomatal closure results in reduced CO_2 movement for carboxylation within the chloroplast[10]. Several studies have been focused on physiology of other moringa species accessions under different watering interval[7,9,12]. But in Ethiopia, despite of the versatility use of moringa, research on physiological response of *M. stenepetala* accessions seedling under different drought stress as not been conducted and documented[13,14]. The main objective of this work was, to study physiological responses of *Moringa stenopetala* seedlings to drought stress.

MATERIALS AND METHODS

Description of the study area: The present experiment was carried out under greenhouse condition during October, 2018 to January, 2018 at Hawassa University, College of Agriculture campus. The area is located in the Sidama zone, Southern Nations, Nationalities, Peoples Region that is 275 km far from Addis Ababa, capital city of Ethiopia. The site lies at 7°05 N latitude, 38°47 E longitude with average altitude of 1750 m above sea level. According to last 11 years (2007-2018) data obtained from the weather station, the average annual rain fall and temperature (maximum and minimum) of the area is 971.9 mm and 27.9 and 13.8°C, respectively (NMA)[15].

Experimental materials: Three *Moringa stenopetala* accessions seed was supplied by Arbaminch Agricultural Research Center (AARC), Ethiopia. Brief description of the accessions is presented under Table 1.

Experimental procedures: The top soil up to 20 cm depth was collected from Hawassa University, College of Agriculture, Plant and Horticultural sciences research site, Ethiopia. Then after, compost and river sand was collected and filled with 3:2:1(ratio of top soil, compost and river sand) in the perforated black polyethylene tube with 22×16 cm length and width size, respectively as suggested by Ede *et al*.[16]. About 1 g of the experimental media was sampled and analyzed according to method described by Van Reeuwijk[17], for most important physical and chemical properties (except media moisture content at field capacity) in Hawassa University, College of Agriculture, Soil sciences laboratory, Ethiopia. Soil moisture content at field capacity was determined using pressure plate apparatus[18] at Addis Ababa, national soil service laboratory, Ethiopia (Table 2). Then, the perforated black polyethylene tubes filled with media was arranged under greenhouse. The amount of water required applying in the perforated black polythene tubes to field capacity was calculated as:

$$\text{Amount of water to be applied} = (\theta_{FC}-\theta_{AMC})\times\text{Depth of the perforated black polythene tubes (cm)}\times\text{Perforated black polythene tubes area (cm}^2)$$

Table 1: Description of *Moringa stenopetala* accessions used for the current experiment

Accession code	Origin of accession	Growing altitude	Sources
Konso	Konso	1320 masl	AARC
Arbaminch Zuria	Arbaminch Zuria	1222 masl	AARC
Humbo	Humbo	1432 masl	AARC

Table 2: Physical and chemical properties of the present experimental media

Properties	Determined values
Sand (%)	84.68
Silt (%)	8.00
Clay (%)	7.32
Soil textural class	Sandy loam
pH	7.30
Organic matter	9.076
Organic carbon	5.265
Total nitrogen	0.4538
Bulk density (g cm^{-2})	1.02
Electrical conductivity (mS cm^{-1})	0.52
Moisture content at FC (v/v %)	31.90

where, θ_{FC} is the volumetric moisture content at FC and θ_{AMC} is the actual volumetric moisture content.

Then, 2 seeds of each *Moringa stenopetala* accessions were sown during October/09/2018 in 2 cm depth/each perforated black polyethylene tubes and then thinned after germination. All Experimental units were watered well to field capacity up to October/29/2018 (until the commencements of the drought stress treatments made sure that, the seedlings established well. Thereafter, during October/30/2018, the seedlings were subjected to drought stress treatment and the seedlings were maintained in those drought stresses for 2 month while subsequent measurement and observations were made on their morphological and physiological attributes.

Experimental treatments and design: The experiment was laid out in a factorial scheme of 4×3, 4 drought stress [daily (Control), 5 day interval, 10 days interval and 15 days interval] and 3 *Moringa stenopetala* accessions (Konso, Arbaminch Zuria and Humbo). Totally 12 treatments arranged in completely randomized design with 3 replications (36 experimental units). Each experimental unit was consisted of 15 perforated black polythene tubes.

Data collection and measurement: At 2 month after treatments applied, the data of physiological parameter's such as stomata number, length and width, chlorophyll concentration (chl a, chl b and total chl), relative water content percentage, gas exchange parameter's (Photosynthesis, transpiration rate and stomata conductance) and instantaneous water use efficiency from the third leaves of the 3 seedlings were recorded and computed as stated below:

- **Stomata number, length and width:** Stomata number, length and width were counted and measured following the procedure of Torre *et al.*[19]. Abaxial side of the leaf surface were covered by thin layer of clear nail polish and waited for 10 min until the nail polish dried to capture the epidermal imprint of the leaves, thereafter, a thin layer covering a surface on the leaves were peeled off using a clear tape and attached on the microscope slide. Then, the imprint were mounted on automatic upright Leica microscope DM5000 with 40×magnification lens fixed with digital Leica DFC425/DFC425C image processing camera (Germany) connected with LAS version 4.8 application
- **Determination of chlorophyll concentration (µg mL^{-1}) (chl a, chl b and total chl):** Chlorophylls are pigments which are used to harvest light energy that contribute for the photosynthesis which is very important for carbon assimilation. Thus, in the present experiment, it was determined according to Porra *et al.*[20]. In the laboratory dark room (to secure disintegration of chlorophylls' by light), circular leaf discs (200 mm^2 surface) were cut out by using 16 mm diameter cork disc cutter. Then after, immediately, the leaf discs were grinded with 2 mL of cold methanol (99%) in mortal and pestle, the homogenate, combined with a further 3 washings of the pestle and mortal (each of 1.5 mL) with cold methanol (99%). Thereafter, the mixture was homogenized and centrifuged at 2500 revolution per minute (rpm) for 10 min. After filtering, the supernatant was decanted into 2 mL cuvette and absorbance of the centrifuged extracts was recorded at 652.0 and 665.2 nm (as this was a simultaneous extraction of chlorophyll) using spectrophotometer of 6300 (Jenway), made with by Varian Techtron Pty limited, Australia. Finally, Chlorophyll a, chlorophyll b and total chlorophyll was computed with using equation recommended by Porra *et al.*[20]:

$$\text{Chl a} = 16.29\ A_{665} - 8.54\ A_{652}$$

$$\text{Chl b} = 30.66\ A_{652} - 13.58\ A_{665}$$

$$\text{Chl a+b} = 22.12\ A_{652} - 2.71\ A_{665}$$

Where, chl_a is the chlorophyll a, Chl b is the chlorophyll b, chl a+b is the total chlorophylls and $A_{665.2}$ is the absorbance at wave length 652.0 nm.

- **Leaf relative water content percentage (LRWC %):** It was determined by method stated by Chandrasekar *et al.*[21]. Leaf discs (200 mm diameter) were cut out by using cork disc cutter, which was immediately weighed as fresh mass (FW) leaves, then the samples were floated on distilled water inside a closed petri dish kept at room temperature for 24 h. After removing superficially adhering water droplets by tissue paper, the turgid weight (TW) was taken. Afterwards, the leaf discs were dried at 70°C for 72 h until to have a dried weight (DW). Values of fresh, turgid and dry mass were weighed using analytical balance with 0.00001 g precision to calculate relative water content percentage as follows:

$$\text{LRWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where, RWC is the relative water content, FW is the fresh weight, DM is the dry weight and TM is the turgid weight.

- **Gas exchange and instantaneous water use efficiency:** Gas exchange parameters (Photosynthesis, transpiration and stomata conductance) were measured at 2 months after water stress treatments applied using portable infrared gas exchange analyzer LCA-4 ADC (analytical development company, Hoddesdon, England). The measurements was done of the during the time between 2:00 pm and 4:00 pm h local time with other adjustments were as follow: leaf surface temperature varied from 32.44 and 40.88°C, average photosynthetic active radiation (PAR) at the leaf surface 1300 μmol m^{-2} sec^{-1} atmospheric pressure 834 mbar and leaf chamber molar gas flow rate was between 250-253.7 μmol sec^{-1}. Then, instantaneous water use efficiency was calculated based on the data [photosynthesis (Ps) and transpiration rate (E)] provided by the portable infrared gas exchange analyzer. It was the ratio of carbon gain in photosynthesis (Ps) and loss of water transpiration (E), mathematically expressed as:

$$\text{IWUE} = \frac{\text{Ps}}{\text{E}}$$

where, IWUE is the instantaneous water use efficiency, Ps is the photosynthesis and E is the transpiration rate

Statistical analysis: Data collected from physiological traits were subjected to analysis of variance (ANOVA) using proc General Linear Model procedure[22] of SAS version 9.3. Tukey's HSD test was used to separate means at the 5% level of significance.

RESULTS

The soil Laboratory report was indicated that, the experimental media is sandy loam in texture with pH value 7.3, the organic carbon content of the media is 9.076%, consisted 5.265 and 0.4538% of organic carbon and total nitrogen respectively (Table 2).

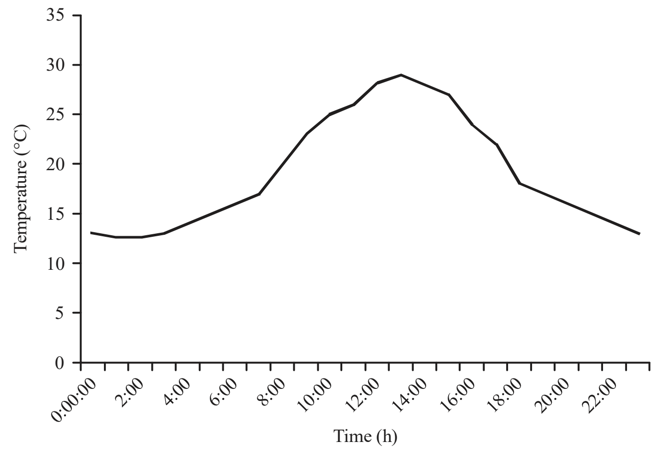

Fig. 1: Average daily greenhouse temperature during experimental period (October to January, 2018)

Description of the greenhouse and its condition: The greenhouse used for present experiment was fenced with metal wire to protect the entrance of any undesirable body in to the greenhouse. The top of the greenhouse was covered by transparent corrugated tin. The daily maximum and minimum air temperatures for the greenhouse were recorded on randomly selected 45 days using mini data logger during the experimental period from October to January, 2018. Data logger was placed inside an open bucket to avoid direct sunlight and hanged close to the seedling canopy. The data logger recorded the climate data every hour for 45 days. The average value of 45 days measurements is represented by Fig. 1.

Mean maximum and minimum air temperature in the greenhouse ranged 28.9 and 12.6°C, respectively (Fig. 1).

Stomata traits

Effect of drought stress on stomata number, length and aperture of moringa accessions seedling: Analysis of variance revealed that, number of stomata was significantly ($p>0.05$ and $p<0.01$) influenced by accessions and drought stress, respectively. While, stomata length and aperture of the *Moringa stenopetala* was significantly ($p<0.01$) influenced by both accession and drought stress. But, there was no significant ($p>0.05$) influence by interaction effect on stomata traits (Table 3).

The highest stomata number was recorded from accession "Humbo", conversely, the lowest stomata number

Table 3: Mean square values of stomata number (SN), stomata length (SL) and stomata width (SW) of *Moringa stenopetala* accessions under different drought stress

		Mean square		
Source of variation	Df	SN	SL	SW
Accession (A)	2	20.0277*	0.0190**	0.0177**
Drought stress (DS)	3	35.657**	0.0749**	0.0255**
A×DS	6	1.546^{ns}	0.0033^{ns}	0.00117^{ns}
Error	24	4.361	0.0017	0.0010
CV (%)		12.63	10.96	14.67

^{ns}Non-significant difference at p≤0.05%, *Significant difference at p≤0.05%, **Significant difference at p≤0.01% probability levels, Df: Degree of freedom, A×DS: Interaction among accessions and drought stress

Table 4: Effect of drought stress on stomata number, length and width of different *Moringa stenopetala* accessions seedling

	Parameters		
Treatments Accessions	Stomata number	Stomata length (μm)	Stomata width (μm)
Konso	16.50±0.85^{ab}	0.39±0.01^{a}	0.22±0.01^{a}
Arbaminch Zuria	15.25±0.85^{b}	0.40±0.01^{a}	0.25±0.01^{a}
Humbo	17.83±0.85^{a}	0.33±0.01^{b}	0.18±0.01^{c}
Turkey/HSD	2.12	0.04	0.03
Drought stress			
Daily (control)	13.88±0.98^{b}	0.49±0.01^{a}	0.29±0.01^{a}
5 days interval	16.44±0.98^{ab}	0.40±0.01^{b}	0.23±0.01^{b}
10 days interval	17.11±0.98^{a}	0.32±0.01^{c}	0.19±0.01^{bc}
15 days interval	18.66±0.98^{a}	0.28±0.01^{c}	0.16±0.01^{c}
Tukey/HSD	2.71	0.05	0.04
CV (%)	12.63	10.96	14.67

Means with different letter in each column of each factor are statistically significant at p-values<0.05, <0.01%

was counted on the accession "Arbaminch Zuria" (Table 4). The longest and widest stomata were observed from the accession "Arbaminch Zuria". Meanwhile, accession "Arbaminch Zuria" and "Konso" statistically similar in stomata length, but they differ each other in stomata aperture (Table 4).

Regarding to water stress, maximum number of stomata were recorded from the seedling grown under 15 days interval watering, conversely, minimum number of stomata were obtained from seedling exposed to daily watering condition (Table 4). In terms of stomata length and width as influenced by water stress, the longest and widest stomata were observed from the seedlings grown under daily watering. But, the shortest and narrowest stomata were observed on the seedling exposed to fifteen days interval watering condition (Table 4).

Correlation analysis indicated that, stomata length and width had positive significant correlation (r = 0.78***, 0.82*** and r = 0.79***, 0.82***) with photosynthetic and transpiration rate, respectively (Table 5). Chlorophyll concentration (a, b and total) had a significant positive relationship (r = 0.74***, 0.83*** and 0.79***) with photosynthetic rate, respectively (Table 5).

Chlorophylls concentration

Effect of drought stress of chlorophylls concentration (μg mL^{-1}) of *Moringa stenopetala* accessions seedling: Different accessions and drought stress used in the present study had a significant (p<0.001) influence on chlorophylls concentration (a, b and total). In contrast, interaction of the 2 main factors had not significantly (p>0.05) influenced chlorophylls concentration (a, b and total) of *Moringa stenopetala* (Table 6).

Maximum chlorophylls centration (a, b and total) were recorded from accession "Arbaminch Zuria". On the other hand, the minimum chlorophylls concentration (a, b and total) was obtained from accession "Humbo" (Table 7).

The highest chlorophyll concentration (a, b and total) was observed from the seedlings grown under daily watering condition. However, the lowest concentration of chlorophyll a was observed from the seedlings subjected to 15 days interval watering condition (Table 7).

Leaf gas exchanges traits

Impact of drought stress on gas exchange traits [photosynthetic rate (μmol m^{-2} sec^{-1}), transpiration rate (mmol m^{-2} sec^{-1}) and stomatal conductance (mol m^{-2} sec^{-1})] of the *Moringa stenopetala* accessions seedlings: The present study revealed that photosynthetic rate, transpiration rate and stomatal conductance of *Moringa stenopetala* were significantly affected by accessions, water stress and interaction of the 2 factors (Table 6).

Figure 2a explains that Accession "Arbaminch Zuria" undergone the highest photosynthetic rate when watered daily. Numerically, the lowest rate of photosynthesis was done by accession "Konso" at 15 days watering interval. The result indicated that variability among accessions for the rate of photosynthesis is narrowed down under much stressed conditions (Fig. 2a). Accession "Humbo" undergone similar rate of photosynthesis with accession "Konso" at all drought stress. However, under daily watered condition, the great difference was showed among accession "Arbaminch Zuria" and the remained accessions (Konso and Humbo).

Similar to photosynthetic rate, the highest transpiration rate was recorded from the accession "Arbaminch Zuria" when grown under daily watering condition. Numerically, the lowest rate of transpiration was observed when accession "Konso"

Table 5: Correlation coefficients among the physiological traits of *Moringa stenopetala* accessions seedlings grown under different drought stress

Parameters	SN	SL	SW	Chl a	Chl b	Ch (a+b)	RWC (%)	Ps	E	IWUE	Gs
SN	-										
SL	-0.69	-									
SW	-0.65	0.76	-								
Chl a	-0.66	0.78	0.85	-							
Chl b	-0.70	0.83	0.88	0.92	-						
Ch (a+b)	-0.69	0.81	0.88	0.98	0.97	-					
RWC (%)	-0.64	0.82	0.79	0.77	0.88	0.83	-				
Ps	-0.70	0.78	0.79	0.74	0.83	0.79	0.82	-			
E	-0.67	0.82	0.82	0.71	0.81	0.77	0.79	0.93	-		
IWUE	-0.68	0.74	0.68	0.74	0.80	0.78	0.82	0.88	0.73	-	
Gs	-0.79	0.79	0.80	0.84	0.87	0.87	0.77	0.89	0.88	0.76	-

SN: Stomata number, SL: Stomata length, SW: Stomata width, Chl a: Chlorophyll a, Chl b: Chlorophyll b, Chl (a+b): Total chlorophylls, RWC (%): Relative water content percentage, Ps: Photosynthetic rate, E: Transpiration rate, IWUE: Instantaneous water use efficiency, Gs: Stomatal conductance, all r-values of the traits were significant at $p \leq 0.01\%$

Table 6: Mean square values of chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll [chl (a+b)] concentration, photosynthetic rate (Ps) and transpiration rate (E) of *Moringa stenopetala* accessions under different drought stress

		Parameters				
Source of variation	Df	Chl a	Chl b	Chl (a+b)	Ps	E
Accession (A)	2	6.829***	1.644***	15.059***	17.584***	0.464***
Drought stress (DS)	3	3.942***	3.394***	14.564***	58.766***	2.344***
A×DS	6	0.028^{ns}	0.042^{ns}	0.0739^{ns}	3.684***	0.181***
Error	24	0.043	0.034	0.149	0.215	0.016
CV		3.89	6.53	4.74	11.36	8.18

^{ns}Non-significant difference at $p \leq 0.5\%$, ***Significant difference at $p \leq 0.1\%$ probability levels, df: Degree of freedom, A×DS: Interaction among accessions and drought stress

Table 7: Impact of drought stress on chlorophyll a, chlorophyll b and total chlorophyll (a+b) concentration of different *Moringa stenopetala* accessions seedling

Treatments	Parameters (μg mL^{-1})		
Accessions	Chl a	Chl b	Chl (a+b)
Konso	5.49 ± 0.08^{b}	2.85 ± 0.07^{b}	8.34 ± 0.15^{b}
Arbaminch Zuria	6.00 ± 0.08^{a}	3.15 ± 0.07^{a}	9.15 ± 0.15^{a}
Humbo	4.48 ± 0.08^{c}	2.45 ± 0.07^{c}	6.93 ± 0.15^{c}
Turkey/HSD	0.21	0.18	0.39
Drought stress			
Daily (control)	6.05 ± 0.09^{a}	3.55 ± 0.08^{a}	9.60 ± 0.18^{a}
5 days interval	5.60 ± 0.09^{b}	3.10 ± 0.08^{b}	8.70 ± 0.18^{b}
10 days interval	5.06 ± 0.09^{c}	2.47 ± 0.08^{c}	7.53 ± 0.18^{c}
15 days interval	4.53 ± 0.09^{d}	2.19 ± 0.08^{d}	6.72 ± 0.18^{d}
Tukey/HSD	0.26	0.24	0.50
CV (%)	3.89	6.53	4.74

Chl a: Concentration of chlorophyll a, Chl b: Concentration of chlorophyll b, Chl (a+b): Concentration of total chlorophyll, means with different letter in each columns are statistically significant at p-values <0.1%

subjected to 15 days watering interval (Fig. 2b). The rate of transpiration of accessions vary only at daily watering but water stress even at 15 days interval resulted in similar response of accessions among themselves.

With regards to stomata conductance, the present report revealed that, under daily watering condition, all the accessions studied was statistically different from each other. As it is repeatedly told so from most of the parameters, the highest stomatal conductance was observed from accession "Arbaminch Zuria" on daily watering. The lowest stomatal conductance was recorded from accession "Humbo" at 15 days interval watering. Meanwhile, at each of five, ten and fifteen days interval watering, statistically all accession responded similar. Even though they did not shown a significant difference among them, the accession "Arbaminch Zuria" was scored the highest stomatal conductance followed by accession "Konso", whereas the lowest stomatal conductance was recorded from the accession "Humbo" (Fig. 2c).

Leaf water related parameters

Impact of drought stress on leaf relative water content (%) of *Moringa stenopetala* accessions seedling: The result indicated that, the maximum leaf relative water content of *Moringa stenopetala* was found from accession "Arbaminch Zuria" when exposed to daily watering condition. On the other hand, the minimum leaf relative water content of *Moringa stenopetala* was observed from the accession "Humbo" when they exposed to 15 days interval watering condition (Fig. 2d). Instantaneous water use efficiency.

Impact of drought stress on leaf relative water content (%) of *Moringa stenopetala* accessions seedling: The highest water use efficiency was observed from the accession

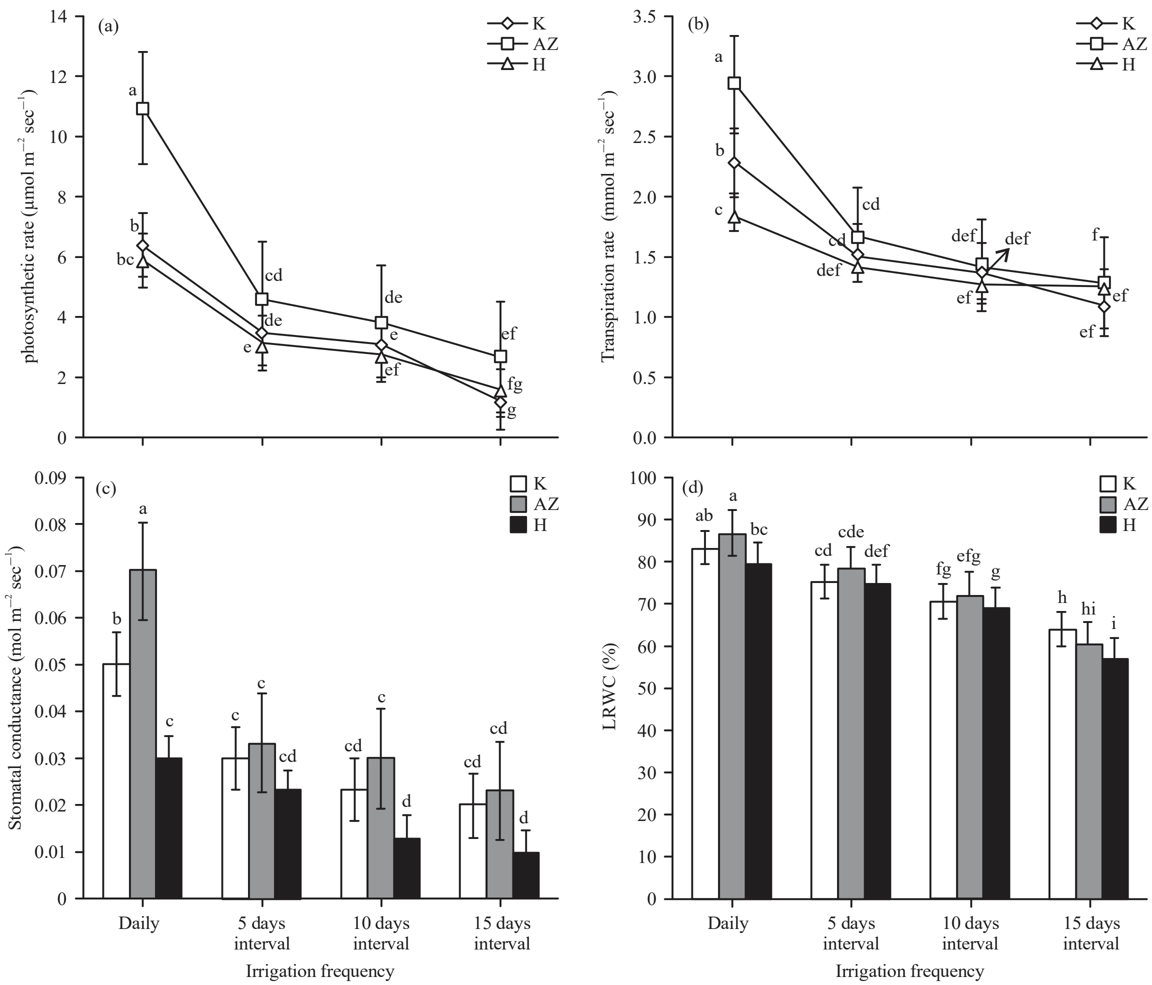

Fig. 2(a-d): Effect of drought stress on (a) Photosynthetic rate, (b) Transpiration rate, (c) Stomatal conductance and (d) Leaf relative water content percentage (LRWC %) of the 3 *Moringa stenpetala* accessions

K: Konso, AZ: Arbaminch Zuria, H: Humbo seedling, means with different letter on the figures are statistically significant, Tukey/HSD (0.05): 1.36^{A}, 0.38^{B}, 0.0163^{C} and 4.370^{D}, CV (%): 11.36^{A}, 8.18^{B}, 18.59^{C} and 2.05^{D}, (Supper scribed a, b, c and d stands for photosynthetic rate, transpiration rate, stomatal conductance, LRWC (%): Leaf relative water content percentage

"Arbaminch Zuria". Conversely, the lowest water use efficiency was observed from the accession "Konso" (Fig. 3). With regard to water stress, the highest water use efficiency was observed from the daily watered condition.

DISCUSSION

The current experiment indicated that, drought stress affected stomata traits (stomata number, length and width). The highest stomata number was recorded from accession "Humbo", conversely, the lowest stomata number was counted on the accession "Arbaminch Zuria" (Table 4). The longest and widest stomata were observed from the accession "Arbaminch Zuria". Meanwhile, accession "Arbaminch Zuria" and "Konso" were statistically similar in stomata length, but they differ each other in stomata aperture (Table 4). This might be associated with leaf water status and gas exchange parameters like photosynthesis, transpiration, stomata conductance. Hence, they determine the size of stomata/given leaf area that, the highest stomata number due to small sized stomata's and lowest stomata number due to larger size of stomata/leaf area provided by genetic makeup of the accessions[23]. Accession with longest and widest stomata seems to the highest photosynthetic, transpiring and metabolic rate resulting high production of dry matter. However, the shortest and narrowest stomata were from accession "H" resulting in least transpiration and photosynthetic rate followed by reduced growth of the seedling[23].

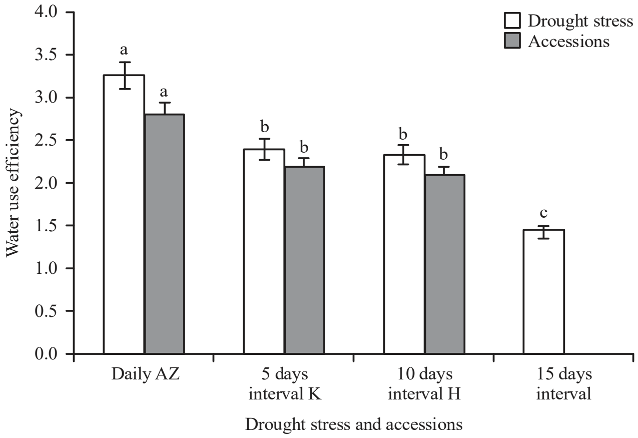

Fig. 3: Water use efficiency of 3 *Moringa stenpetala* accessions

K: Konso, AZ: Arbaminch Zuria, H: Humbo seedling under different drought stress, Tukey (HSD) for accessions and water stress are 0.317 and 0.404, respectively, CV: 13.26, means with different letter at each bar of each factor is statistically significant at p-values $\leq$0.1%

In terms of drought stress, maximum number of stomata was recorded from the seedling grown under 15 days interval watering, conversely, minimum number of stomata were obtained from seedling exposed to daily watering condition (Table 4). Stomata number is increased parallel with increased water stress which is in agreement with study reported by Belhadj *et al.*[23], Bosabalidis and Kofidis[24] and Hetherington and Woodward[25]. This might be due to the size of stomata/given leaf area that, the highest stomata number due to small sized stomata's and lowest stomata number due to larger size of stomata/leaf area influenced by water deficit[23,26]. The present work indicated the negative significant correlation (r = -0.67***) among stomata density and transpiration rate. Not only transpiration rate, but also it associated negatively with all traits (Table 4). Moreover, the higher stomata density is indicator for higher transpiration rate, highest metabolism and absorption of water or not[27]. However, in our present work, the highest stomata density that produced when seedlings subjected to increased water stress had not increased transpiration rate of the *Moringa stenopetala*. Even though more stomata density is produced, the stomata holes was reduced (narrowed) due to increased water deficit and then, tends to reduce the photosynthetic and transpiration rate of the seedling. In terms of stomata length and width as influenced by water stress, the longest and widest stomata were observed from the seedlings grown under daily watering. But, the shortest and narrowest stomata were observed on the seedling exposed to 15 days interval watering condition (Table 4). In line with Belhadj *et al.*[23], Bosabalidis and Kofidis[24] and Hetherington and Woodward[25], the result revealed that, stomata length and width of *Moringa stenopetla* reduced significantly with increased deficit level. Generally, reduced water availability triggers a series of stomata responses in plants[11,28]. As soon as the roots detect a decrease in soil moisture, water potential and cell turgor are reduced. The roots produce abscisic acid (ABA) which is carried to the leaves which tends to promotes reduction of stomata length and width thereby narrowing the stomata. This limit gas exchange and resulted in reduced stomatal conductance, photosynthetic and transpiration rates as well as reduction in leaf traits followed by restriction of entire seedling growth and development[28,29].

Maximum chlorophylls concentration (a, b and total) were recorded from accession "Arbaminch Zuria". While, the minimum chlorophylls concentration (a, b and total) was obtained from accession "Humbo" (Table 7). Genetic makeup of the accessions contributed for the variation. The different amount of chlorophylls (a, b and total) in the accession could be associated with leaf water status of the accessions. This tends to influence gas exchange capacity like stomatal conductance, transpiration and photosynthetic rate of particular accessions based on their natural gift which seems to capture solar radiation. This could result in seedling growth and development[30]. Moreover, total chlorophyll concentration might be associated with chlorophyll a (r = 0.98) and chlorophyll b (r = 0.97) concentration which has been explained by correlation analysis (Table 5).

The highest chlorophyll concentration (a, b and total) was observed from the seedlings grown under daily watering condition. However, the lowest concentration of chlorophylls was observed from the seedlings subjected to 15 days interval watering condition (Table 7). Chlorophylls decreased with increasing drought stress. A decrease of chlorophylls with increased water deficit implies a degradation of chlorophyll due to insufficient of water in the soil[30]. The reduction of chlorophylls under wide drought stress might be regarded as a drought response mechanism associated with minimization of light absorption by chloroplasts. As a whole, the decrease in total chlorophyll concentration as water stress increased led to inactivation of photosynthesis that limits plant growth and productivity[31]. The present study corroborated with study reported by Jaleel *et al.*[32] and Pirzad *et al.*[33] who stated that, the concentration of chlorophyll a was significantly decreased with increased water deficit. The photosynthetic rate that reduced under much stressed condition is due to the reduction of chlorophyll concentration. Moreover, it might be related with stomata traits and stomatal conductance. Hence photosynthesis and stomatal conductance had strongly correlated. As a result, during water stress condition, as stomatal conductance reduced photosynthetic rate also reduced. So that, the decreased in chlorophylls concentration as water stress increased has been considered a typical symptom of oxidative stress and may be the result of decreased pigment photo-oxidation and chlorophyll degradation[34]. Furthermore, the decreased chlorophyll concentration under stressed conditions could be due to increased chlorophyllase activity, although a stimulated activity under water stress might not be purely hydrolytic[2,35,36].

The result indicated that variability among accessions for the rate of gas exchange is narrowed down under much stressed conditions (Fig. 2a). The genetic potential of accessions is determining factor for the rate of photosynthesis but environmental conditions (eg. availability of moisture) is crucial to express full potential of the accessions. According to the work done on *Moringa oleifera* and *Moringa peregrine* by Badran[5] and Abaker[12], wide drought stress has been significantly decreased photosynthetic rate which is in line with present work. Seemingly, this indicates that a reduction in photosynthetic rate was caused by both a decrease in stomatal conductance derived from water stress effect on photosynthetic apparatus as suggested in previous studies by Dos Santos *et al.*[37], Chaves and Oliveira[38], Flexas *et al.*[39] and Warren[40]. In similar pattern Rivas *et al.*[35] reported that, transpiration rate was decreased with increased water stress in moringa seedlings. This could be due to the reduced amount of water in the cell which leads turgor loss and closure of stomata[3]. In line with Abaker[12] and Rivas *et al.*[35], the result indicated that, water deficit decreased stomatal conductance of all Moringa accessions. This could be a major cause of water deficit induced decreases in CO_2 assimilation capacity which results in decline of the growth[38-40].

Relative water content of leaves (LRWC) decreased in all the accessions as the seedlings were subjected to progressively reduced soil moisture conditions. However, the different rate of decline at each stress level is might be due to the ability of different accession with respect to stress level (each accession may have their own mechanism coping against different level of water stress). In other expression, it is the ability of the accession to maintain higher leaf water status to maintain turgidity and other essential physiological process during the stress period[41]. As a result, accession "Arbaminch Zuria" maintain high RWC at all water stress level except at fifteen day interval watering condition. This might be associated with leaf area and gas exchange parameters like stomata conductance, photosynthetic and transpiration rate. The higher leaf area and photosynthetic rate character of the accession "Arbaminch Zuria" than accession "Humbo" and 'Konso" leads accession "Arbaminch Zuria" efficient water use accession. Because, water use efficiency is calculated from photosynthesis and transpiration rate. However, the process of photosynthesis could be affected by stomatal and non-stomatal cases. Restricting CO_2 entry into leaves (stomatal case) and variations in leaf biochemistry that results in inhibition or down regulation of photosynthesis (non-stomatal limitation)[42]. In our study, therefore the observed variations might be stomatal case or non stomatal case that made accession "Arbaminch Zuria" superior than accession "Humbo" and 'Konso". Accordingly, Maswada *et al.*[43] reported that the highest water use efficiency had reported on unstressed conditions. In addition, Farooq *et al.*[10] revealed that the decrease in water use efficiency in drought-stressed treatments might be attributed to less leaf area and rate of photosynthesis and transpiration. Furthermore, when plants are subjected to water deficit, the stomatal pores progressively close, decreasing the stomatal conductance to water vapour and thus slowing transpiration. Thereby, the rate at which water deficits develop is reduced, but CO_2 entry into leaves is also potentially limited. Hence under more water stressed conditions the rate of CO_2 entry in to the leaves could be less than rate of transpiration when compared to unstressed condition. This concept might be a reason for the decline in water use efficiency parallel to increased water stress.

CONCLUSION

It can be concluded that variations in physiological responses of 3 *Moringa stenoptala* accessions (Konso, Arbaminch Zuria and Humbo) to different drought stress were observed. Accordingly, prolonging drought stress reduced physiological traits except stomata number. All the accessions of *Moringa stenopetla* seedlings produced more values in physiological traits except stomata, when the seedlings watered daily to field capacity. But accession "Arbaminch Zuria" produced the largest values for those physiological traits except stomata number. Contrarily, except stomata number, the lowest value in physiological traits was observed from accession "Humbo", when subjected to fifteen days interval watering. Therefore, Arbaminch Zuria can be taken as a better accession both under stressed and unstressed conditions for production of moringa seedling. Furthermore, study is still needed on more indigenous and exotic accession under different drought stress for longer periods of time as a perennial crop.

SIGNIFICANCE STATEMENT

This study discovered effect drought stress on chlorophyll concentration, leaf gas exchanges, stomata traits and leaf water status of moringa (*Moringa stenopetala* L.) accessions seedlings under greenhouse conditions. The information how different moringa accessions responded to different drought stress levels is critically important to identify more tolerant accession that can be recommended for breeding programs for further improvement.

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