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Interactive effects of seed priming and watering frequency on *Acacia tortilis* seedlings growth performance in arid saline field

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Abstract: First year growth and survival is very crucial for successful planting in arid lands. Seed priming proved to enhance seedling performance under various growth conditions. In this study, seed samples of *Acacia tortilis* were primed in different osmo-conditioning and hormonal priming solutions, sown in the nursery, and then the produced seedlings were transplanted into the open field under different levels of watering frequencies.

The results obtained revealed significant effects of irrigation and seed priming treatments and their interaction in all growth traits measured, indicating that priming treatments respond differently under different irrigation. The interactive effects produced better growth performance of treated seedlings under all watering treatments. However, treating the seedlings with PEG osmo-priming at the rate of 10% followed by hormonal priming at the rates of 200 and 300 ppm produced better values across all irrigation treatments, with PEG10 ranked better in most treatments. While the trend in growth rate showed that during the early growth stages all the treatments obtained close results and were started to separate with increasing time. In regards to irrigation treatment, irrigating the seedlings twice a week and once every week revealed no significant difference in all growth traits, indicating no need to irrigate twice a week. The analysis of antioxidant compounds (total phenols and total flavonoids) showed similar trend with high values in primed seedlings. In the same way, PEG 10% produced higher phenols and flavonoids across all watering treatments. In another hand, with in the irrigation treatment both phenols and flavonoids were increased with increasing watering frequency. The results concluded that priming the seeds would improve early growth performance for this species under arid saline conditions.

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Introduction

A. tortilis is an important arid and semi-arid tree providing valuable requirements to the local population and animals (Yadav et al., 2013). It plays a beneficial role in human, animal and other plant species lives (Abd El Rahman and Krzywinski, 2008). Also, provide firewood, local charcoal and livestock feed (Timberlake, 1980) as well as some medicinal values such as treatment of various diseases like skin allergy, cough, and inflammatory reaction (Yadav et al., 2013). This vulnerable species facing serious threats due to a combination of many factors including urban expansion, infrastructure development and climate change (GAMEP, 2017).

Drought has been a major problem affecting plant growth and ecosystem productivity in arid and semi-arid areas (Zhenzhu et al., 2010). The reduction of plant productivity happen as the result of inhibiting growth factors (Singh et al., 2014) and by inducing stomatal closure, and thus reduces photosynthesis (Németh et al., 2002). Whereas, global warming lead to water deficit through evapotranspiration, thus increasing the frequency and density of drought (Fischlin et al., 2007).

The process of seed priming includes the exposure of the seeds to an external factor making the germinated seedlings more tolerant to future stress (Tanou et al., 2012). Therefore, the process of seed priming improves germination by empowering the seeds to withstand environmental stresses that happen during germination (Farooq et al., 2009).

Osmo-priming which is known as osmo-conditioning of the seeds in order to reduce the osmotic potential of the sowing media by soaking the seeds for a specific period of time in osmotic solutions such as sugar, polyethylene glycol (PEG), glycerol, sorbitol, or mannitol (Nawaz et al., 2013). The osmo-regulation may decrease plant growth, however, it significantly increased the tolerance of the plants to water stress (Meher et al., 2018).

While hormonal priming with Gibberellic acid (GA3) improves photosynthetic capacity, antioxidant system and seedling emergence (Galhaut et al., 2014) and enhances vegetative growth of the plants (Ashraf et al. 2002). It also plays an important role in stem or internode elongation and stimulates cell division and expansion (Gupta and Chakrabarty, 2013).

The aim of this study was to test the interactive effects of seed priming and reduced water frequency on *Acacia tortilis* field performance under arid saline soils of Kingdom of Saudi Arabia.

Materials and Methods

Experimental site

This experiment was carried in King Abdulaziz University Experimental Farm at Hada Al-Sham area approximately 100 km North-East of Jeddah city. The farm is located in Latitude 21º 48' 3" N, Longitude 39º 43' 25" E and 240 masl elevation).

Experimental design and treatments

For this experiment, a split plot design with three replicates was used. The main plots treatments were represented by three watering frequencies (twice a week, once every week and once every two weeks). While the subplots were fitted with seed priming treatments: PEG-5%, PEG-10% and PEG-20% as asomo-priming and GA3 200 ppm, GA3 300 ppm and GA3 500 ppm, as hormonal priming, plus control untreated seeds. The primed seedlings were allowed to grow in the nursery for four months before been transplanted into the open field. In the field, the irrigation treatments were applied and the primed seedlings were fitted in each irrigation treatment. Drip irrigation system was use for irrigation. The seedlings in the open field were allowed to grow for one year.

Measurements of growth parameters in the field

Four consecutive measurements of growth parameters (height "HT", root collar diameter "RCD" were made at interval of every three months. Then relative diameter (RDGR) and height (RMGR) growth rates were analyzed. The relative growth rate was calculated through the following equations of (Waston, 1958) as RHGR = $(H_{last} - \text{In } H_{initial}) / (t_{last} - t_{initial})$, H in cm where t in months. While RDGR Diameter = $(\text{In } D_{last} - D_{initial}) / (t_{last} - t_{initial})$; D in mm where t in months.

Determination of total phenols (TP) and total flavonoids (TF)

For determination of TP and TF fresh leaves from each treatment in each block were collected and kept in liquid nitrogen and transferred to the Department for analysis. In the Department Lab the samples were stored under -30 C$^{o.}$ Later the samples were extracted with methanol, then TP was determined according to the method described Hoff and Singleton (1977) and TF was determined using a modified colorimetric method as described by Zhishen et al. (1999).

Results and discussion

Growth traits

In this study analysis of variance showed that the effects of irrigation and seed priming treatments as well as their interaction was significantly differed in all growth traits measured (root collar diameter, seedlings height, relative diameter growth rate and relative height growth rate) (Table 1). The positive effects of seed priming produced under different irrigation regimes in the open field highlights the importance of priming the seeds under such harsh environments. While the significant irrigation*seed priming interaction indicates that the seed priming respond differently under different irrigation. In another hand the results of Duncan's multiple range mean separation test revealed that seedlings performance in the open field after one year of growth were not significantly varied between whether watering the seedlings at the rate of twice a week or once every one week and both were significantly higher than irrigating the seedlings once every two weeks (Table 1). This may indicate that as general seedlings can be irrigated once every week in these areas. In regards to priming treatments among the irrigation most of the treatments were significantly produced higher growth values compared to the control (Table 2). This reflects similar results to Sisodia et al. (2018) in that seed priming can enhance positive effects in improving seedlings growth. Within the three irrigations the treatments of PEG10, GA300 and GA200 obtained better values in all growth traits measured. However, in general treating the seedlings with PEG 10% produced better growth values, followed by GA3 at the rates of 300 and 200 ppm most of the irrigations. This trend which was obtained in the field was similar to the trend in the nursery after four months of growth. This may be explained by the fact that either irrigating the seedlings every two weeks is not enough to screen the water stress adaptive response and hence reflects the treatments with better adaptive enhancement or may be one year is not enough for the seedlings to overcome the residual effects of the treatments obtained in the nursery.

The trends of diameter and height growth rate over time were demonstrated in figures 1a, b and c & 2a, b and c. From the graphs it is clear that diameter and height growth revealed that all the treatments were clustered during the first measurements at the age of 3 month and 6 month measurements, where it start to separate in the third and fourth measurements (at the age of 9 and 12 months, respectively). However, the best performance of seedlings height and diameter relative growth rate produced by PEG 10% under all irrigation treatments, may highlight the significance of osmo priming under water stress and saline conditions. Improvement of germination and seedlings growth under water stress was reported by (Topacoglu et al., 2016). While Hur, (1991) reported that osmo-priming enhanced general crop performance under saline conditions.

On the other hand, GA3 300ppm and 200ppm achieved high results in all irrigation periods. This confirms that Gibberellic acid (GA3) improvement of photosynthetic properties, antioxidant system and seedling emergence (Galhaut et al., 2014) and enhancement of vegetative growth (Ashraf et al. 2002).

Total phenols and total flavonoids

Analysis of variance showed that *A. tortilis* leaves total phenols content were significantly differed between irrigation frequency as well as irrigation*seed priming interaction, but was not significantly differed among seed priming treatment. In regards to total flavonoids the data analyzed showed a very high significant differences between irrigation and seed priming treatments as well as the interaction between them (Table 3). The positive irrigation*seed priming interaction shown in this study for both total phenols and total flavonoids indicates that seed priming effects respond differently under different water stress as earlier reported by (Espanany et al., 2016).

In regards, to irrigation frequency effects the data obtained showed that both total phenols and total flavonoids were increased with increased water frequency (Vosoughi, et al., 2018). While for the seed priming effects within irrigation the data showed that seedlings treated with PEG at different levels produced higher total phenols and total flavonoids values than seedlings treated with GA3 hormonal solutions (Table 4). Our explanation to this may be due to the fact that PEG osmo-priming normally induces more negative osmotic potential leading to seedlings more tolerant to adverse conditions (Pradhan, et al., 2014). Like seedlings growth, PEG treatment at the rate of 10% obtained better TP and TF values.

Table1. Effects of irrigation frequency on root collar diameter (RCD), height (HT), relative diameter growth rate (RDGR) and relative height growth rate (RHGR) in *Acacia tortilis* grown under arid saline soil

Irrigation	RCD	HT	RDGR	RHGR
Twice a week	20.71 ^{a}	128.48 ^{a}	1.97 ^{a}	10.96 ^{a}
Once a week	20.42 ^{a}	130.21 ^{a}	1.84 ^{a}	10.99 ^{a}
Once every 2 weeks	14.47 ^{b}	103.37 ^{b}	1.22 ^{b}	7.94 ^{b}
Irrigation effect (MS+P)	211.99**	4294.62**	2.63**	49.38**
Priming effect (MS+P)	37.15**	3357.79**	0.56**	44.39**
priming * Irrigation effect (MS+P)	19.59**	924.21*	0.25*	17.08*

* Means with the same letter in the same column are not significantly different at p= 0.05 using new Duncan's multiple range test.
* P≤ 0.05 ** P≤ 0.01 ns = Not significant

Table 2. Effects of PEG and GA3 seed priming within irrigation on seedlings root collar diameter (RCD), height (HT), relative diameter growth rate (RDGR) and relative height growth rate (RHGR) in *Acacia tortilis* grown under arid saline

Irrigation	Treated	RCD	HIT	DRGR	HRGR
Twice a week	PEG 5%	18.3 ^{bc}	120.6 ^{bcd}	1.8 ^{b}	11.3 a^{b}
	PEG 10%	25.6 ^{a}	168.6 ^{a}	2.5 ^{a}	15.1 ^{a}
	PEG 20%	21.3 a^{b}	117.3 ^{cd}	2.12 a^{b}	10.2 ^{bc}
	GA3 200 ppm	23.0 a^{b}	161.3 a^{b}	2.14 a^{b}	14.1 a^{b}
	GA3 300 ppm	21.3 a^{b}	150.6 ^{abc}	1.9 ^{b}	12.8 a^{b}
	GA3 500 ppm	20.3 ^{b}	88.6 ^{d}	1.8 ^{ab}	6.4 ^{c}
	Control	15.0 ^{c}	92.0 ^{d}	1.2 ^{c}	6.6 ^{c}
Once a week	PEG 5%	17.3 ^{bc}	102.6 ^{b}	1.5 ^{ab}	8.4 ^{b}
	PEG 10%	23.5 a^{b}	135.0 ^{ab}	2.3 ^{a}	13.1 ^{a}
	PEG 20%	22.0 ^{abc}	126.6 ^{ab}	1.8 ^{ab}	8.7 ^{b}
	GA3 200 ppm	21.0 ^{abc}	146.0 ^{ab}	1.9 ^{ab}	13.8 ^{a}
	GA3 300 ppm	24.0 ^{a}	150.6 ^{a}	2.2 ^{a}	12.3 ^{ab}
	GA3 500 ppm	19.5 ^{abc}	133.0 ^{ab}	1.8 ^{ab}	12.6 ^{ab}
	Control	16.3 ^{bc}	120.0 ^{ab}	1.3 ^{b}	8.7 ^{b}
Once every 2 weeks	PEG 5%	15.0 ^{b}	75.6 ^{c}	1.1 ^{b}	4.2 ^{b}
	PEG 10%	20.5 ^{a}	120.5 ^{abc}	1.9 ^{a}	12.6 ^{a}
	PEG 20%	11.5 ^{c}	90.0 ^{abc}	0.9 ^{b}	7.5 ^{ab}
	GA3 200 ppm	13.6 ^{bc}	124.6 ^{ab}	1.0 ^{b}	9.7 ^{ab}
	GA3 300 ppm	14.0 ^{bc}	135.3 ^{a}	1.2 ^{b}	11.3 ^{ab}
	GA3 500 ppm	13.6 ^{bc}	84.5 ^{bc}	1.1 ^{b}	5.7 ^{ab}
	Control	14.0 ^{bc}	92.3 ^{abc}	1.1 ^{b}	5.2 ^{b}

*Means with the same letter in the same column are not significantly different at p= 0.05 using new Duncan's multiple range test.

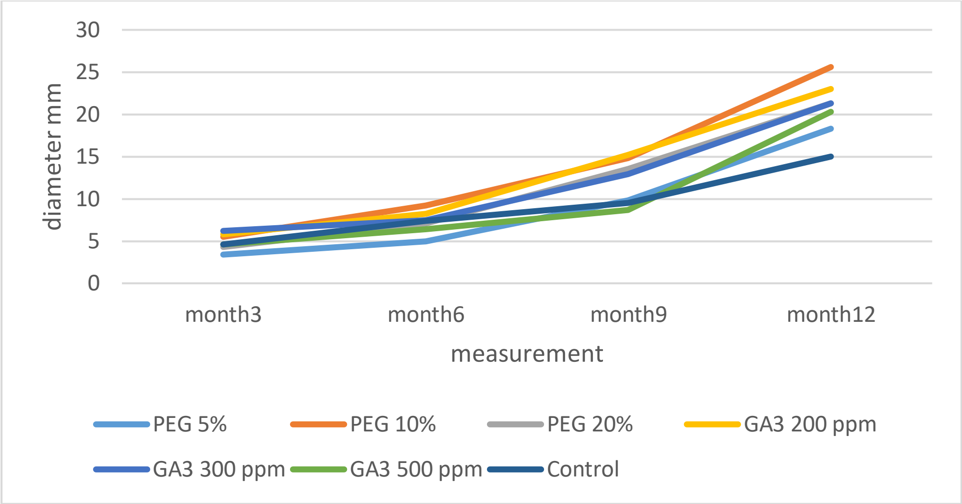

Fig. 1a. Trend in *Acacia tortilis* root collar diameter growth as affected by PEG and GA3 seed priming treatment under irrigation twice a week

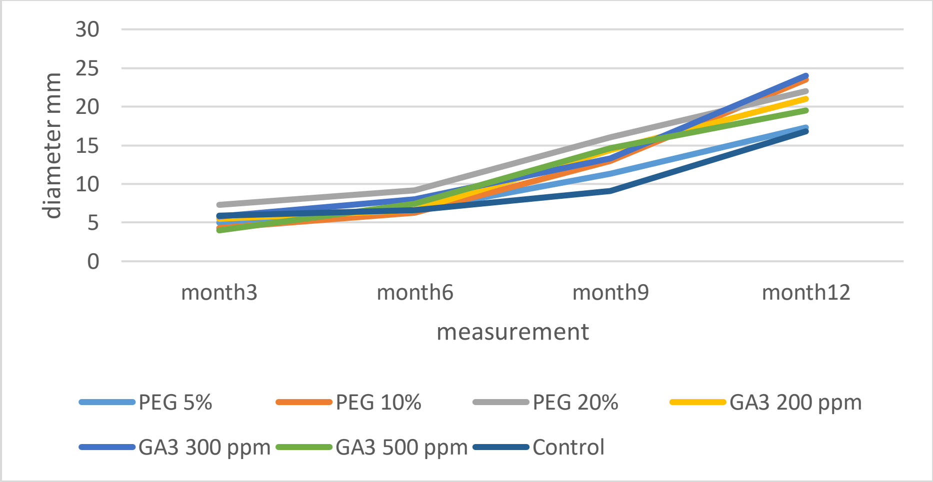

Fig. 1b. Trend in *Acacia tortilis* root collar diameter growth as affected by PEG and GA3 seed priming treatment under irrigation once a week

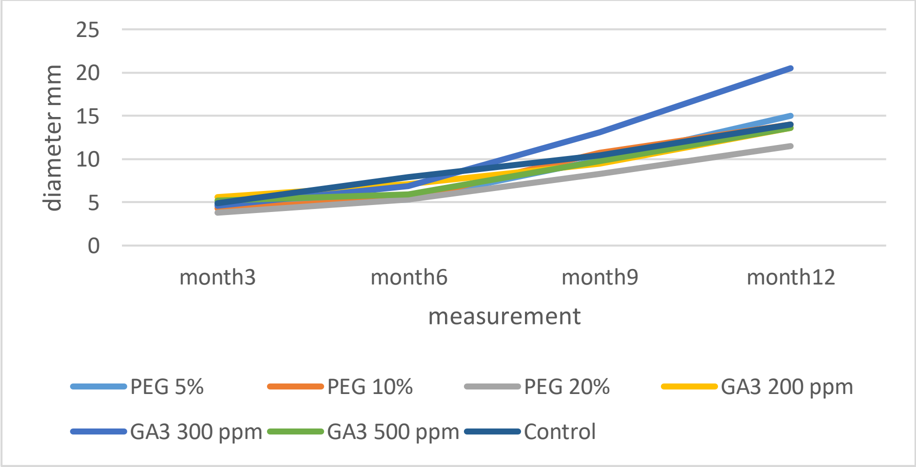

Fig. 1c. Trend in *Acacia tortilis* root collar diameter growth as affected by PEG and GA3 seed priming treatment under irrigation once every two weeks

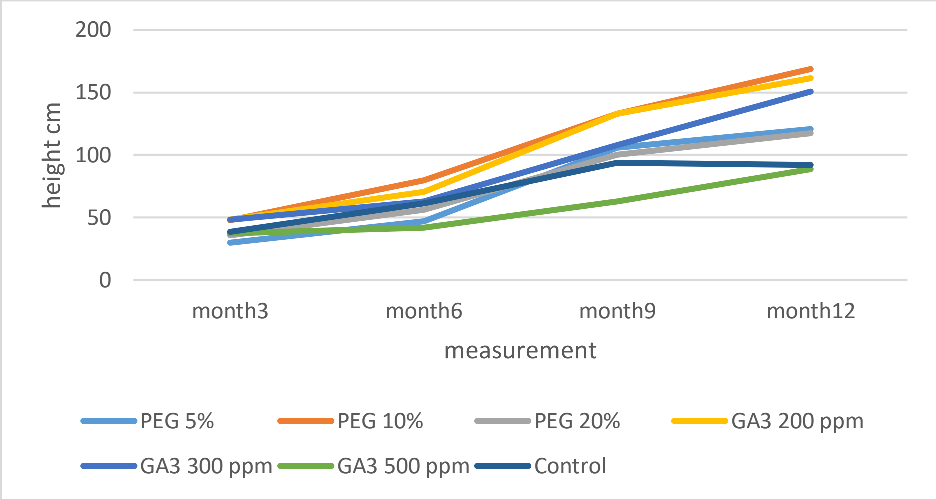

Fig. 2a. Trend in *Acacia tortilis* height growth as affected by PEG and GA3 seed priming treatment under irrigation twice a week

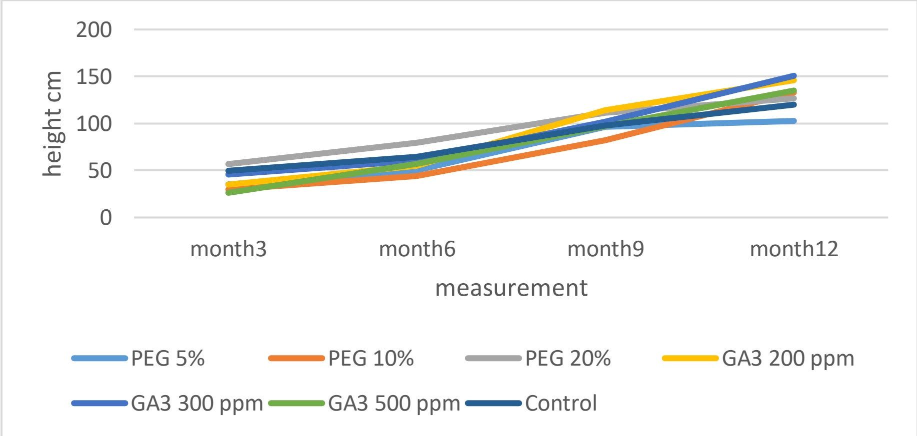

Fig. 2b. Trend in *Acacia tortilis* height growth as affected by PEG and GA3 seed priming treatment under irrigation once a week

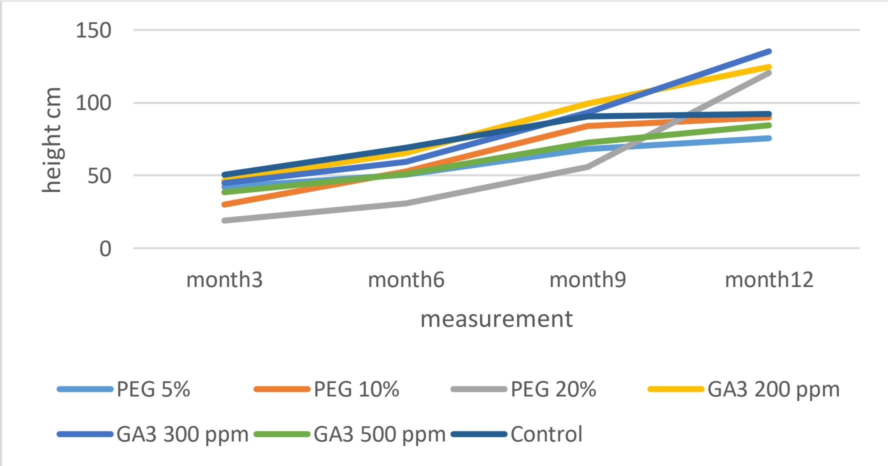

Fig. 2c. Trend in *Acacia tortilis* height growth as affected by PEG and GA3 seed priming treatment under irrigation once every two weeks

Table 3. Effects of Irrigation of irrigation frequency on total phenols (TP) and total flavonoids (TF) in *Acacia tortilis* grown under arid saline soil

Irrigation	TP	TF
Twice a week	1.02 [a]	2.64 [a]
Once a week	0.89 [a b]	1.85 [b]
Once every 2 weeks	0.68 [b]	1.69 [b]
Irrigation effect (MS+P)	0.615*	5.367**
Priming effect (MS+P)	0.268ns	0.239*
priming * Irrigation effect (MS+P)	0.563**	0.920**

* Means with the same letter in the same column are not significantly different at p= 0.05 using new Duncan's multiple range test.

* P≤ 0.05 ** P≤ 0.01 ns = Not significant

Table 4. Effects of PEG and GA3 seed priming within irrigation on *Acacia tortilis* leaves total phenols (TP) and total flavonoids (TF)

Irrigation	Treated	TP	TF
Twice a week	PEG 5%	1.83a	3.03 [ab]
	PEG 10%	1.39a	3.26 [a]
	PEG 20%	0.50a	2.28 [bc]
	GA3 200 ppm	1.07a	2.83 [ab]
	GA3 300 ppm	0.47a	2.46 [bc]
	GA3 500 ppm	1.30a	2.85 [ab]
	Control	1.04a	1.73 [c]
Once a week	PEG 5%	0.97 a	2.07a
	PEG 10%	1.14a	2.17a
	PEG 20%	0.42a	1.57a
	GA3 200 ppm	1.11a	1.80a
	GA3 300 ppm	0.63a	1.52a
	GA3 500 ppm	1.29a	2.15a
	Control	0.64a	1.62a
Once every 2 weeks	PEG 5%	0.33 [c]	1.20 [bc]
	PEG 10%	1.40 [a]	2.43 [a]
	PEG 20%	1.38 [a]	2.42 [a]
	GA3 200 ppm	0.62 [bc]	1.78 [b]
	GA3 300 ppm	0.44 [c]	1.28 [bc]
	GA3 500 ppm	0.32 [c]	0.95 [c]
	Control	1.27 [ab]	1.80 [b]

*Means with the same letter in the same column are not significantly different at p= 0.05 using new Duncan's multiple range test.

Conclusions

The results obtained in this study showed that seeds priming by PEG and GA3 improved growth performance and enhanced antioxidant production for this species under arid saline conditions. Osm-priming resulted in better growth values followed by hormonal priming. Treating the seedlings with osmo-priming PEG 10% was resulted in better growth performance followed by GA3 300ppm and GA3 200 ppm. In regards, to antioxidant compounds (total phenols and total flavonoids contents) osmo-priming obtained better values with PGE 10% as better treatment. Concerning the irrigation frequencies used both total phenols and total flavonoids were increased with increasing irrigation frequency.

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