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ECOLOGICAL DIVERSITY OF *ZIZIPHUS SPINA-CHRISTI* (L.) WILLD. OF FOUR PROVENANCES IN SAUDI ARABIA: DIFFERENTIAL RESPONSES TO DROUGHT STRESS AND CHARACTERIZATION OF GROWTH, BIOMASS AND PROLINE CONTENTS

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Abstract - In the present study, four provenances were studied and (i) soil parameters by localities, (ii) Morphology of fruits, seeds and leaves, (iii) Germination percentage under temperature and osmotic potential, (iv) Growth and biomass production under drought stress, (v) proline and total soluble sugars contents under drought stress were determined. The germination responses of seeds were determined over a wide range of constant temperatures (15 to 50 °C) and polyethylene glycol (PEG_{6000}) solutions of different osmotic potentials (0 to -1,2 MPa). The effect of drought on several physiological parameters are studied by submitted plants to water deficit (10 and 20 days of withheld irrigation). Morphological study indicating a large-scale diversity among the provenances. Germination was inhibited by either an increase or decrease in temperature from the most suitable temperature found (40 °C). The highest germination percentages (95%) were obtained under control conditions without PEG, and increasing moisture stress progressively inhibited seed germination, which was less than 18% at -1.2 MPa. The results showed that growth parameters and biomass production in leaves, stem and roots were not impacted under medium drought stress stress. The applied drought stresses increased the proline and soluble sugars contents of the plant. Cultivating *Ziziphus spina-christi* under drought or salt stresses could be a good alternative to valorize antioxidant molecules from this species in industrial applications.

Keywords: soils parameters; morphology; diversity; drought stress; plant growth, biomass; proline; soluble sugars.

Introduction

The genus *Ziziphus* belongs to the family Rhamnaceae with about 85 species. *Ziziphus spina-christi* (L.) Willd. (sidr) is one of the species of *Ziziphus* found in Saudi Arabia. It is a wild and cultivated plant distributed in the Middle East, Pakistan and in the North and East of Africa (Alansi et al., 2016). The plant *Zizyphus spina-christi* is readily distributed in the Sahara and Sahel, where the annual rainfall is about 50-300 mm or on periodically inundated sites (Mohammed et al., 2013; Mohammed et al., 2012). Z. *spina-christi* can grow either as a tree or as a shrub. The leaves are short, the flowers are pedunculated and the yellow or red fruits are edible (Chaudhary et al., 2001). *Ziziphus spina-christi* (sidr) is a shrub, sometimes a tree, native to a vast area of Africa stretching from Mauritania to West Africa. In the Kingdom of Saudi Arabia, *Ziziphus spina-christi* is a medicinal plant for many diseases and have wide genetic diversity (Alansi at al., 2016). The genus *Zizyphus* has medicinal importance as all parts of the plant are used by the local Arab people tohelp maintain a healthy life style. *Zizyphus spina-christi* has been reported to have activity against bacterial and fungal pathogens that are normally quite resistant to modern medications (Mohammed et al., 2011; Mohammed et al., 2012). The medicinal properties of *Ziziphus* tree, also known as nabq or sidr, were also recognized and have been in use

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in pharaonic carpentry, diet, and medicine (El-Ansary et al., 2018). It is used extensively for the treatment of ulcers, wounds, eye diseases, bronchitis febrifuge, diuretic and as anti-inflammatory agent for healing skin diseases such as atopic dermatitis (Mohammed et al., 2011). Similarly, different parts of the plant are used for various medicinal purposes among the local populace of Northern Nigeria (Bukar et al., 2015). It is used for the treatment of wounds, burns, stomach discomfort and urinary infections (Adzu et al., 2003). Honeys originating from Sidr (*Ziziphus spina-christi* L.) trees in Saudi Arabia exhibited substantial antimicrobial activity against pathogenic gram-positive bacteria (Owayss et al., 2020). The antimicrobial activity of water-diluted honeys (Sidr) was high than that of broad-spectrum antibacterial antibiotics against bacterial strains, but these honeys were relativity less potent than antifungal antibiotics against a fungal strain (Owayss et al., 2020). Z. *spina-christi* is a shrubby tree growing up to 3 m tall; leafy branches are thorny; leaves are small and shiny; and it has flower 3 mm wide, greenish in clusters and have no scent. While, Z. *spina-christi* var. inermis is a tree growing up to 12 m high, leafy, without spines; leaves are long; flowers have a musty smell, pale greenish and 4 mm wide, mature fruit sweet tasting and apricot coloured (Almalki & Alzahrani, 2018). Several species of the genus *Ziziphus* were reported to be used in traditional medicine as well used in treatment of various diseases; they are used as an immune system stimulant, anti-inflammatory, antitumor, antioxidant, antimicrobial, anti hypoglycaemic, anti hypotensive and liver protective agent in different countries around the world (Miller & Morris, 1988; Said et al., 2006; Obeed et al., 2008; Godini et al., 2009). There is a great diversity of vegetation in Saudi Arabia due to different climatic regimes, ranging from Mediterranean, arid and semiarid climates (Thomas, 2010) and a wide variety of a habitat including high mountains, deep valleys, rocky deserts, meadows and coastal plains (Almalki & Alzahrani, 2018). Later, Migahid (1988) recorded three species of *Ziziphus* in Saudi Arabia (Z. *spina-christi*, Z. *mucronata* and Z. *lotus*). The author mentioned that Z. *spina-christi* species is widely distributed in north Hijaz, south Hijaz, southern region, eastern region, eastern and western Najd, north region, including Tabuk, Al Jauf and Sakakah areas, Nefud region, including the great northern Nefud area, Dahna' and Al-Qasim areas and Al-Rub' Al-Khali, representing most of the southern and south-eastern parts of Saudi Arabia. In Saudi arabia, *Ziziphus spina-christi* is a wild tree used as folk medicine. *Z. spina-christi* is frequently available in Saudi Arabia and is locally known as (Sidr) Ebid (2015). This author indicated that the ethanolic extracts of *Ziziphus spina-christi* exhibited antibacterial activity. In Saudi arabia, Al-Ali et al. (2019) and Badr et al. (2020) found the importance of the antifungal and antimicrobial activities of *Z. spina-christi* extracts for novel drug development that inhabited the biochemical activities. As well, Almeer et al. (2019) confirmed the antioxidant, anti-inflammatory, and antiapoptotic activities of Z. *spina-christi*. Seed germination of *Ziziphus* species is affected by the initial percentage viability at the time of seed collection, and by storage conditions, environmental conditions at sowing time, and treatments applied to break dormancy (Pareek, 2001). As indicated by Pareek (2001), the storage at reduced temperatures of 4.5 ± 0.5 °C in perforated polythene bags result in retention of viability for longer periods. Applying three different cold stratification durations (20, 40 and 60 days) at 5 ± 1 °C, Olmez et al. (2007) observed that the highest germination percentage was 14.1% for *Z. jujuba* seeds without removal of the endocarp when stratified for 20 days and sown under greenhouse conditions. Studying the germination rate on Z. *mauritiana*, Grice (1996) found that seeds collected from the soil surface declined from a rate of 56% in the control (fresh dehulled seeds) to 31 and 20% after 6 and 12 months, respectively. The establishment of plants in arid regions is often limited by temperature when moisture conditions are favourable (Evans & Etherington, 1990). Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range species (Jordan & Haferkamp, 1989). Both seedgermination rate and final germination percentage decrease with reduced soil water potential (Tobe et al., 2006; Daws et al., 2007; Gorai et al., 2009). Besides temperature and moisture, burial depth plays an important role in seed germination for plants living in sandy desert where seeds are often deeply buried or exposed to air due to sand movement (Tobe et al., 2006). Germination of seeds was directly related to the seed size and the depth at which seeds were buried (Bond et al., 1999; Ren et al., 2002). Plants are subjected to a multitude of environmental stresses that include biotic and abiotic ones in arid and semi-arid areas, such as water defiit, high salinity, floding, extremes of temperature, radiation, and deficient or in excess of essential mineral (Ashraf, 2009). During their evolution, plants synthesize several known diffrent secondary metabolites to face environmental conditions (Piccolella et al., 2018). According to the literature, drought and salinity are considered the most important stresses. In fact, these two stresses affcted the plants growth in many countries (Ashraf, 2009). These stresses affect plant developmental crop productivity, physiological processes, molecular biology and genetics (Zandalinasa et al., 2018).

There has been little experimental research done on Z. *spina-christi* and we attempt to investigate four provenances of this specie: (i) soil parameters by localities, (ii) Morphology of fruits, seeds and leaves, (iii) Germination percentage under temperature and osmotic potential, (iv) Growth and biomass production under drought stress, (v) proline and total soluble sugars contents under drought stress. Information from this study provides basic knowledge about germination and growth that can be used for re-establishing projects.

Materials and Methods

Soil analysis in the localities of *Ziziphus spina-christi*

Soil samples were collected from four localities: At-taif, Riyadh, Jizan and Jeddah (triplicates), representing a profie at a depth of 0-50 cm (Abd El-Gawad, 2014). Soil texture, water holding capacity, soil porosity, organic carbon, and sulfate were determined according to Piper (1947). Calcium carbonate was determined by titration against 1 N NaOH and expressed as percentage (Jackson, 1962). A soil solution (1:5) was prepared for each soil sample. The electrical conductivity (EC), pH, and chloride were determined according to Jackson (1962). Carbonate and bicarbonate were determined by titration method using 0.1 N HCl (Pierce et al., 1958). The cations Na^+ and K^+ were determined using a flame photometer, while Ca^{++} and Mg^{++} were estimated according to Allen et al. (1974) using an atomic absorption spectrometer.

Morphology of Ziziphus fruits, seeds and leaves

Fruits and leaves of Z. *spina-christi* were obtained from wild plants which were collected from four localities: At-taif, Riyadh, Jizan and Jeddah. Fruits of *Ziziphus spina-christi* were collected from these provenances on Septembre-Decembre 2017, where collected samples were stored in cotton sacks. Fruits and leaves were collected from five trees from each provenance: 10 leaves per tree, 50 fruits per tree and 50 seeds per tree. Fruit, seed, leaves and petiole length and width (mm) was measured by calipher and length/width rate were calculated. A ruler was used to measure leaf characters.

Effects of constant temperatures on seed germination and recovery

Fruits of Z. *spina-christi* were obtained from wild plants which were collected from four localities: At-taif, Riyadh, Jizan and Jeddah. Fruits were cleaned and stored for three months in the conservation room in which relative humidity was set at 30% and temperature was maintained at 20 °C. When experiments were carried out, fruit seeds were removed and endocarps were cracked using a manual peeler (Maraghni et al., 2010). Seeds were surface sterilized in 0.58% sodium hypochlorite for 1 min, subsequently washed with deionized water and airdried before being used in experiments to avoid fungus attack. To determine the most suitable temperature for germination, seeds were sown on two layers of filter paper in a 90 mm glass Petri dish with 5 ml of deionized water and incubated at: 15, 20, 25, 30, 35, 40, 45 and 50 °C in darkness. A completely randomised design was used in the germination tests. For each treatment, five replicates of 20 seeds each were used. During 20 days the number of germinated seeds were counted and removed every 2 days. A seed was considered to have germinated when the emerging radicle elongated to 2 mm (Maraghni et al., 2010). Three characteristics of seed germination were determined: final germination percentage, number of days to first germination (delay of germination) and mean time to germination (MTG). MTG was estimated according to the formula: $MTG=\Sigma (ni\times di)/N$, where ni is the number of germinated seeds at day i, d the incubation period in days and N the total number of germinated seeds in the treatment.

Effects of osmotic potential on seed germination

The effects of osmotic potential on seed germination were examined by incubating seeds, as described in the first experiment, at the most suitable temperature found with PEG_{6000} solutions of known osmotic potential: 0, -0.2, -0.4, -0.6, -0.8, -1.0 and -1.2 MPa (Michel & Kaufmann, 1973). PEG_{6000} solutions were renewed every 48 h under sterile conditions to ensure relatively constant osmotic potential in the treatments. For each treatment, five replicates of 20 seeds each were used (Maraghni et al., 2010).

Plant growth conditions

Seeds were surface sterilized in 0.58% sodium hypochlorite for 1 min, subsequently washed with deionized water and airdried before being used in experiments to avoid fungus attack. They were sown in 5 L plastic pots in a 2:1 mixture of sandy soil and peat in a growth chamber at 25/18 C day/ night temperatures, at 65-85% relative humidity, with 16/8 h photoperiod. Initially, 5 seeds were planted in each pot. Plants were watered using tap water twice weekly to maintain soil moisture close to field capacity before initiation of water treatment. Potted 90-day-old plants were subjected to drought stress (irrigation with held) or continuously grown under field capacity conditions (Control) for 20 days. The pots were arranged in a randomized complete block design with four replicates per treatment and three plants per pot for every date of harvest corresponding to 10 and 20 days of drought. Water logging was avoided by drainage holes in the bottom of the pot, which permitted soil aeration and drainage of excess water. Day 0 of the experiment was considered as the beginning of the drought period. Every 5 days, plants (control and stressed) were harvested and divided into aerial part and roots prior to use for analyses of proline and total soluble sugars contents.

Growth and biomass production

Plant height (using a meter tape) and stem diameter (using digital vernier callipers) were recorded on each individual. Each individual was carefully removed from the soil substrate, roots were carefully washed, and each individual was excised into three sections: leaves, stem and roots. Leaf fresh weight, stem fresh weight and root fresh weight were recorded using a digital scale for each individual in each treatment. Subsequently, each plant section (leaves, stem and roots) was oven dried at 70 °C until constant weight, and the corresponding data of leaf biomass, stem biomass, root biomass and total biomass were recorded. These data were used to calculate water contents and biomass allocation to leaves, stem and roots as well as root to shoot ratio.

Proline and total soluble sugars contents

Organic solutes contents were determined in leaves of four plants per treatment. Dry plant material (25 mg) was extracted with 80% ethanol at 80 C. The solution was filtered and concentration of total soluble sugars was determined by the anthrone colorimetric method (Savouré, 1980). Proline was also determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973). Approximately, 300 mg of dry tissue was homogenized in 10 mL of 3% aqueous sulphosalicylic acid and filtered. To 2 mL of the filtrate, 2 mL of acid ninhydrin were added, followed by the addition of 2 mL of glacial acetic acid and boiling for 60 min. The mixture was extracted with toluene, and the free proline was quantified spectrophotometrically at 520 nm.

Statistical analysis

Data were analysed using SPSS statistical software version 23. All measurements were subjected to analysis of variance (ANOVA) and differences among treatments were compared by Tukey's HSD test at the level of significance P = 0.05.

Results

Soil analysis in the localities of *Ziziphus spina-christi*

Soil parameters where it exists the provenances of *Ziziphus spina-christi* are presented in Table 1. The soil texture was sandy loam in four localities studied with pH value is between 7.25 at 7.86. The CaCO$_3$ and OM (Organic matter) percentage attained values of about 8.69 to 21.63 and 1.95 to 3.15 respectively. Additionally, cations attained considerable values.

Table 1. Mean value and standard error of the different soil parameters at depths of 0-50 cm in the four localities of *Zizyphus spina-christi*.

Parameters	**At-taif**	**Riyadh**	**Jizan**	**Jeddah**
pH	7.43±1.23^{a}	7.35±0.09^{ab}	7.86±1.95^{ab}	7.25±0.35^{b}
EC (mmhos cm^{1})	3.62±1.23^{bc}	5.32±1.98^{a}	3.48±0.89^{bc}	2.36±0.68^{d}
OM (%)	2.41±0.72b	3.15±0.68a	2.15±0.48c	1.95±0.07cd
OC (%)	1.62±0.04c	2.36±0.45b	1.26±0.42d	3.26±1.26a
CaCO$_3$ (%)	21.63±5.62a	15.36±3.15b	12.35±2.35c	8.69±1.25d
P+++ (mg/100 g dry soil)	1.32±0.75bc	1.29±0.7d8	2.06±0.85a	1.45±0.08bc
K+ (mg/100 g dry soil)	68±7.25a	35.45±5.45c	48.26±4.15b	23.58±3.25d
Ca++ (mg/100 g dry soil)	25.48±4.12b	28.25±2.45a	21.65±3.25c	15.68±1.48d
Mg++ (mg/100 g dry soil)	24.64±3.72b	18.25±2.48c	29.65±3.24a	12.38±1.27d
Na+ (mg/100 g dry soil)	88.63±7.15d	132.55±6.35b	96.45±5.64c	105.47±4.29a
Cl^{-} (mg/100 g dry soil)	104.25±8.07b	95.15±5.12c	106.85±3.54a	75.68±3.09d
HCO$_3^{-}$ (mg/100 g dry soil)	8.42±3.04b	9.36±2.68a	8.19±1.07c	7.45±1.36d
Sand (%)	68.26±1.26d	73.27±1.95c	82.68±3.25b	91.56±2.35a
Silt (%)	9.58±2.35b	21.17±2.15a	7.07±0.92c	2.01±0.39d
Clay (%)	4.16±0.24d	5.56±0.84c	10.25±0.35a	6.34±0.45b

Values of each parameter (mean ±95% confidence limits), having the same letter are not significant different ($P > 0.05$) from each other (Tukey test).

Table 2. Mean value and standard error of the fruits, seeds and leaves parameters of *Zizyphus spina-christi.*

Parameters (mm)	**At-taif**	**Riyadh**	**Jizan**	**Jeddah**
Leaf length 16 at 65 mm	38.62±2.35c	41.54±4.26ab	36.48±3.15d	42.25±2.48a
Leaf width 11 at 51 mm	28.35±1.20d	33.36±2.37ab	31.25±1.42c	34.12±3.16a
Leaf L/W rate	1.36±0.09d	1.24±0.73ab	1.16±0.70c	1.23±0.12a
Petiole length 2 at 19 mm	7.32±1.02c	9.35±2.34ab	7.25±1.95d	9.56±1.78a
Fruit length 11 at 28 mm	15.36±2.15cd	18.36±3.17ab	14.25±2.04c	17.35±2.74a
Fruit width 10 at 25 mm	14.25±1.96d	16.35±2.34ab	13.45±3.84c	16.02±2.37a
Fruit L/W rate	1.07±0.07d	1.12±0.21a	1.05±0.08c	1.08±0.07b
Seed length 7 at 17	9.25±1.03c	12.35±2.42ab	10.25±3.37d	14.36±1.73a
Seed width 6 at 12	8.35±2.35d	10.98±2.13ab	9.36±1.08c	12.75±2.31a
Seed L/W rate	1.10±0.04cd	1.12±0.07ab	1.09±0.34c	1.12±0.21a

Values of each parameter (mean ±95% confidence limits), having the same letter are not significant different (P > 0.05) from each other (Tukey test).

Morphology of fruits, seeds and leaves of *Ziziphus spina-christi*

The fruits, seeds and leaves parameters of *Ziziphus spina-christi* of four localities is presented in Table 2. The leaf length varied between 36.48 and 42.25 mm. The leaf widht varied between 28.35 and 34.12 mm. Provenances of Riadh and Jeddah have the most important dimensions of lenght and widh leaf. The petiole lenght varied betwen 7.25 and 9.56 mm. Provenances of Riadh and Jeddah have the most important dimensions of lenght petiole. The fruit length varied between 14.25 and 18.36 mm. The fruit widht varied between 13.45 and 16.35 mm. Provenances of Riadh and Jeddah have the most important dimensions of lenght and widh fruit. The seed length varied between 9.25 and 14.36 mm. The seed widht varied between 8.35 and 12.75 mm. Provenances of Riadh and Jeddah have the most important dimensions of lenght and widh seeds. The plot correation (Fig. 1) indicated that soil parameters is corrolated to the morphological parameters of the fruits, seed, leaves and petiole of leaves. The lenght and widht of fruit are correlated to lenght and widh of seeds and leaves and petiole leaves. The size and dimensions of the fruits, seeds, leaves and petiole influenced by soils parameters.

Effects of temperatures on seed germination

In response to the tested constant temperatures, most germination (80 to 95%) of Z. *spina-christi* occurred at 40 °C and none at 10 °C (Table 3). Temperature significantly affected the final percentages of germination (Table 3). During the first four days, most germination, and fastest germination, occurred at 40 °C. Provenances of Riadh and Jeddah have the highest final germination percentage at 40 °C from 95 and 94%, respectively.

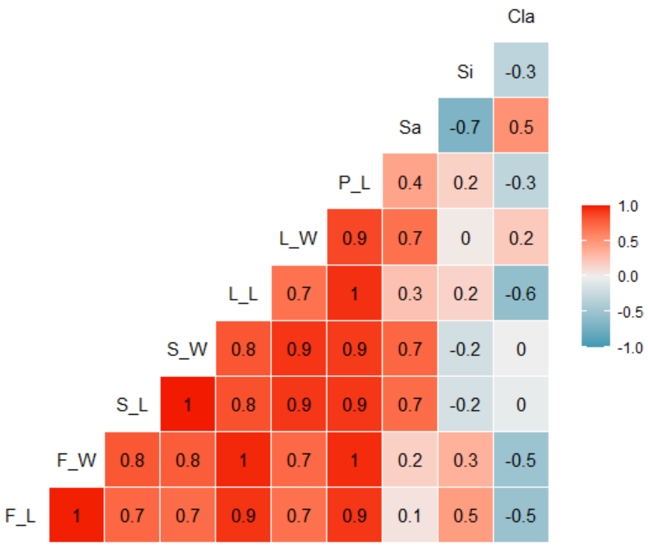

Figure 1. Correlations between soil parameters (Sa: sand; Si: silt; Cla: clay) and leaves, fruits, seeds and Petiole parameters (F_L: fruit lenght; F_W: fruit widht; S_L: seeds lenght; S_W: seeds widht; P_L: petiole lenght; L_L: leaves lenght; L_W: leaves widht), significant correlations ($P < 0.05$).

Effects of osmotic potential on seed germination

The means time of germination (MTG) was significantly affected by temperature and PEG_{6000} treatment based on the results of one-way ANOVA (Table 4). Germination rate increased with increasing temperature from 15 to 40 °C (Table 3) and decreased with decreasing water potential from 0 to -1.2 MPa (Table 4). Provenances of Riadh and Jeddah have the highest final germination percentage under -1.2 MPa from 16 and 18%, respectively.

Table 3. Variation of the final germination percentages (%) and the mean time to germination (MTG, days) of Ziziphus spina-christi seeds during 20 days at different temperatures (15-45 °C). At 15 and 50 °C seed germination was completely inhibited.

Parameters (mm)	Temperature	At-taif	Riyadh	Jizan	Jeddah
Final germination (%)	15	36±10.24a	28±8.25c	33±16.37b	21±72.24d
	20	41±22.14b	39±5.24c	43±12.34a	38±14.25d
	25	65±24.58a	45±3.24d	63±14.25b	51±16.34c
	30	75±18.24a	69±2.15c	74±14.09b	64±12.24d
	35	61±18.25d	77±14.25ab	76±10.36c	78±8.69a
	40	80±9.35d	95±12.35a	81±9.64c	94±12.35ab
	45	63±21.25c	75±15.48a	69±10.25b	58±6.35d
	50	15±2.35a	14±4.15b	12±2.34c	10±1.28d
Mean time of germination MTG (Days)	15	10.25±5.24d	11.75±3.37bc	12.34±3.64a	11.25±3.48bc
	20	8.24±2.76d	9.35±4.12c	10.25±3.18a	9.37±2.79b
	25	7.24±3.17c	8.25±3.48a	8.24±3.15b	7.13±2.13d
	30	5.24±1.78d	7.25±2.43a	6.34±2.46b	5.12±2.14
	35	4.24±2.11c	5.26±1.63a	3.24±2.13d	4.26±1.07b
	40	3.25±1.26ab	2.68±1.05c	2.48±0.95d	3.24±1.03a
	45	5.26±2.35b	4.25±1.20c	3.18±1.95d	5.63±3.15a
	50	7.25±2.98a	6.35±2.34b	5.26±2.09d	6.25±2.45c

Values of each parameter (mean ±95% confidence limits), having the same letter are not significant different (P > 0.05) from each other (Tukey test).

Table 4. Variation of the final germination percentages (%) and the mean time to germination (MTG, days) of Ziziphus spina-christi seeds during 20 days at different PEG_{6000} solutions of different osmotic potentials (0 to -1 MPa).

Parameters (mm)	PEG 6000 (-MPa)	At-taif	Riyadh	Jizan	Jeddah
Final germination (%)	0.0	95±3.25c	100±2.34a	97±8.67cd	98±9.35ab
	0.2	90±3.24cd	95±8.35a	91±4.26cd	91±2.69b
	0.4	88±7.24ab	86±1.25cd	87±8.12a	85±5.24c
	0.6	73±2.4d	79±6.45c	81±7.24bc	82±4.86a
	0.8	56±6.48d	62±3.45c	49±8.37b	75±3.26a
	1.0	23±4.12c	56±2.78ab	21±9.36	63±1.24a
	1.2	8±2.34c	16±3.33b	4±4.12d	18±3.78a
Mean time of germination MTG (Days)	0.0	2.35±1.02a	3.25±1.02ab	2.68±0.59d	3.18±2.34c
	0.2	3.48±1.34cd	5.24±1.27a	4.85±2.34b	4.15±1.27c
	0.4	4.19±2.34d	6.34±3.63ab	5.24±2.42c	6.31±2.13ab
	0.6	5.42±1.23cd	7.15±2.45b	7.12±3.48c	7.23±6.35a
	0.8	7.15±3.45d	8.25±3.15c	8.35±3.25ab	8.34±2.34ab
	1.0	11.25±4.25a	10.25±3.24b	10.23±4.25bc	9.36±3.64d
	1.2	16.32±3.12a	12.24±6.34d	14.02±4.24b	13.25±3.26c

Values of each parameter (mean ±95% confidence limits), having the same letter are not significant different (P > 0.05) from each other (Tukey test).

Growth and biomass production

Drought stress had a negative impact on various growth parameters measured during the experiment for four provenance. Plant height systematically decreased with increasing drought stress (Table 5). Plant height decreased from 31.5 to 22.3 cm for At-taif provenance from 36.24 to 29.70 cm for Riyadh provenance, 29.3 to 26.5 cm for Jizan provenance and 38.2 to 31.2 cm for Jeddah provenance, respectively. The two most drought stress-resistant are Riyadh and Jeddah provenanaces. Stem diameter decreased significantly under drought stress, 3.12 to 2.88 mm for At-taif provenance, 4.23 to 4.18 mm for Riyadh provenance, 3.56 to 3.12 mm for Jizan provenance and 3.69 to 3.45 mm for Jeddah provenance, respectively. The provenances which are less resistant to drought stress are At-taif and Jizan provenences. Significant decreases in leaf, stem and root fresh weight were observed across the drought stress treatments (Table 5). The fresh weight was found to be highest in all plant sections for all provenances under control condition followed by 10 and 20 days of treatment. Leaf and stem moisture content also decreased significantly across the treatments (Table 5), with highest moisture contents in the control condition followed by 10 and 20 days of treatment. Drought stress had a significant negative impact on the leaf biomass, stem biomass and root biomass. As a result, total biomass also remained not similar between 10 and 20 days of treatment and decreased significantly under drought treatment (Table 5).

Table 5. Growth parameters studied of *Ziziphus spina-christi* of four provenances.

Parameters	Treatment (days)	At-taif	Riyadh	Jizan	Jeddah
Plant height (cm)	Control	31.5±2.35c	36.24±3.12ab	29.3±4.16d	38.2±6.42a
	10 days	22.3±1.26d	29.7±5.61b	26.5±6.31c	31.2±3.54a
	20 days	20.59±2.35d	26.35±3.32b	24.15±5.24c	29.35±9.37a
Stem diameter (mm)	Control	3.12±2.03cd	4.23±1.24ab	3.56±0.95c	3.69±1.08b
	10 days	2.88±3.24d	4.18±2.13a	3.12±0.34c	3.45±1.24ab
	20 days	2.04±1.26cd	3.65±1.73a	2.95±1.02c	3.12±0.97ab
Leaf fresh weight (g)	Control	3.79±0.95cd	5.27±1.08a	4.26±1.04c	4.95±1.23ab
	10 days	2.35±0.08d	4.33±2.05ab	3.15±0.34c	3.52±0.43b
	20 days	2.12±0.07cd	3.96±0.93a	2.78±0.42c	3.14±0.83b
Stem fresh weight (g)	Control	3.63±1.02d	4.98±1.42a	4.17±1.39c	4.85±1.49b
	10 days	2.37±1.12d	4.12±0.93b	3.65±0.07c	4.33±0.08ab
	20 days	2.11±0.23cd	3.96±0.85b	3.32±1.04c	3.99±0.96a
Root fresh weight (g)	Control	3.45±0.86d	4.52±1.02ab	3.88±0.72c	4.73±1.34b
	10 days	2.69±0.75cd	4.13±0.97b	2.99±0.34c	4.23±1.05a
	20 days	2.16±0.24d	3.67±0.82ab	2.43±0.94cd	3.97±1.34a
Leaf moisture contents (%)	Control	32.12±5.36d	40.25±6.37b	35.26±3.82c	48.35±11.24a
	10 days	22.35±3.45cd	37.25±5.19b	28.35±5.28c	41.25±10.24a
	20 days	20.13±1.76d	35.16±4.23b	27.32±4.16c	38.67±7.19a
Stem moisture contents (%)	Control	29.35±8.35d	35.24±3.94aa	31.24±6.78c	34.24±8.37b
	10 days	24.18±4.15cd	30.42±4.67a	26.49±6.47c	29.37±7.45ab
	20 days	22.16±3.24d	39.01±6.87a	24.16±3.45c	27.15±3.78b
Root moisture contents (%)	Control	26.35±4.10c	29.34±6.79a	25.34±4.32d	28.47±4.19b
	10 days	22.15±4.68cd	25.76±4.03a	20.54±3.24c	24.15±3.24ab
	20 days	20.11±2.13c	24.15±1.23a	19.65±2.38d	22.54±4.23b

Values of each parameter (mean ±95% confidence), having the same letter are not significant different (P > 0.05) from each other (Tukey test).

Table 6. Effects of 10 and 20 days of water withheld on the soluble sugars and proline in leaves of of *Ziziphyus spina-christi*.

Parameters	Treatment (days)	Treatment	At-taif	Riyadh	Jizan	Jeddah
Soluble sugars (mg g^{-1} FM)	10	Control	7.36±2.35c	7.45±0.39b	7.13±2.48d	7.46±5.24a
		Stressed	7.51±3.45b	7.85±2.35a	7.39±4.97dd	7.96±3.19bc
	20	Control	7.26±6.34b	7.36±1.45a	7.09±3.16d	7.12±4.16c
		Stressed	6.95±1.45c	7.18±2.48a	6.87±0.58d	6.98±2.73ab
Proline (mg g^{-1} FM)	10	Control	45.6±3.48c	46.3±3.19b	44.2±5.16d	48.2±3.15a
		Stressed	47.1±7.37c	48.4±4.12ab	46.3±4.57cd	50.4±2.75a
	20	Control	44.7±6.74c	45.3±3.64b	43.4±2.17d	47.2±6.34a
		Stressed	41.2±4.19d	42.1±4.19bc	42.3±3.14b	45.2±5.48a

Values of each parameter (mean ±95% confidence), having the same letter are not significant different ($P > 0.05$) from each other (Tukey test).

Proline and total soluble sugars contents

Drought stress had a positive impact on soluble sugars and proline for 10 days of treatment and have a negative impact for same parameters for 20 days of treatment (Table 6). For soluble sugars, at 10 days of water withheld, the provenances which produced better are Riyadh and Jeddah provenanaces (7.45 to 7.85 and 7.46 to 7.96 mg g^{-1} FM). For proline, at 10 days of water withheld, the provenances which produced better are Riyadh and Jeddah provenanaces (46.3 to 48.4 and 48.2 to 50.40 mg g^{-1} FM). At 20 days of water withheld, the provenances of At-taif and Jizan are the least productive of soluble sugars and proline (Table 6) therefore the least resistant.

DISCUSSION

Soil analysis in the localities of *Ziziphus spina-christi*

Our analyses of soil revealed that the soil texture was sandy loam in four localities of the provenances of *Ziziphus spina-christi*. Our results is in accordance with Algandaby and El-Darier (2018). The community of *Ziziphus spina-christi* expressed high signifiant correlation with sand, calcium carbonate, water holding capacity, and magnesium. In the present study, *Ziziphus spina-christi* survived in sandy soil, and it appears to be highly susceptible to salinity; this is in harmony with the fidings of other investigators (Laamouri et al., 2008; Laamouri, 2005).

Morphology of fruits, seeds and leaves of *Ziziphus spina-christi*

Our results showed that the fruits, seeds and leaves parameters of *Ziziphus spina-christi* of four provenances vary according localities. Provenances of Riadh and Jeddah have the most important dimensions of lenght petiole and have the most important dimensions of lenght and widh fruit and the most important dimensions of lenght and widh seeds. Similarities observed among some populations, despite their distinctiveness of geographic origin, suggest a narrow genetic base. These results accord with Bina et al. (2012) work. Result of mean comparisons showed that *Ziziphus Mauritiana* Ghafari genotype had highest fruit length 39.4 mm and fruit diameter 23.9 mm. In our results, the leaf length varied between 36.48 and 42.25 mm. The leaf widht varied between 28.35 and 34.12 mm. Fruit length varied between 14.25 and 18.36 mm. The fruit widht varied between 13.45 and 16.35 mm. In Baghazadeh-Daryaii (2017), the leaf length and width varied between 16 and 65.6 and 11.9 to 51.5 mm respectively. In similar research, the amounts of these traits were different 19 to 30 and 9 to 17.5 mm respectively (Khakdaman et al., 2007). The highest ratio of fruit weight to seed weight was 9.625, In similar studies on fruit flesh/stone ratio of jujube, was found to be about 16.22 (Ecevit et al., 2008) and 15.54 (Ghazaeian, 2015). In this present study the thorn length were between 0 and 19.1 mm. The thorn length on jujube varied from 1.06 to 2.02 cm (Khakdaman et al., 2007) and 2-5 cm (Ghazaeian, 2015). Saied et al. (2007) found that, leaves are simple, alternate, narrowly ovatelanceolate, varying from 1 to 9 cm in length and 1 to 3.5 cm in width, are glabrous above, minutely and densely pubescent beneath, have three basal, conspicuous veins running up to the apex and around 0.5 to 1 cm long petioles. Fruit is a globose drupe about 1 to 1.5 cm in diameter, red-brown, with a hard stone surrounded by a sweet edible pulp. Fruiting time extends from October until April (Saied et al., 2007). We suggest that climate can affect these traits as genetics and physiological and morphological quality can compatible with unfavourable desert conditions. Our resultats showed that soil parameters is corrolated to the morphological parameters of the fruits, seed, leaves and petiole of leaves. The study concur with earlier report (Laamouri et al., 2008).

Effects of temperatures on seed germination

In our study, response to the tested constant temperatures, most germination (80 to 95%) of Z. *spina-christi* occurred at 40 °C and none at 10 °C. It is evident that germination and emergence of *Ziziphus* seeds are inhibited by a stony endocarp. Unfortunately, similar to several other species, the seeds exhibit mechanical scarification to overcome physical dormancy. The impermeable endocarps of Z. *lotus* fruits were artificially broken to release seeds that can achieve 100% germination at 35 °C (Maraghni et al., 2010). Our results are similar to other studies which found that the of the endocarp can achieve higher percentages of germination and emergence on jujube species than those of intact stones such as Z. *mauritiana* (Prins & Maghembe, 1994; Grice, 1996), *Z. nummularia* (Hussain et al., 1993), Z. *abyssinica* (Prins & Maghembe, 1994), *Z. mucronata* (Hassen et al., 2005; Griffiths & Lawes, 2006), Z. *spina-christi* (Saied et al., 2008) and Z. *joazeiro* (Alves et al., 2008). Our results showed that temperature significantly affected the final percentages of germination. During the first four days, most germination, and fastest germination, occurred at 40 °C. Provenances of Riadh and Jeddah have the highest final germination percentage at 40 °C from 95 and 94%, respectively. Laamouri & Zine El Abidine (2000) found high percentages of germination of *Ziziphus lotus* at 25 °C and Maraghni et al. (2010) found high percentages of germination of *Ziziphus lotus* at 35 °C (100%). higher percentages of germination were observed from 30 to 35 °C (Danthu et al., 1993).

Effects of osmotic potential on seed germination

In our study, Provenances of Riadh and Jeddah have the highest final germination percentage under -1.2 MPa from 16 and 18%, respectively. We found that germination rate with decreasing water potential from 0 to -1.2 MPa. Desert shrubs vary in their ability to germinate in the presence of moisture stress. Maraghani et al. (2010) noted that Seeds of Z. *lotus* germinated to 95 and less than 5% in PEG_{6000} solutions of -0.4 and -1 MPa. According to Scifres and Brock (1969), seeds of *Prosopis juliflora* are more tolerant of moisture stress at an optimum germination temperature (29 °C) than they are at temperatures above and below it. By increasing moisture stress, similar results were found on germination of *Diospyros texana* seeds that decreased from about 95 to 45% at 0 and -0.6 MPa, respectively (Everitt, 1984), whereas germination of three deciduous semishrubs of genus Artemisia was inhibited severely in PEG_{6000} solutions at -1.2 MPa (Tobe et al., 2006).

Growth and biomass production

Drought is one of the major factors that could influence morphological and physiological characteristics of plants (Ren et al., 2007; Xiangwen et al., 2009). In our results we found that drought stress had a negative impact on various growth parameters measured during the experiment for four provenance. Plant height systematically decreased with increasing drought stress, stem diameter decreased significantly under drought stress. The provenances which are less resistant to drought stress are At-taif and Jizan provenences. Significant decreases in leaf, stem and root fresh weight were observed across the drought stress treatments, leaf and stem moisture content also decreased significantly across the treatments. Zafar et al. (2019) found that the effects of salt stress on plant growth include changes in morphological characteristics such as stem height, stem diameter and number of leaves of *Terminalia Arjuna*. Others have found 60% reductions in shoot growth in seedlings of *Leucaena leucocephala* when subjected to 100 mM NaCl, while in seedlings of *Prosopis juliflora* shoot growth reductions was just 15% under the same salt conditions, the negative changes in growth traits have been linked to the loss of turgor pressure due to decreases in plant water potential as well as decrease in division and cell expansion (Mguis et al., 2012). Drought stress had a significant negative impact on the leaf biomass, stem biomass and root biomass. The effects of drought stress on plant growth include changes in morphological characteristics such as stem height and stem diameter. The present study shows that the drought stress reduced the growth of Z. *spina-christi* by restricting leaf formation and biomass production. This response has been reported in several species subjected to drought, like Z. *lotus* (Maraghni et al., 2013) Z. *mauritiana* (Clifford et al., 1998), Z. *rotundifolia* (Arndt et al., 2001), *Olea europaea* (Bacelar et al., 2007) and *Adansonia digitata* (De Smedt et al., 2012). The drought-induced growth reduction might be related to accelerated senescence of older leaves and reduction of their size (Maraghni et al., 2013). Fresh biomass and moisture contents systematically decreased in salt stress of T. *arjuna* (Zafar et al., 2019). Similar results have been reported in previous studies where a decrease in biomass has been recognized as the capability to survive under high salt stress environments species, for example, *Eucalyptus* (Isla et al., 2014), *poplar* (Vaario et al., 2011) and *Acacia* (Nguyen et al., 2004). A reduction in the plant water potential can result in a reduction in cell turgor, which will negatively affect biomass production (Gorai et al., 2010). According to Munns (1993), this sensitivity could be explained due to an imbalance among cations as a result of the complex interaction with the xylem transport system. The NaCl salinity affcted development and growth of seedlings of *Ziziphus spina-christi* at the highest concentration (200 mM NaCl), which was associated with the appearance of foliar necrosis (Gorai et al., 2019). This was in agreement with previous reports on *Ziziphus* species including Z. *spina-christi* at 180 and 220 mM (Sohail et al., 2009; Shekafandeh & Takhti, 2013, respectively), Z. *mauritiana* at 131 and 176 mM (Bhatt et al., 2008; Agrawal et al., 2013, respectively), Z. *mauritiana* grafted on Z. *spina-christi* at 220 mM (Bhat et al., 2009), and Z. *rotundifolia and Z. nummularia* at 220 mM (Gupta et al., 2002). Maraghni et al. (2013) showed that the osmotic potential of the solution induced by PEG reduced the growth of Z. *lotus* by restricting leaf formation and biomass production and The drought-induced growth reduction might be related to accelerated senescence of older leaves and reduction of their size. This response has been reported in several species subjected to drought, like Z. *mauritiana* (Clifford et al., 1998), Z. *rotundifolia* (Arndt et al., 2001), *Olea europaea* (*Bacelar* et al., 2007) and *Adansonia digitata* (De Smedt et al., 2012). Boughalleb et al. (2015) found that shoot dry mass decreased significantly with water drought periods in A. gombiformis and this reduction in biomass accumulation was higher than in the root ones. Thus, at the end of the experiment, dry mass decreased by 23.5 and 11.7% in the shoots and roots, respectively, in comparison to their respective plants under well-watered conditions. Drought caused impaired mitosis, cell elongation, and expansion resulted in reduced growth (Hussain et al., 2008). Furthermore, the plants responded to water deficit by increasing the proportion of water-absorbing root biomass relatively to the water-loosing leaf biomass (Duan et al., 2005). Several authors have found similar results (Liu & Stutzel, 2004), verifying the plant growth adjustment including the reduction of biomass accumulation with the severity or extent of the water stress. In conclusion, *Ziziphus spina-christi* grew and survived under severe saline conditions.

Proline and total soluble sugars contents

Drought stress had a positive impact on soluble sugars and proline for 10 days of treatment and have a negative impact for same parameters for 20 days of treatment. For proline, at 10 days of water withheld, the provenances which produced better are Riyadh and Jeddah provenanaces (46.3 to 48.4 and 48.2 to 50.40 mg g^{-1} FM). *Ziziphus lotus* seedlings in the presence of PEG-enriched nutrient solution accumulated higher amounts of proline in the leaves than in the roots (Maraghni et al., 2013). Leaf proline accumulation was higher than that of control conditions. This is in agreement with findings of Choudhary et al. (1996) and Clifford et al. (1998) who reported a similar response in other species of the genus *Ziziphus* under osmotic stress. Sundaresan & Sudhakaran (1995) mentioned that water stress induced by PEG (-1.65 MPa) caused a 25-fold increase in proline levels in young excised leaves of the susceptible cultivar of cassava, while the increase was about ninefold in the tolerant cultivar. For soluble sugars, at 10 days of water withheld, the provenances which produced better are Riyadh and Jeddah provenanaces. At 20 days of water withheld, the provenances of At-taif and Jizan are the least productive of soluble sugars and proline therefore the least resistant. The unchanged content of proline and soluble sugars measured in *Ziziphus spina-christi* under mild to moderate drought conditions (up to 20 days) could be explained by its comparatively good tolerance towards the imposed water stress, this results in accordance with Boughalleb et al. (2015). According to Arndt et al. (2001), the decreased demand for carbon caused by low photosynthetic rates in drought-stressed leaves contributes to the increase of soluble sugars concentrations. Starch accumulation decreases in leaves of Z. *lotus*, whereas it increases in roots as osmotic stress intensified (Maraghni et al., 2013). Boughalleb et al. (2015) found that under drought conditions, the biosynthesis and accumulation of compatible solutes, such as proline and soluble sugars, seemed to be associated with drought tolerance in many plant species and showed that proline and total soluble sugars content increased significantly only after 20 days of water deficit reaching 1.4 to 1.5 fold higher amounts than that in control, respectively. The higher accumulation of proline was correlated with drought tolerance in cultivars of sugarcane (Cia et al., 2012) and tomato (Ghorbanli et al., 2013). Soluble sugars also contributed to improving drought tolerance of sugar beets (Choluj et al., 2008) and wheat (Loutfy et al., 2012). Boughalleb et al. (2015) suggested that A. *gombiformis* had higher capacity of osmotic adjustment in terms of accumulating proline and soluble sugars, especially under prolonged drought, which could protect plants from damages of dehydration (Chaves et al., 2003; Ashraf & Foolad, 2007).

Conclusions

From the present study, it can be concluded that seeds of *Ziziphus spina-christi* have a mechanical dormancy and germinated better at high temperatures and the most suitable temperature found is 40 °C. Under natural conditions, seed germination is more complicated and influenced by many factors such as salinity, drought, light and temperature. The present study showed an increase in proline and soluble sugars under drought stress. The two provenances of Riyadh and Jeddah showed significant resistance against drought stress and satisfactory growth. Finally, based on the tolerance shown by seeds and plants under medium levels of osmotic potential and drought stress, it is suggested that *Ziziphus spina-christi* especially the two provenances of Riyafh and Jeddah can be planted to reduce secondary salinization.

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