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Influence of Drought Stress on Growth and Nodulation of *Acacia origena* (Hunde) Inoculated with Indigenous Rhizobium Isolated from Saudi Arabia

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Abstract: Drought is the main abiotic factor affecting the survival of soil microorganisms and plant growth. The present study was conducted at the Range and Forestry Applied Research Unit, King Saud University at Dirab, South of Riyadh City to study the effect of drought stress on the growth and nodulation of *Acacia origena* inoculated with indigenous Rhizobium. Three indigenous Rhizobium isolates (KS1, KS2 and KS3) were isolated from the root nodules of seedlings of *Acacia tortilis* (Forssk.), *Leucaena leucocephala* (Lam.) and *Acacia saligna* (Maslin), respectively. The tree seeds were obtained from Abha, Saudi Arabia and planted in a shade house. Drought stress significantly reduced both seedling growth and nodulation of *Acacia origena.* Under this stress, the Rhizobium isolates varied in their response to the drought period; they were able to create nodules on the roots of the *Acacia origena,* while this declined with an increase in the drought period. A treatment that involved withholding irrigation for 21 days was the most effective treatment for growth and nodulation. Further, indigenous Rhizobium isolates KS1 and KS2 are drought tolerant. The inoculation of seedlings with indigenous Rhizobium isolates might therefore improve the drought stress tolerance of *Acacia origena* seedlings under these conditions.

Key words: Drought stress • Indigenous Rhizobium • *Acacia origena* • Nodulation

INTRODUCTION

Drought is the main abiotic factor that affects the survival of soil microorganisms and plant growth and drought stress has an adverse influence on water relations, mineral nutrition, metabolism and photosynthesis [1]. Water is one of the most important resources for agricultural production. In many parts of the world, water availability is economically and/or technically limited; 28 and of the Earth's land surface is considered to be too dry for crop production [2-4]. Drought and salinity are considered by agronomic researchers to be challenges for nodulating legume crops. However, there are many wild legumes that grow in deserts, some of which are saline- and/or high pH-tolerant [5]. Studies on the nodulation of legumes have been conducted in many of arid and semi-arid areas of Africa [6-10]. There are many potential nodulated legumes native to arid and semi-arid areas that have been studied for their tolerance to drought or salinity, such as *Prosopis* spp. in the Americas, *Mimosa* spp. in the Caatinga biome of Brazil, pasture species and trees, especially African acacias [11-13]. Some of these species were described in a review of nine major arid and semi-arid areas around the world. Studies of root nodule bacteria under stress conditions in soil might mimic their survival in natural habitats. This is because bacterial strains under carbon and energy limitations in soil may be more sensitive to environmental stress than they would be in rich laboratory media. In addition, rhizobia may withstand and successfully multiply under these conditions, but their infectibility and nodulating ability may be changed [14]. Selection of rhizobia that can withstand drought stress is important for improving plant productivity for reforestation applications in desert and semi-desert areas. In Saudi Arabia, acacia populations play a major role in the productivity and stability of the desert environment, but they are threatened due to indiscriminate cutting, extreme environmental conditions, such as drought and salinity and low density. There are much recent interest in the tolerance of wild legumes and their symbionts to severe drought, salinity and elevated temperatures. However, natural symbiotic rhizobia with legumes establish effective symbioses under these conditions [6]. Knowledge of the symbioses of woody

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legume species in natural ecosystems and the distributions of root nodule bacteria under drought and salt stress conditions is limited. Because salinity is often associated with drought in semi-arid and arid regions, the present study aims to examine the effect of drought stress on the growth and nodulation of *Acacia origena* inoculated with indigenous rhizobia.

MATERIALS AND METHODS

Tree Species and Soil: *Acacia origena* are medium-sized trees (6 meters height), native to Ethiopia, West Eritrea and to regions across the Red Sea in Yemen and Saudi Arabia. The bark is white to greyish-white in thick-papery layers; younger branches have yellow to yellowish-brown bark. Leaves on reduced axillary shoots; pinnae usually 2-4 pairs per leaf; leaflets with 14-34 pairs per pinna, glabrous, 6 mm × 2 mm. Inflorescence globular heads, creamy-white, 1 cm in diameter, pedunculate, 2 cm long. Pods are straight, purplish-green to red, 8 cm × 2 cm, obtuse or falcate, longitudinally and obliquely veined. The trees are restricted to only the eastern slopes of the Asir and Abha regions and extend to an elevation of 2700 meters together with *Juniperus procera*. The physical and chemical characteristics of soil used in the study were as follows: the soil texture was sandy loam with pH 8.2 and E.C 1.01 mmhos/m; total soluble cations (Na^+, K^+ and Ca^{++}) were 1.04,. 52 and 0.25 meq/l, respectively; organic matter 1.0 and and total nitrogen 29.2 ppm. The soil was air-dried for 1 week, thoroughly mixed and autoclaved twice for 2 h (121 ~) on two consecutive days.

Bacterial Strains and Isolation of Root-nodule Bacteria: Rhizobium isolates KS1, KS2 and KS3 were isolated from root nodules of seedlings of *Acacia tortilis* (Forssk.), *Leucaena leucocephala* (Lam.) and *Acacia saligna* (Maslin), respectively, which were grown in the Dirab Valley, South of Riyadh City. The isolates were collected from healthy, unbroken pink root nodules collected in the field. The method used to isolate root-nodulating bacteria from the seedlings was described by Vincent [15].

Growth and Plant Material: The *Acacia origena* seeds used in this experiment were obtained from the Abha region (South of the Kingdom) by the Range and Forestry Applied Research Unit, Faculty of Food and Agriculture Sciences, King Saud University. The seeds were immersed in hot water (100°C) for 15 min and in cool water for 24 h, then sown in plastic tray troughs containing a mixture of sterilized vermiculate and sand (1:1 by volume). Germination began at day 7. After four weeks, the seedlings were transplanted into plastic pots (15 cm diameter) containing a sterilized sand: vermiculate mix (2:1 v: v). The unstressed seedlings were watered every 3 days, while the drought-stressed seedlings were watered every 7, 14 and 21 days. Each treatment had three replicates. Seedlings were inoculated with 10 ml of *Rhizobium* isolates KS1, KS2, or KS3 containing approximately 1×10^9. *Rhizobium* cells grown in yeast extract mannitol broth with shaking (200 rpm). Pots were arranged in factorial experiment in complete randomized design with three replicates in the greenhouse and the experiment was conducted from October 2012 to the first week of March 2013 and repeated from October 2013 to the first week of March 2014. Seedlings were maintained at 28°C. At the end of the experiment, seedlings were harvested and analyzed for their height, diameter, nodule numbers, nodule dry weight and stem and root dry weight.

Extraction and Analysis of Chlorophyll: Chlorophyll *a, b* and *a+b* were determined in fresh leaves by using the method described by Porra *et al.* [16]. The leaf samples were weighed and ground with 1 ml of solvent (*N, N*-dimethylformamide (DMF)) using a pestle and mortar. The homogenate, combined from a further three washings of the pestle and mortar (each 1.5 ml) with the same solvent, was centrifuged at 2500 rpm in a bench centrifuge for 10 min. The pellet was extracted with 1 ml of solvent in a homogenizer and the pooled supernatants were adjusted to a final volume of 8 ml. The spectrum was recorded between 750 and 600 nm and the major red absorption peak was automatically determined using a UV-VIS spectrophotometer (T80 UV/VIS Spectrophotometer, PG instruments Ltd- USA) with the recording zeroed at 750 nm. The Chl. *a, b* and Chl. *a+b* contents in µmol/l were calculated using the equations of Porra *et al*. [16].

Statistical Analysis: The obtained data were submitted to analysis of variance according to the method of Snedecor and Cochran [17] using ANOVA and LSD procedures available in the SAS software package (version 9.13, 2008).

RESULTS

Effect of Drought Stress on Growth Parameters and Nodulation of *Acacia origena*: Drought is the main abiotic factor affecting the survival of soil microorganisms and plant growth. There were significant differences between the Rhizobium isolates and period of drought stress on seedling growth and nodulation of *Acacia origena* over the two seasons. The differences in height, diameter and number of nodules per plant between

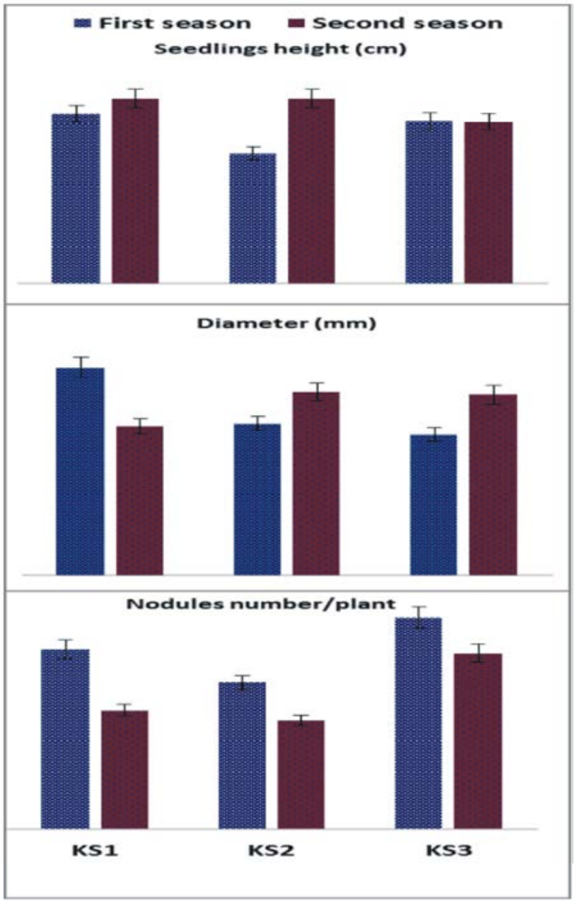

Fig. 1: The mean effect of Rhizobium isolates on height, diameter and nodules number of *Acacia origena* under drought period over two seasons.

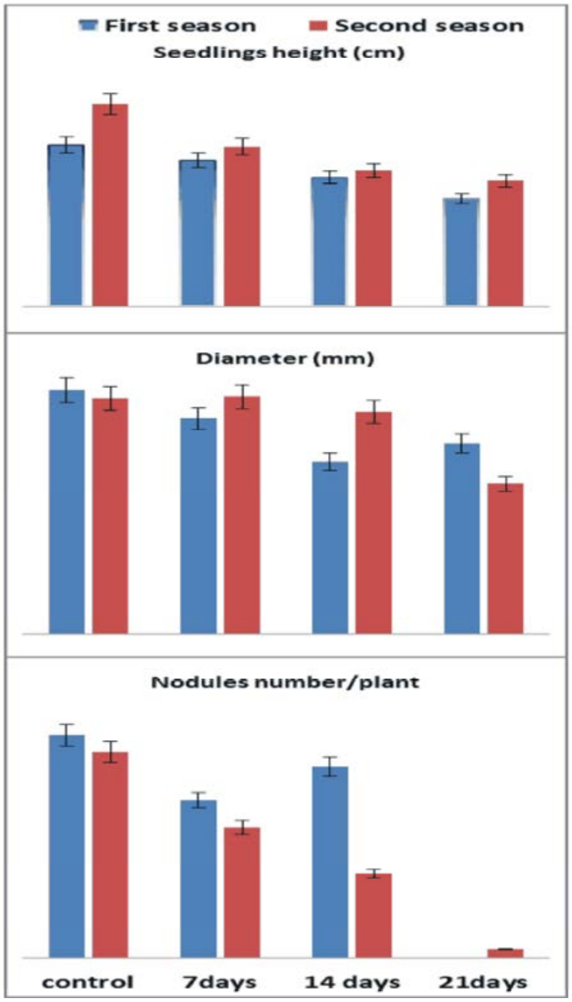

Fig. 2: The mean effect of drought period on height, diameter and nodules number of *Acacia origena* over two seasons.

Rhizobium isolates, drought stress and their interactions were highly significant at ($P < 0.01$) in both seasons, while the other parameters varied between significant and not significant in both seasons. The main effect of Rhizobium isolates varied in their influence on seedling height and diameter between KS1 and KS2 in the two seasons and isolate KS3 produced the largest number of nodules (Fig. 1). The main effect of drought period followed the same trend as for Rhizobium isolates; the treatment irrigated every 21 days was the most influential treatment with regard to seedling height, diameter and the nodule numbers (Fig. 2).

The interaction between rhizobium isolates and drought period was significantly different for growth and nodulation. Under drought stress, the isolates were able to form nodules on the roots of the *Acacia origena,* but this declined with an increase in drought period (Table 1). Rhizobium isolates KS2 and KS3 were the most competitive under drought stress compared to KS1 in the two seasons and showed improvement in some growth parameters. The seedlings in the 21 day drought period had the most impact compared with the control seedlings in both growing seasons (Table 1). The seedlings inoculated with the Rhizobium isolate KS3 were the most tolerant to the drought treatments for height; the percentage reduction for the two seasons was lower than for KS1 and KS2; the reductions were 11% and 12% after 7 days of withholding irrigation, respectively. After 14 days withholding irrigation, the height reductions were 37% and 15%, while at 21 days the height reductions were 52% and 15%, respectively (Table 1). The same trend was found for nodule numbers; the Rhizobium isolate KS3 was the most tolerant to drought in both seasons. The percentage reductions in number of nodules per plant were 38%, 39% and 100% in the first season and 42%, 51% and 100% in the second season for 7, 14 and 21 days of withholding irrigation

Table 1: Interaction between Rhizobium isolates and drought period on the growth and nodulation of *Acacia origena* over two seasons

		Seedling growth					
		First Season			Second Season		
Rhizobium isolates	Drought period (days)	Height (cm)	Diameter (mm)	No Nod	Height (cm)	Diameter (mm)	No Nod
KS1	Control	24.7 ±1.5a	1.62±0.37a	15.0±3.6a	31.0 ±3.6a	1.27±0.30b	11.0±1.0b
	7	20.3±2.0b	1.57±0.05a	8.3±1.5bc	24.3±1.2b	1.20±0.19b	5.7±102d
	14	20.0±2.6b	1.45±0.13a	7.0±1.0c	19.7±0.6bc	1.11±0.11b	3.0±1.0e
	21	20.0±2.0b	1.44±0.16a	0.0±0.0d	17.7±1.5c	0.80±0.04c	0.3±0.2f
KS2	Control	20.0±2.0b	1.43±0.09a	10.3±1.5b	32.7±4.0a	1.57±0.15a	7.3±1.2cd
	7	17.7±2.1bc	1.06±0.14b	7.0±1.0c	24.0±1.0b	1.53±0.08ab	6.7±1.5cd
	14	14.7±1.6c	1.02±0.05b	7.3±1.5bc	19.0±2.6c	1.47±0.20ab	3.3±1.2de
	21	13.0±0.9c	0.94±0.12bc	0.0±0.0d	17.0±4.3c	0.80±0.04c	1.0±0.5ef
KS3	Control	27.3±1.5a	1.30±0.13ab	16.0±3.0a	22.8±3.0bc	1.56±0.04a	14.3±4.0a
	7	24.3±3.8a	1.29±0.12ab	10.0±2.6bc	20.0±2.6bc	1.47±0.20ab	8.3±1.5c
	14	17.3±2.4ab	0.88±0.11bc	9.7±1.2bc	19.3±3.1bc	1.20±0.18b	7.0±1.0cd
	21	13.0±0.9c	0.61±0.42c	0.0±0.0d	19.0±0.5c	1.06±0.07bc	0.0±0.0f

Means followed by the same superscript letter in each column are not significantly different at $P<0.05$ according to the LSD test. KS1, *Rhizobium* isolated from *Acacia tortilis* seedlings; KS2, *Rhizobium* isolated from *Leucaena leucocephala* seedlings; and KS3, *Rhizobium* isolated from *Acacia saligna* seedlings. No Nod: nodules number.

Table 2: Interaction effect between Rhizobium isolates and drought period on seedlings biomass of *Acacia origena* over two seasons

		Seedlings biomass					
		First Season			Second Season		
Rhizobium isolates	Drought period (days)	Nod Dry(mg)	SD(g)	RD(g)	Nod Dry(mg)	SD(g)	RD(g)
KS1	Control	0.092±0.01a	0.24±0.01a	0.08±0.01a	0.034±0.010a	0.16±0.01a	0.063±0.010ab
	7	0.023±0.004b	0.20±0.02b	0.07±0.01a	0.022±0.003b	0.10±0.07a	0.037±0.000c
	14	0.022±0.007b	0.19±0.04bc	0.07±0.02a	0.016±0.005b	0.08±0.01a	0.030±0.005c
	21	0.00±00c	0.17±0.01bc	0.07±0.02a	0.00±0.000c	0.07±0.01a	0.026±0.003c
KS2	Control	0.010±0.003c	0.07±0.02d	0.06±0.02b	0.094±0.012a	0.21±0.04a	0.083±0.006a
	7	0.06±0.003c	0.03±0.0e	0.04±0.03cd	0.026±0.006b	0.20±0.02a	0.073±0.007abc
	14	0.005±0.002c	0.02±0.01e	0.02±0.01e	0.024±0.022b	0.14±0.06a	0.070±0.022abc
	21	0.00±00c	0.02±0.0e	0.03±0.01de	0.017±0.007b	0.11±0.05a	0.050±0.007abc
KS3	Control	0.023±0.005b	0.18±0.02b	0.07±0.01a	0.031±0.009b	0.19±0.04a	0.077±0.006a
	7	0.018±0.009bc	0.17±0.03bc	0.06±0.02bc	0.026±0.006b	0.17±0.01a	0.060±0.000abc
	14	0.013±0.002c	0.16±0.04c	0.05±0.00bc	0.022±0.007b	0.16±0.07a	0.053±0.012abc
	21	0.00±00c	0.03±0.03e	0.03±0.01de	0.000±0.000c	0.12±0.09a	0.040±0.009b

Means followed by the same superscript letter in each column are not significantly different at $P<0.05$ according to the LSD test. KS1, *Rhizobium* isolated from *Acacia tortilis* seedlings; KS2, *Rhizobium* isolated from *Leucaena leucocephala* seedlings; and KS3, *Rhizobium* isolated from *Acacia saligna* seedlings. SD, Shoot dry weight (g); RD, Root dry weight (g); Nod D, nodular dry weight (mg).

compared with control seedlings, respectively. The seedlings diameter varied between KS1 and KS2 (Table 1).

Effect of Drought on Seedlings Biomass of *Acacia origena*: The interaction between Rhizobium isolates and drought stress periods was significant for shoot and root dry weight in both seasons. Table 2 shows the mean values of the interaction between the rhizobium isolates and drought stress periods during the 1st and 2nd seasons with regard to seedling biomass. The nodule dry weight and shoot and root dry weight were reduced with increasing drought treatments and 21 days of withholding irrigation was the treatment with the greatest effect on seedling biomass (Table 2). The Rhizobium isolates varied in their tolerance to drought; the seedlings inoculated with rhizobium KS1 produced the highest number of nodules, shoot and root dry weight in the first season, while the Rhizobium isolate KSA2 had the highest number of nodules, shoot and root dry weight in the second season.

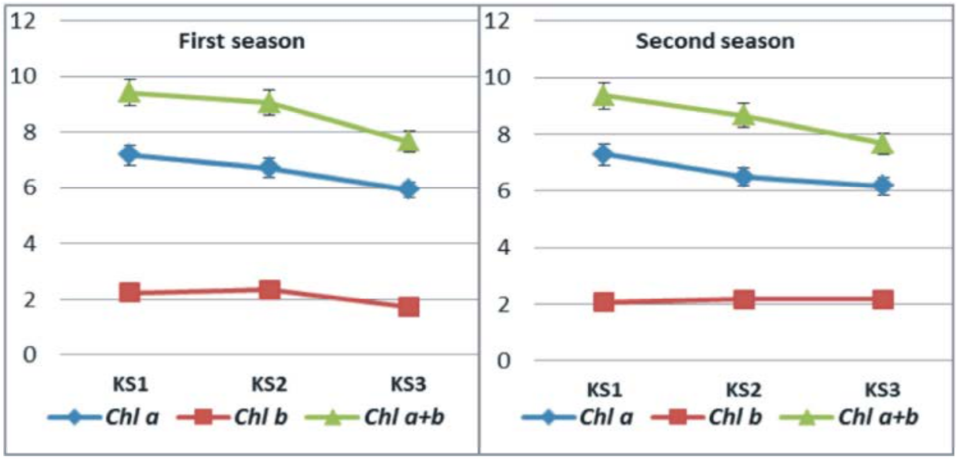

Fig. 3: Mean effect of Rhizobium isolates on chlorophyll content over two seasons.

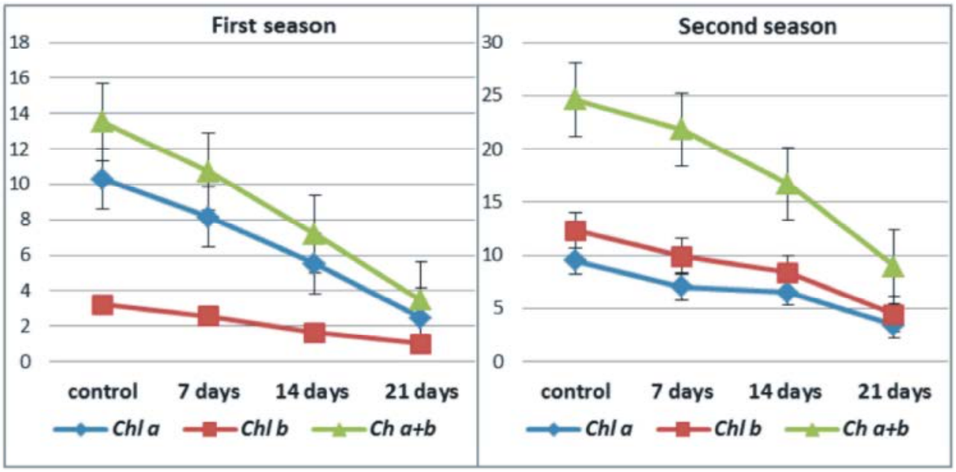

Fig. 4: Mean effect of drought period on chlorophyll content over two seasons.

Table 3: Interaction effect between Rhizobium isolates and drought period on chlorophyll content of *Acacia origena* over two seasons

		Chlorophyll contents (µml/l)					
		First season			Second Season		
Rhizobium isolates	Drought period (Days)	*Chl. a*	*Chl. b*	*Chl. a+b*	*Chl. a*	*Chl. b*	*Chl. a+b*
KS1	Control	10.6±0.9a	3.47±0.29a	14.05±1.2a	10.3±1.13a	2.89±0.25ab	13.12±1.39a
	7	8.36±0.46bc	2.71±0.11ab	11.06±0.55bc	9.65±1.28a	2.73±0.41ab	12.37±1.67ab
	14	7.47±0.67c	2.07±0.09b	9.54±0.75c	4.65±0.80b	1.47±0.74bc	6.11±0.07c
	21	2.30±1.0f	0.73±0.27c	2.03±1.2f	4.60±0.39b	1.18±0.09c	5.79±0.37c
KS3	Control	10.6±0.97a	3.47±0.29a	14.05±1.2a	7.95±4.47ab	3.10±0.44a	11.04±4.71ab
	7	8.61±0.58b	2.88±0.17ab	11.46±0.73bc	7.94±4.27a	2.71±1.48ab	10.66±5.95ab
	14	4.64±0.80d	1.47±0.74bc	6.11±0.06de	7.44±0.63bc	1.94±0.14bc	9.38±0.52bc
	21	3.01±0.11ef	1.56±0.89b	4.57±0.9ef	2.64±0.65c	0.91±0.18c	3.55±0.83c
KS3	Control	9.72±1.68ab	2.73±0.46ab	12.45±2.1ab	10.23±1.14ab	2.89±0.26ab	13.12±1.39a
	7	7.47±0.67c	2.07±0.09b	9.54±0.75c	9.63±01.31ab	2.70±0.45ab	12.32±1.75ab
	14	4.46±0.70de	1.40±0.79bc	5.87±0.37e	7.49±0.70b	2.10±0.14b	9.59±0.83bc
	21	2.08±0.30f	0.69±0.22c	2.77±0.52f	3.05±0.0.66c	0.99±0.12c	4.04±0.76c

Means followed by the same superscript letter in each column are not significantly different at *P*<0.05 according to the LSD test. KS1, *Rhizobium* isolated from *Acacia tortillis* seedlings; KS2, *Rhizobium* isolated from *Leucaena leucocephala* seedlings; and KS3, *Rhizobium* isolated from *Acacia saligna* seedlings.

Effect of Drought Stress on Chlorophyll Content of *Acacia origena:* The chlorophyll content is one of the most significant parameters related to the physiological status of plants and estimations of chlorophyll content and related chlorophyll parameters can be used as indexes of nutrient status, physiological stress and changes in abiotic factors. The analysis of variance for *Acacia origena* detected variation between Rhizobium isolates, drought period and their interaction in both seasons. Chlorophyll *a* and *a+b* were significantly different, whereas chlorophyll *b* was not significantly different in the first season, while in the second season, the drought period differed significantly. The mean effect of chlorophyll content in the seedlings inoculated with Rhizobium isolate KS1 was high compared with KS2 and KS3 in both seasons (Fig. 3). The drought period had a significant effect on the chlorophyll content of the seedlings. The chlorophyll content was reduced with increasing irrigation intervals (21 days) (Fig. 4). The interaction between Rhizobium isolates and drought stress periods for chlorophyll *a* and *a+b* was significant in the first season, while in the second season was not significant. Rhizobium isolate KS2 was most tolerant to drought stress, followed by KS1 and KS3 was most sensitive to drought in the 1st and 2nd growing seasons (Table 3). The reduction percentages in the chlorophyll content of seedlings inoculated with KS2 in the first season were 18.8%, 17% and 18.4% for chlorophyll *a, b* and *a+b*, respectively. The same trend was found in the second season; the reduction in chlorophyll *a, b* and *a+b* was lower than the in first season, the percentage reductions were 1%, 13% and 3.4%, respectively (Table 3).

DISCUSSION AND CONCLUSION

The reduction in the growth of the seedlings due to drought encompassed all growth characteristics measured in the present study. Many aspects of plant growth are sensitive to drought stress in both cellular and whole-plant [18]. Decreased stem height and diameter of woody trees under drought stress conditions was reported also by El-Juhany and Aref [19]. The results of this study indicated that drought period significantly reduced the seedling height and shoot and root dry weight in *Acacia origena*. A decrease in shoot dry weight due to drought stress was inevitable such as the reduction in both height and diameter. Similar observations were reported by Pokhiryal *et al.* [20] on *Acacia nilotica*, Aref and El Guhany [21, 22] on *Acacia tortilis* and *Acacia gerrardii* subsp *negevensis* and Ramos *et al.* [23] on common bean. The mechanisms for conserving water adopted by acacia trees to endure water stress in this study seem to be related to reducing absorption *via* thin roots and decreased leaf area. The results show that under different drought stress periods, stem diameter was reduced with increasing the period of withholding irrigation compared with normal irrigation. This is in agreement with those obtained by Aref and El- Juhany [21, 22]. The decreased in root dry weight under drought stress conditions presented in our study concurs with other results for woody species in the seedling stage, such as reported by Ibrahim [24] on poplar, Aref and El-Juhany [22], on some acacia species and El-Juhany and Aref [19] on *Leucaena* spp. However, the lower percentage of reduction in shoot and root dry weight may reflect the morphological plasticity of root systems that enable them to cope with variable soil conditions.

The reduction in chlorophyll content during seedling exposed to different drought stress periods may be attributed to the decrease in number of leaves. The reduction of chlorophyll content related to the reduction of leaf area as a result of drought stress. This result is in agreement with the results obtained by Kozlowski *et al.* [25], who indicated that water deficits reduced leaf area by inhibiting the initiation of leaves, as well as their subsequent enlargement. Also, Aref and El-Juhany [22] reported that water stress reduced leaf area, as well as reduced the surface conducts photosynthesis, thus decreasing the quantity of photosynthate available for plant growth. In non-irrigated soil, the seedlings were not able to produce a new root or root tips because the water deficiency lowered the growth activity of the seedling after the nursery phase. Additionally, the root nodules become thickened, larger and more resistant to infection with Rhizobium [26]. Furthermore, results indicated that Rhizobium isolates varied in their response to drought period, but the treatment of withholding irrigation for 21 days had the most impact on nodulation. Additionally, it was found that the indigenous rhizobium isolates can be more tolerant to drought stress. The most tolerant rhizobium isolates were KS1 and KS2. This result was in contrast with the findings of Swaine *et al.* [27], who reported that the symbiosis of legume-Rhizobium, in general, is known to be more sensitive to environmental stress (especially drought) than the uninfected legume Rhizobium. Drought tolerant strains of the bacterium have been documented for both crop species and agroforestry tree species by several studies. Nodulation and nitrogen fixation in legume-*Rhizobium* associations are adversely affected by salinity and drought, which can preclude legume establishment and growth or reduce crop yield. Moreover, commercial strains of *Rhizobium* typically cannot tolerate or function under high levels of osmotic stress caused by salinity or drought [28]. In contrast, the present results showed that *Acacia origena* seedlings inoculated with Rhizobium were able to tolerate drought stress and this is in agreement with the results of Mrema *et al.* [29] and Wurzburger and Miniat [30], who indicated that growth was declined in trees under drought stress conditions, but the growth and physiological responses did not correspond to the rhizobium species and their ability to fix nitrogen.

This study demonstrated that drought stress had negative effects on the growth of *Acacia* trees. Acacia species are able to survive under stress conditions but their behaviour differed in terms of tolerance to different levels of drought stress. Under the conditions of this study, drought stress significantly reduced both the seedling growth and nodulation of Acacia *origena.* However, the Rhizobium isolates were able to produce nodules on the seedlings of *Acacia origena* under drought stress. Thus, indigenous Rhizobium strains can help the Acacia tress to withstand the stress conditions found in the field. Our results suggest that the selection of Rhizobium isolates with tolerance to drought stress is essential for reforestation and improving the productivity of Acacia trees under the natural conditions of Saudi Arabia. Even if these abiotic factors do not act alone in natural conditions, it is necessary to test the efficiency of the Rhizobium isolates under shade house conditions prior to using them as inoculants in the field.

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REFERENCES

1. Fall, D., M. Ourarhi, M. Missbah, E.N. Bakhoum, A.M. Zoubeirou and H. Abelmoumem, 2011. The efficiency and competitiveness of three *Mesorhizobium* sp. strain nodulating *Acacia senegal* (L.) Willd under water deficiency conditions in greenhouse. Symbiosis, 54: 87-94.
2. Bray, E.A., 2004. Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*. Journal of Experimental Botany, 55: 2331-2341.
3. Fereres, E. and M.A. Soriano, 2007. Deficit irrigation for reducing agricultural water use. Journal of Experimental Botany, 58(2): 147-159.
4. Ruíz-Lozano, J.M., M.C. Perálvarez, R. Aroca and R. Azcón, 2011. The application of a treated sugar beet waste residue to soil modifies the responses of mycorrhizal and non mycorrhizal lettuce plants to drought stress. Plant and Soil, 346: 153-166.
5. Schimel, D.S., 2010. Dry lands in the earth system. Science, 327: 418-419.
6. Zahran, H.H., 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. Journal of Biotechnology, 91: 43-53.
7. Zakhia, F., H. Jederz, O. Domerguei, A. Willems, J.C. Cleyet-Mareh, M. Gillis, B. Dreyfus and P. de Lajudie, 2004. Characterisation of wild legume nodulating bacteria (LNB) in the infra-arid zone of Tunisia. Systematic and Applied Microbiology, 27: 380-395.
8. Wolde-meskel, E., T. Berg, N.K. Peters and A. Frostegård, 2004. Nodulation status of native woody legumes and phenotypic characteristics of associated rhizobia in soils of Southern Ethiopia. Biology and Fertility of Soils, 40: 55-66.
9. Odee, D.W., J.M. Sutherland, E.T. Makatiani, S.G. McInroy and J.I. Sprent, 1997. Phenotypic characterization and composition of rhizobia associated with woody legumes growing in diverse Kenyan conditions. Plant Soil, 188: 65-75.
10. Nick, G., P. de Lajudie, B.D. Eardly, S. Soumalainen, L. Paulin, X. Zhang, M. Gillis and K. Lindström, 1999. *Sinorhizobiu marboris* sp. nov and *Sinorhizobium kostiense* sp. nov., isolated from leguminous trees in Sudan and Kenya. International Journal of Systematic Bacteriology, 49: 1359-1368.
11. Sprent, J.I., 2009. Legume Nodulation: A global Perspective. Wiley-Blackwell, Oxford.
12. Sprent, J.I. and H.S. Gehlot, 2010. Nodulated legumes in arid and semi-arid environments: are they important?. Plant Ecology and Diversity, 3: 211-219.
13. dos Reis, F.B.Jr., M.F. Simon, E. Gross, R.M. Boddey, G.N. Elliott, N.E. Neto, M.F. Loureiro, L.P. de Queiroz, M.R. Scotti, W.M. Chen, A. Noren, M.C. Rubio, S.M. de Faria, C. Bontemps, S.R. Goi, J.P.W. Young, J.I. Sprent and E.K. James, 2010. Nodulation and nitrogen fixation by *Mimosa* spp. in the Cerrado and Caatinga Biomes of Brazil. New Phytologist, 186: 934-946.

14. Abd-Alla, M.H. and A.M. Abdel Wahab, 1995. Survival of *Rhizobium leguminosarum* biovar *viceae* subjected to heat, drought and salinity in soil. Biologia Plantarum, 37(1): 131-137.
15. Vincent, J.M., 1970. A Manual for the Practical Study of Root-Nodule Bacteria. Blackwell Scientific, Oxford.
16. Porra, R.J., W.A. Thompson and P.E. Kriedemann, 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA) Bioenergetics, 975(3): 384-394.
17. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods.7th Ed., Iowa State Univ. Press, Ames, Iowa.
18. El-Juhany, L.I. and I.M. Aref, 2005. Interactive effects of low water supply and high salt concentration on the growth and dry matter partitioning of *Conocarpus erectus* seedlings. Saudi Journal of Biological Sciences, 12(2): 147-157.
19. El-Juhany, L.I. and I.M. Aref, 1999. Growth and dry matter partitioning of *Leucaena leucocephala* (Lam.) de Wit. trees as affected by water stress. Alexandria Journal of Agricultural Research, 44(2): 237-259.
20. Pokhriyal, T., C. Uma-Singh, S.P. Chukiyal and U. Sing, 1997. Effects of water stress treatments on growth parameters and nitrogenase activity in *Acacia nilotica*. Indian Journal of Plant Physiology, 2(1): 72-74.
21. Aref, I.M. and L.I. El-Juhany, 1999. Effects of drought stress on the growth of *Acacia asak* (Forssk.), *A. tortilis* (Forssk.) and *A. gerrardii* (Benth) ssp. *Negevensis* (Zoh.). Journal of Agriculture Sciences, Mansoura University, 24(10): 5627-5636.
22. Aref, I.M. and L.I. El-Juhany, 2001. Impact of sudden water stress on the growth of eight acacia species. Alexandria Science Exchange Journal, 22(4): 413-422.
23. Ramos, M.L.G., R. Parsons, J.I. Sprnt and E.K. James, 2003. Effect of water stress on nitrogen fixations and nodule structure of common bean. Pesquisa Agropecuária Brasileira, 38: 339-347.
24. Ibrahim, L., M.F. Proe and A.D. Cameron, 1997. Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. Canadian Journal of Forest Research, 27(9): 1413-1419.
25. Kozlowski, T.T., P.J. Kramer and S.G. Pallady, 1991. The Physiological Ecology of Woody Plants. Academic Press, INC., San Diego, New York, Boston, London, Sydney, Tokyo, Toronto, pp: 796.
26. Miettinen, P., I.O. Luukkanen, S. Johansson, E. Eklundand and J.N. Mulatya, 1988. 3Rhizobium nodulation in *Prosopis juliflora* seedlings at different irrigation levels in eastern Kenya. Plant and Soil, 112: 233-238.
27. Swaine, E.K., M.D. Swaine and K. Killham, 2007. Effects of drought on isolates of *Bradyrhizobium elkanii* cultured from *Albizia adianthifolia* seedlings of different provenances. Agroforestry Systems, 69: 135-145.
28. Mohammad, R.M., M.Z. Akhavan-Kharazian, W.F. Campbell and M.D. Rumbaugh, 1991. Identification of salt-and drought-tolerant *Rhizobium meliloti* L. strains. Plant and Soil, 134: 271-276.
29. Mrema, A.F., U. Granhall and L. Sennerby-Forsse, 1997. Plant growth, leaf water potential, nitrogenase activity and nodule anatomy in *Leucaena leucocephala* as affected by water stress and nitrogen availability. Trees, 12: 42-48.
30. Wurzburger, N. and C.F. Miniat, 2014. Drought enhances symbiotic dinitrogen fixation and competitive ability of a temperate forest tree. Oecologia, 174: 1117-1126.