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#### Research Article

# Seed priming improved germination, seedling quality and response to imposed drought stress in Vachellia tortilis subsp. spirocarpa (Hochst. Ex A. Rich.)

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#### Abstract

Successful afforestation in arid lands requires rapid, uniform seed germination and good quality seedlings. Seed priming is a pre-treatment of seeds to improve germination and produce seedlings that withstand changing environmental conditions. Therefore, the present study aimed to test the effects of seed priming on Vachellia tortilis seed germination, nursery seedlings quality and the response of produced seedlings to imposed drought stress. Seed samples of V. tortilis were primed in three concentrations of osmo-priming solutions (polyethylene glycol (PEG-6000)) and three concentrations of hormonal priming solutions (Gibberellic acid (GA3)). Then, the effects were monitored during seed germination, nursery seedling development, and in the greenhouse under three successive cycles of drying and rewatering treatments. During germination, seed priming resulted in a higher total germination percentage (TGP), accelerated uniform germination (higher germination index (GI) and lower mean germination time (MGT)), and increased seedling vigour index (SVI). The recorded improvement in germination was associated with stimulation of antioxidant enzymes (peroxidase (POD) and polyphenol oxidase (PPO)) and hydrolytic enzymes (Polygalacturonase (PG) and α-amylase) activities in primed germinated seeds. While in the nursery, priming significantly improved the quality traits of seedlings, including root collar diameter, shoot-to-root ratio, sturdiness quotient, and Dickson's quality index. GA3-300ppm, GA3-200ppm, and PEG-10% treatments resulted in increases in total dry weight of 160%, 121%, and 64%, respectively. The high-quality seedlings produced resulted in better growth and survival under imposed drought stress in the greenhouse. The findings concluded that seed priming is crucial for enhancing the germination and quality of V. tortilis

Keywords: Antioxidant and hydrolytic enzymes, Dry out and rewatering, GA3, PEG-6000, Seed germination, Seed priming, Seedlings quality

# INTRODUCTION

Rehabilitation of degraded forests requires the best seed germination characteristics and high-quality seedling development that can pass the first-year survival test, a common challenge in harsh environments. The process of seed germination consists of three successive phases to complete: the first phase is the imbibition phase, during which water is absorbed; the second

phase is the lag phase, during which various biochemical processes occur; and the third phase is the germination of seeds, recognised by the emergence of the radicle. Seed priming is a method used to pretreat seeds during germination and before sowing, involving the controlled hydration of seeds in specific solutions for a certain period to activate germination without allowing the emergence of the radicle (Ibrahim, 2016). This process normally takes place during the lag phase

of the germination. This treatment aims to induce physiological, biochemical and molecular changes such as cell division and elongation, stress response proteins, and antioxidant and hydrolytic enzyme activities (Nawaz et al., 2013). The stimulating process enables pre-germinated seeds to overcome environmental stresses that occur when they are later sown (Faroog et al., 2009). The effects will extend to improving the development of early seedlings (Blunk et al., 2019) and field performance (AlZubaidi et al., 2021). Thus, this method is now widely used to break seed dormancy, enhance rapid and uniform germination, and produce better quality seedlings under various conditions (Sisodia et al., 2018). It promotes speedy seed germination and better plant growth performance (Espanany et al., 2016). Moreover, the process of seed priming can enhance certain signalling factors, making plants more tolerant to future environmental stress (Tanou et al., 2012). Antioxidant enzymes play a major function in breaking down and removing free radicals from cells (Govindaraj et al., 2017). Many of these enzymes such as peroxidase (POD) and polyphenol oxidase (PPO), can be induced through seed priming treatment (Bogdanovic et al., 2008). While hydrolytic enzymes are group of hydrolases that break the chemical bonds of the molecules in the presence of water. The  $\alpha$ amylase enzyme plays a vital role in hydrolysing starch in seeds, which enhances germination and early seedling growth (Nawaz et al., 2013). While Polygalacturonase (PG) is involved in various plant biochemical processes, such as seed germination and embryo development (Wang et al., 2016).

Osmo-conditioning of seeds with PEG-6000 is used to shorten germination time and produce more uniform seedlings (Carvalho *et al.*, 2020). It can also enhance enzyme activities and improve germination or early seedling growth under stress conditions (AlZubaidi *et al.*, 2021). Moreover, it can affect the glyoxylate pathway, which transforms lipids into sugars, a process crucial for early embryo development (Taiz and Zeiger, 2002). Therefore, osmo-priming contributes to the improvement of the germination rate by increasing various enzymatic activities and activating processes related to the cell cycle (Nawaz *et al.*, 2013).

It is known that Gibberellic Acid (GA3) is involved in seedling emergence, the photosynthetic process, and the antioxidant system (Galhaut *et al.*, 2014). Ashraf *et al.* (2002) reported that the application of GA3 enhances vegetative growth and precipitates Na and CI- in roots and shoots, thus leading to a significant increase in photosynthetic capacity. Not only does GA3 affect seed germination, but it also influences stem elongation, the development of meristematic tissues, and the differentiation of floral organs. It contributes to the development of stems or internodes and enhances cell division and expansion (Gupta and Chakrabarty, 2013).

In dry land environments, recurrent droughts that occur in multiple cycles of drought-recovery events are more common than prolonged droughts (Zheng et al., 2017). Consequently, trees in arid lands have developed a wide range of morphological, physiological, and biochemical adaptive mechanisms to tolerate drought stress (Kozlowski and Pallardy, 2002). The effects of seed priming can be extended to enhance adaptive traits in response to cyclic drought and recovery (Brito et al., 2018) or later in the field (AlZubaidi et al., 2021). Vachellia tortilis, Umbrella Thorn (common name) and Sumur (Arabic name) are true arid land leguminous thorny tree species (FAO, 2009). It is a multifunctional and valuable tree (Jamal et al., 2013). It forms one of the most important vegetation communities in South-Western Saudi Arabia (Chaudhary and Aljowaid 1999). The tree is one of the main plant communities in the Makkah Region (AlZubaidi et al., 2022). However, most of these communities deteriorated due to various biological and environmental factors (Wahbi et al., 2013; Hosny et al., 2018). This urgently calls for the conservation and rehabilitation of these degraded areas. Successful rehabilitation requires rapid seed germination and high-quality nursery seedling stock. Therefore, this study aimed to investigate the effect of seed priming on improving *V. tortilis* seed germination, nursery seedling quality, and its adaptive response to drought. Under the hypothesis that seed priming will signal adaptive traits during the lag phase of germination.

# **MATERIALS AND METHODS**

## Seed priming and germination

In this study, seeds of *V. tortilis* subspecies spirocarpa were collected from the natural range of the species in the East Makkah Region, Kingdom of Saudi Arabia. The seeds were collected from over 25 trees, and the seeds were extracted in the field. The seeds were stored in the laboratory fridge before the working sample for the study was drawn. The seed samples were treated with three osmo-priming solutions (polyethylene glycol (PEG-6000); at the rates of 5%, 10% and 20%, respectively, corresponding to -0.27, -0.54 and -1.09 MPa osmotic potential) and three concentrations of hormonal priming solutions (Gibberellic acid (GA3) at the rates of 200 ppm, 300 ppm and 500 ppm), in addition to distilled water as control. The seed samples were soaked in their respective solutions for 24 hours, then removed and dried on a sterile surface. To carry out the germination test four replicates of twenty seeds were used in each treatment. The seeds were sown in filter paper, placed in sterilized petri dishes and hydrated with distilled water. The petri dishes were then placed under controlled conditions in an incubator. A germination count was performed daily throughout the entire germination period. At the end of the germination count, the following germination parameters were analysed: total germination percentage (TGP), Germination index (GI), mean germination time (MGT), First day of germination (FDG), and last day of germination (LDG). Then the seedlings were allowed to grow for one month before total length was measured for the assessment of the seedlings vigor index (SVI) (total seedling length\* total germination percentage) (Kader 2005).

#### **Enzymes activities assays**

For the analysis of enzymatic activities, 2 g samples of germinated seeds were weighed and homogenised in 20 mM Tris–HCl buffer with a pH of 7.2 (Ali *et al.*, 2019). Then we centrifuged the homogenate at the speed of 10,000 rpm for 10 min at 4°C and the supernatant (crude extract) produced was stored at -20 °C for later peroxidase (POD), polyphenoloxidase (PPO), polygalaturonase (PG) and  $\alpha$ -amylase assays.

The enzymatic activities of the two antioxidant enzymes (peroxidase (POD) and polyphenol oxidase (PPO)) and two hydrolytic enzymes (Polygalacturonase (PG) and  $\alpha$ -amylase) were assayed according to the methods described by Jie et al. (2002). In POD and PPO enzymes, the change in absorbance (at 470 nm for POD and 400 nm for PPO) was followed using a spectrophotometer. While PG and  $\alpha$ -amylase activities were assayed by determining the liberated reducing end products using galacturonic acid and maltose, respectively. Then, one unit of enzyme activity was defined as the amount of enzyme that liberated 1  $\mu$ M of reducing sugar per minute under standard assay conditions.

## Nursery seedlings quality

Uniform seedlings from each treatment were selected and allowed to grow in the nursery for three months. The selected seedlings were transferred in polythene bags (20X30 cm when flat), filled with beat moss and clay soil (1: 1 ratio). The seedlings were placed on a nursery bench with an overhead sprinkler irrigation system. At the end of the three months, destructive samples of three seedlings were taken from each treatment. Seedling height, root collar diameter, and root length were measured and the number of branches per seedling was counted. Then seedlings were separated into shoot and roots and fresh weights were immediately weighed. The samples were then oven-dried at 65°C for 48 hours, after which their dry weights were measured. Seedling quality parameters were analysed according to Elfeel and Abohassan (2015). These included shoot-to-shoot ratio (Sh/Rt ratio) ( ratio of shoot dry weight to dry root weight); Sturdiness quotient (SQ) as seedlings height in (cm) divided by root collar diameter in (mm); Dickson's quality index (DQI) is total seedling dry weight divided by the sum of SQ and Sh/Rt ratio (DQI= Total dry weight/(SQ+ Sh/Rt ratio). Also, the percentage increase of total dry weight obtained by

each treatment was calculated ((total dry weight of treatment X -total dry weight of the control)/total dry of the control\*100).

## Drying out and rewatering experiment

After completing the nursery experiment, homogeneous seedlings were selected and transferred to the greenhouse for an imposed drought stress experiment (drying out and rewatering). The experiment was set up as a split-plot design with three randomised complete replicates. The main plot was represented by irrigation treatments (normal watering and drying out and rewatering), while the subplots were occupied by priming treatments. The irrigation treatments are T1 (normal irrigation) and T2 (drying out and rewatering). The seedlings in T1 were watered normally every two days to field capacity, while those in T2 were subjected to three successive cycles of drying out and rewatering, with a 5-day recovery period between the cycles. In every cycle, the water was completely withheld from the seedlings until some seedlings started to wilt, and then rewatering recovery was applied. At the end of cycle three and the final recovery, the water was fully withheld from both T1 and T2 treatments, and the survival count was monitored for six weeks. At the end, the relative growth rate (RGR) was calculated using the following equation: (W2 - W1) / (t2- t1). Where W1 and W2 are the total dry weights of seedlings at times t1 and t2 (Elfeel et al., 2013). While dry matter content (DMC) was calculated as the ratio of dry mass to fresh mass. Regarding seedling mortality, we recorded the first day on which mortality commenced and the final day when mortality reached 100%. Seedling was counted dead when all its leaves were completely wilted.

## Data analysis

Analysis of variance (ANOVA) was conducted to determine the effects of seed priming treatment on all germination, seedling quality, and drought experiment parameters studied. The means were separated with Duncan's multiple Range Test (p = 0.05). The data were analyzed with SAS Statistical Analysis Software, University Edition, version (9.4), (SAS 2018).

## **RESULTS AND DISCUSSION**

## Effect of seed priming on germination

Seed germination is a three-phase process: absorption of water (imbibition), biochemical processes (lag phase) and the emergence of the radicle (germination). Seed priming is involved in activating various germination processes during the lag phase, preceding radicle emergence (Ibrahim, 2016). The results of the present study revealed that seed priming significantly improved all germination parameters compared with control un-

primed seeds (Table 1). The positive effects of seed priming on improving germination parameters have been reported in numerous studies (Naguib, 2019; Alvarado-Lopez, 2014). This can largely be attributed to the effects of priming, which signals many biochemical processes during seed germination (Ibrahim, 2016). Priming the seeds with PEG-6000 at a rate of 10% and GA3 at a concentration of 300 ppm increased the total germination percentage to 97.5%, compared to the control, which had an unprimed seed germination percentage of approximately 82.5%. Regarding germination properties, most seed priming treatments accelerated germination speed and uniformity by increasing germination index (GI) and reducing maximum germination time (MGT). Rapid and uniform seed germination is crucial for producing better seedlings (Sharma et al., 2014). In this study we analyzed GI and MGT as indicators of seed vigor (speed and uniformity). Higher GI and lower (MGT) indicates better speedy germination quality (kader, 2005). The results of this study showed that treating Samur seeds with 10% PEG-600 resulted in better GI, followed by GA-300. In contrast, seeds treated with PEG and GA3 at various concentrations showed a better germination speed compared to the control, untreated seeds. This finding is similar to that of Espanany et al. (2016), who concluded that seed priming promotes speedy seed germination. A similar trend was observed in the mean germination time (MGT), where the seeds treated with PEG at 10% and GA3 at the rate of 300ppm obtained lower (MGT) values than those treated with other concentrations. The enhancement of MGT caused by the application of PEG-6000 at a dose of 10% compared to other doses was also reported by Carvalho et al. (2020). The observed data showed that treating V. trotilis seeds with GA3 at a concentration of 300 pm resulted in the best seedling vigour index, compared to the control and all other treatments. It was observed that GA3 at a concentration of 300 mm was not only significantly improved GI, but also improved cumulative germination per cent, increased GI, and lowered MGT. The highly positive effect of GA-300 ppm on all germination parameters may be attributed to the positive induction of enzymatic activities caused by GA3-300 Ppm, as assayed in the germinated seeds (Figs. 1 & 2). Improvement of seed germination quality as the result of GA3 application was also reported in other studies (Aiello *et al.*, 2017).

#### **Enzymes activities**

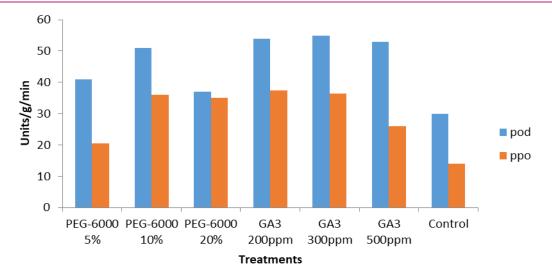
As illustrated in the Figs. 1 and 2, the enzymatic activities (POD, PPO, PG, and α-amylase) in germinated primed seeds were higher compared to the control. Regarding the antioxidant enzymes, the data showed that treating the seeds with GA3 at concentrations of 500 ppm, 300 ppm, and 200 ppm resulted in the best (POD) enzymatic activity compared to the PEG-6000 and control untreated seeds. However, PEG-600 produced better POD activity compared to the control, with the best values observed in PEG-6000 at 10%. The data showed that GA3 at 300pp increased the POD activity to 55 units/g/min compared to the value of 30 units/g/ min in the control. While PEG-6000 10% increased the value from 30 units/a/min in the control to 51 units/a/ min. In turn, (PPO) enzyme activity was higher in GA3 200ppm followed by GA3 300pp and PEG 10%. The Improvement of antioxidant activities by seed priming in this species may result in better regulation of reactive oxygen species (ROS), which enhances better germination characteristics.

On the other hand, the hydrolytic enzymatic activities (PG and  $\alpha$ -amylase) were higher in seeds treated with various concentrations of GA3, followed by PEG treatments (Fig. 2). GA3 at 300 pp obtained better results compared to the control and other treatments. For PEG, a concentration of 10% resulted in better values com-

**Table 1.** Effect of PEG and GA3 seed priming on germination parameter (Total germination percentage (TGP), Germination index (GI), mean germination time (MGT), First day of germination (FDG), last day of germination (LDG) and seed-lings vigor index (SVI))

0 0 17						
Treated	TGP	GI	MGT	FDG	LDG	SVI
PEG-6000 5%	96.25	10.05 abc	2.91	2	6	1828.8ab
PEG-6000 10%	97.50	12.07	2.31 b	2	7	1830.6ab
PEG-6000 20%	95.00	9.91 a b <sup>c</sup>	2.42 b	2	7	1448.1 °
GA3 200ppm	96.25	8.67 bc	2.84ab	3	7	1815.6ab
GA3 300ppm	97.50	10.97 <sup>ab</sup>	2.35 <sup>a</sup>	2	6	1987.5
GA3 500ppm	95.00	8.50 <sup>b</sup> c	2.88 <sup>ab</sup>	3	7	1795.6ab
Control	82.50 b	7.39 °	3.53 <sup>a</sup>	3	7	1527.5 bc
Р	*	*	*			*
C.V.	5.29	2.02	0.51			20.40

<sup>\* =</sup> p  $\leq$  0.05 , \*\* = p  $\leq$  0.01, ns = not significant; \* Means with the same letter in the same column are not significantly different at p= 0.05 using Duncan's multiple range test



**Fig. 1.** Effect of PEG-6000 and GA3 priming on antioxidant enzymatic activities (peroxidase (POD) and polyphenol oxidase (PPO) enzymes) in Vachellia tortilis germinated seeds

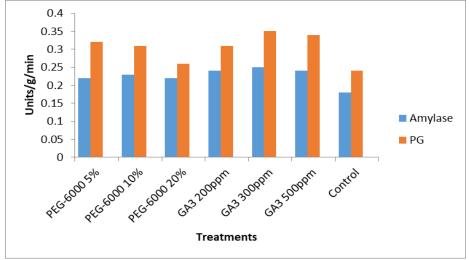
pared to the control and other concentrations. The higher induction of PG and  $\alpha$ -amylase enzyme activities, analysed in this study, obtained by all treatments of GA, may be related to the fact that GA represents a major regulator involved in inducing hydrolytic enzymes in germinating seeds (Gupta and Chakrabarty, 2013).

An observed relationship existed between germination quality (especially total germination per cent and seedling vigour) and the enzymatic activities of primed seeds. The priming treatments activated enzymatic activities, leading to enhanced germination. For instance, comparing of germination data in (Table 1) and POD activity in (Fig. 1), it was found that priming the seeds with PEG-6000 10% resulted in high POD activity as well as very high total germination percent and seedlings vigor. This may be attributed to the fact that priming increased POD activity, which helped to reduce oxidative stress and improved the metabolic process during germination. Regarding priming the seeds with

GA3, it was observed that all levels of GA3 (with the Best values for GA3 at 300ppm) produced higher hydrolytic enzymatic activities, which in turn resulted in high total germination percentages and seedling vigour. This may be due to the fact GA3 induced the hydrolytic enzymes which enhanced better germination characteristics. It is known that the  $\alpha$ -amylase enzyme plays a vital role in hydrolysing starch in seeds, which enhances germination and early seedling growth (Nawaz *et al.*, 2013). While PG contributes to many plant biochemical processes during germination and embryo development (Wang *et al.*, 2016).

# **Nursery seedlings quality**

The current study revealed significant effects of seed priming on all seedlings quality traits studied (Table 2). It was observed that when the seeds were treated with GA3 and PEG-6000, they exhibited better growth values across most traits compared to the control. The



**Fig. 2.** Effect of PEG-6000 and GA3 priming on hydrolytic enzymatic activities (Polygalacturonase (PG) and α-amylase enzymes) in Vachellia tortilis germinated seeds

**Table 2.** Effect of PEG and GA3 seed priming on *Vachellia tortilis* Seedlings growth: root collar diameter (RCD), shoot length (SHLG), root length (RTLG), number of branches (NOBR), shoot/root ratio (SH/RT), sturdiness quotient (SQ) and Dickson's quality index (DQI). Then percentage increase in total dry weight (TDW) was calculated as compared to the control.

Treated	RCD (mm)	SHLG (cm)	RTLG (cm)	NOBR	SH/RT ratio	SQ	DQI	Increase in TDW %
PEG 5%	1.79 b	17.33 b	15.50 <sup>ab</sup>	67.33 c	5.54	10.03	0.018 <sup>b</sup>	-12
PEG 10%	3.02	27.67 b	21.67 <sup>ab</sup>	188.33	2.89	9.60	0.040 <sup>ab</sup>	64
PEG 20%	2.22ab	20.83 b	15.17 b	115.33 b	5.84	9.76	0.023 b	3
GA3 200ppm	1.99 b	27.17 <sup>b</sup>	22.00ab	152.33	4.84	16.12	0.048ab	121
GA3 300ppm	3.01	32.83	26.00	181.67	2.95	11.02	0.064	160
GA3 500ppm	1.68 b	16.83 b	19.83ab	73.00 ℃	3.55	10.39	0.017 b	-42
Control	1.90 b	20.83 b	17.67ab	69.00 c	4.84	10.85	0.020 b	-
Р	*	**	*	**	ns	ns	*	
C.V.	0.49	3.36	5.35	19.46	2.08	4.17	0.017	

<sup>\* =</sup> p  $\leq$  0.05, \*\* = p  $\leq$  0.01, ns = not significant; \* Means with the same letter in the same column are not significantly different at p= 0.05 using new Duncan's multiple range test

priming growth traits included root collar diameter (RCD), shoot length (SHLG), root length (RTLG) and number of branches (NOBR). However, in almost all traits measured, priming the seeds with GA3 at 300 ppm, GA3 at 200 ppm, and PEG at 10% significantly resulted in higher values. This was reflected in an increase in total seedling dry weight of 160%, 121%, and 64% in the treatments of GA3-300ppm, GA3-200, and PEG-10%, respectively (Table 2). This increase in seedling growth can be attributed to the improvement of the seedling's quality parameters studied (Dickson's quality index (DQI), shoot-to-root ratio (Sh/Rh), and sturdiness quotient (SQ). For the DQI, GA3 at a concentration of 300 ppm had a significantly higher value, followed by GA3 at 200 ppm and PEG 10%. At the same time, priming recorded lowest value in Sh/Rt. In arid land plants, a lower shoot to root ratio was a very important indicator of seedlings' fitness quality (Elfeel and Abohassan, 2015). This allows for the allocation of a reasonable amount of carbohydrates for optimal root development. The revealed very low Sh/Rt in PEG 10% and GA3 300ppm may reflect that these concentrations produced a better balance in photosynthate distribution between the shoot and the root for this species. For the SQ our results revealed that all the three concentrations of PEG accounted for lower SQ compared to the three GA3 concentrations and the control. These lower values in SQ produced as a result of PEG treatment may be attributed to the fact that PEG, as an osmoprimer, is more closely related to stress traits in plants. Several studies have associated PEG with the treatment of stress in three intraspecific taxa of Pinus nigra (Carvalho et al., 2020).

Although, in most parameters analysed, both GA3 at 300 and 200 ppm rates and PEG 10% obtained higher

results than all other treatments, the GA3 concentration of 300 ppm outweighed the other two treatments in some parameters. It is known that GA3 is a crucial factor in seed germination mechanisms and is related to many other important mechanisms. For instance, Galhaut *et al.* (2014) and Ashraf *et al.* (2002) reported that Gibberellic acid (GA3) can improve photosynthetic properties and the antioxidant system and enhance seedling emergence and vegetative growth. GA3 also plays an important role in stem or internode elongation, stimulating cell division and expansion (Gupta and Chakrabarty, 2013).

# Drying out and rewatering

The analysis of the split-plot design, based on imposed drought stress through drying out and rewatering, revealed a non-significant interaction between irrigation and priming treatments in both RGR and DMC (Table 3). The non-significant interaction indicates that the ranking of seed priming treatments is independent of irrigation treatment, suggesting a similar ranking in both normally irrigated seedlings and seedlings subjected to drying-out and rewatering treatments. For the main treatments, irrigation and priming significantly differed in relative growth rate (RGR). While for DMC only irrigation treatments showed significant differences (Table 3). GA 200, GA 300 and PEG 10 obtained better values in RGR and DMC. Regarding seedling survival, the obtained results indicated that treating seeds with PEG at different concentrations resulted in a better survival rate, both in seedlings normally watered and those subjected to drying and rewatering treatment, compared to GA3 and the control. The higher survival of seedlings treated with PEG may be due to the fact that PEG, as an osmo-primer, induces seedlings' response to water

Table 3. Combined effects of imposed drought and PEG and GA3 seed priming on Vachellia tortilis seedlings relative growth rate (RGR), dry matter content (DMC) and seedlings mortality (first day mortality start (1st of M) and last day mortality reached 100% (Last of M)

Treated	RGR		DMC		1 <sup>st</sup> of M		Last of M	
	cycle	normal	cycle	normal	cycle	normal	cycle	normal
PEG 5%	0.32 <sup>b</sup>	0.49 <sup>b</sup>	0.45 <sup>b</sup>	0.46bc	29	14	42	35
PEG 10%	0.42 <sup>a</sup>	0.56 <sup>a</sup>	0.50 <sup>b</sup>	0.44 c	31	14	42	35
PEG 20%	0.31 <sup>b</sup>	0.43 <sup>b</sup>	0.50 <sup>b</sup>	0.47 <sup>a</sup> bc	28	12	41	35
GA3 200ppm	0.34 <sup>b</sup>	0.49 <sup>b</sup>	0.47 <sup>b</sup>	$0.46^{b_C}$	21	7	40	34
GA3 300ppm	0.42 <sup>a</sup>	0.57 <sup>a</sup>	0.59 <sup>a</sup>	0.59	19	7	39	32
GA3 500ppm	$0.32^{b}$	$0.47^{b}$	0.47 <sup>b</sup>	0.57 <sup>ab</sup>	17	7	38	31
Control	0.30 <sup>b</sup>	0.49 <sup>b</sup>	0.49 <sup>b</sup>	0.5 <sup>abc</sup>	21	7	35	28
Block Irrigation	ns **		ns ns					
Block*irrigation	ns		ns					
Priming	*		**					
Irrigation*priming	ns		ns					
C.V.	17.5		10.5					

 $<sup>* =</sup> p \le 0.05$ p= 0.05 using new Duncan's multiple range test

stress (AlZubaidi et al., 2021). However, the application of GA3, especially GA3 at rates of 200 and 300 ppm, revealed better growth and survival than the control.

#### Conclusion

The results of this study demonstrate the significant benefits of seed priming in improving the germination, seedling quality, and drought tolerance of V. tortilis. Priming with GA3 and PEG enhanced germination rate, seedling vigour, and overall seedling quality. The increased activity of antioxidant and hydrolytic enzymes in primed seeds likely contributed to these positive effects. While both GA3 and PEG were effective, GA3 at 300 ppm exhibited the most pronounced impact on germination and seedling growth. The improved seedling quality, characterised by a higher root-to-shoot ratio and a sturdier quotient, translated into enhanced drought tolerance. Seedlings from primed seeds exhibited better survival and growth under cyclic drought stress. These findings have important implications for the successful establishment of V. tortilis in arid and semi-arid regions. Future research could investigate the optimal priming treatments for various environmental conditions and examine the long-term effects of seed priming on plant growth and development.

## **Conflict of interest**

The authors declare that they have no conflict of interest.

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<sup>\*\* =</sup> p ≤ 0.01 ns = not significant; \* Means with the same letter in the same column are not significantly different at

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