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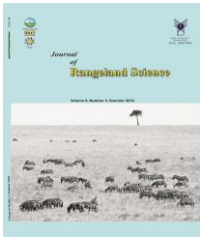

Research and Full Length Article:

The Effects of Seed Priming with Acid Ascorbic on Seed Germination and Morphological Traits of *Taverniera cuneifolia* under Drought Stress

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Abstract. The species of *Taverniera cuneifolia* belonging to the family of legominoseae is a perennial species usually growing in the south of Iran. The objective of this study was to evaluate the effects of the drought stress and seed priming using acid ascorbic (AsA) on seed germination of *Taverniera cuneifolia* in laboratory and seedling morphological traits in greenhouse conditions. This study was conducted in 2015 in Iran. In laboratory, a factorial experiment was conducted using Polyethylene glycol 6000 in drought stress (0, -4, -8, -16 bar) and priming AsA in four levels (0, 50, 100 and 200 mM) for 24 hours and in the greenhouse, experiment AsA in four levels (0, 50, 100 and 200 mM) and drought stress in four irrigation periods (3, 6, 9 and 12 days). Experimental design was a completely randomized design in four replications with 30 seeds per pots per replication. In greenhouse, seeds were sown in plastic pots in soil. All pots were irrigated until germination stage. Then, drought stresses were applied for 64 days and finally, morphological traits were measured. The data were analyzed and means comparisons were made using Duncan test ($P<0.05$). Results showed that increasing drought stress caused significant decreases in the seed germination, and morphological traits of *T. cuneifolia*. The lowest seed germination, shoot length, root length and vigor index were obtained in drought stress of -16 bar. The highest germination rate was obtained in priming seeds in 200 mM AsA and non drought stress treatment. The highest specific leaf area (cm^2/g), leaf area (cm^2), and dry weight biomass were found in priming seeds in 200 mM AsA. In conclusion, priming with AsA improved the seed germination rate and morphological traits in seedling of *T. cuneifolia*.

Key words: *Taverniera cuneifolia*, Drought stress, Seed priming, Acid ascorbic, Morphological traits

Introduction

Drought is one of the serious obstacles for field crops especially in the arid and semi-arid regions of the world. Seed priming is a technique of seed enhancement that improves the germination or seedling growth and rate or uniformity of the seedling establishment (Taylor *et al*., 1998). Seed priming improves seed performance by rapid and uniform germination, and normal and vigorous seedling which resulted in faster and better germination and emergence in different crops (Powell *et al*., 2000; Cantliffe, 2003). Various prehydration (hydropriming) or priming treatments have been employed to increase the speed and synchrony of seed germination (Bradford, 1986; Mohammadi *et al.*, 2014). The general purpose of seed priming is to hydrate partially the seed to a point where germination processes are initiated but not completed. Most priming treatments involve imbibing seed with the restricted amounts of water to allow sufficient hydration and advance of metabolic processes but preventing from the protrusion of the radicle. Treated seeds usually would exhibit rapid germination when water is absorbed under field conditions (Ashraf and Foolad, 2005).

The genus *Taverniera* belonging to the family of Fabaceae includes twelve species and is endemic to the Northeastern African and Southwestern Asian countries (Naik, 1998; Stadler *et al.*, 1994). *Taverniera cuneifolia* is a perennial crop usually growing in the south of Iran. Seed germination and early seedling establishment are the most vulnerable stages to chilling and drought, and it is a challenge in forage production (Ashraf and Foolad, 2005). Imbibitional injury is a major concern during germination; rapid water absorption by dry seeds results in membrane irregularities such as folds, prominences, and circular holes (Hoekstra *et al*., 1999). These perturbations can be exacerbated by chilling due to the inhibition of membrane reorganization that is required during imbibition (Bradford, 1986) and may compromise germination performance manifested by a low final germination percentage, germination rate and uniformity.

Seed priming may also increase the seed or seedling tolerance to drought stress (Demir and Mavi, 2004). Seeds of *Festuca ovina* exhibited the increased final germination percentage in a saline environment after priming with NaCl and polyethylene glycol (Dianati Tilaki *et al*., 2011). Positive effects of priming with L(+)-Ascorbic acid fine have been reported on growth and yield of *Cicer arietinum* plants (Beltagi, 2008). Ascorbic acid (AsA) is also known to provide protection against a number of abiotic stresses by triggering the potential to tolerate stress (Senaratna *et al*., 2000). Drought also disturbs the plant growth owing to loss of turgor (Farooq *et al.*, 2009; Taiz and Zeiger, 2010) as water supply from the xylem to the surrounding elongating cells is interrupted (Nonami, 1998).

The objective of this study was to evaluate the effects of seed priming using acid ascorbic on seed germination and seedling growth of *T. cuneifolia* species under drought stress.

Materials and Methods

Seeds of *T. cuneifolia* as well as soils used in this study were obtained from the surrounding rangelands of Sarchahan Bandar Abbas, Iran in 2015. The climate of Sarchahan is arid with annual mean temperature around 26.8°C and annual precipitation of 167 mm.

Seeds were surface-sterilized by dipping in sodium hypochlorite (5%) solution for 3 min, washed with distilled water and dried on filter paper. The study consisted of two independent experiments. In laboratory, the effects of

AsA priming in four levels (0, 50, 100 and 200 mM for 24 h) were studied on seed germination traits of *T. cuneifolia* in four drought stresses using Polyethylene glycol 6000 (0, -4, -8, -16 bar). The experimental design was a completely randomized design in four replications. The primed seeds were sown in Perti dishes (30 seeds per dish) and were transferred to germination chamber at 22±2°C under a 12h daylight photoperiod and 60% relative humidity.

In the next experiment, the seeds were primed with AsA in four levels (0, 50, 100 and 200 mM). Seeds were sown in plots (30 seeds per plastic pot of 22 cm high and 19 cm diameter filled with 2 kg of natural habitat soil) The soil texture was clay loam with 7.78 pH, 0.258 dsm^{-1} EC, 54% sand, 32% silt, 14% clay, field capacity 13.4% and wilting point 6.4%. Pots were kept in greenhouse with temperature of 30±4°C in day and 25±4°C in night with natural light and all pots were irrigated with distilled water until germination stage. The effects of drought stress were applied on the seedling in four irrigation periods (3, 6, 9 and 12 days) and plants were irrigated for 64 days based on treatments. The greenhouse experiment was as the same as laboratory one using a factorial experiment based on a completely design with four replications.

In laboratory, the germinated seeds were counted one-day interval for 21 days. Germination was considered to occur when the root length was 2 mm. The seedling with short, thick, and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated ones (ISTA, 2003). The germination percent, root length and shoot length were measured on 21st day. Root length and shoot length were measured manually with a ruler. The germination rate was calculated by Gairola *et al.* (2011) method. The Vigor Index (VI) and Mean Germination Time (MGT) were calculated by the equations 1 and 2, respectively (Abdul-Baki and Anderson, 1973):

$$VI = (RL + SL) \times GP \quad (1)$$

$$MGT = \frac{\sum (Gt \times Tt)}{\sum Gt} \quad (2)$$

Where:
GP=germination percent
RL=Root length
SL=Shoot length
Gt = Number of germinated seeds on Day *t*
Tt =Time corresponding to *Gt* in days
In greenhouse, leaf area was measured using leaf area meter instrument. Fresh weight of seedling was taken after immediately cutting the leaves and turgid weight was obtained after 6h soaking in distilled water at room temperature under low light conditions. The dry weight of seedling was obtained after drying the leaf samples at 60°C for 48 h in an oven (Schonfeld *et al.*, 1988). Specific Leaf Area (*SLA*) was measured using (Arias *et al*., 2007) the equation 3:

$$SLA = \frac{S}{Sdw} \quad (3)$$

S= leaf area and
S_{dw} is dry weight of leaves

The experimental design was a two-factor factorial priming treatment with drought levels arranged in a completely randomized design with 4 replications. The data were statistically analyzed by SPSS computer software. The difference between the means was compared using Duncan method ($P< 0.05$).

Results

Laboratory

In laboratory experiment, germination rate was affected significantly by AsA priming treatments (Table 1). There was a significant difference between drought stress levels for all the traits and there was a significant priming × drought stress

interaction for germination rate and mean of germination time (Table 1).

Results showed no significant effects of priming on root length, shoot length and vigor index. Increasing the drought stress delayed mean germination time and reduced the germination percent, root length, shoot length and vigor index (Table 2). The Maximum germination rate was recorded in AsA priming 200mM and non-drought treatment (Table 3). AsA priming significantly shortened the mean germination time as compared to other treatments (Table 3).

Table 1. Analysis of variance for seed germination traits of *T. cuneifolia* using priming treatments with ascorbic acid and drought levels in laboratory.

Source of variation	DF	MS					
		Germination percentage	Germination rate	Mean time germination	Root length	shoot Length	Vigor index
Priming (P)	3	635.36^{ns}	13.61^{**}	98.97^{ns}	0.46^{ns}	0.03^{ns}	0.60^{ns}
Drought (D)	3	10375.63^{**}	92.61^{**}	1492.21^{**}	5.85^{**}	5.77^{**}	7.36^{**}
P×D	9	528.62^{ns}	4.12^{**}	311.30^{**}	0.90^{ns}	0.26^{ns}	0.92^{ns}
Error	48	285.78	0.95	80.27	0.52	0.13	0.49

* and ** significant at 5 and 1%, respectively and ns indicates not significant

Table 2. Influence of seed priming with ascorbic acid on root and shoot lengths, vigor index and germination percent in *T. cuneifolia* in laboratory.

Ascorbic acid (mMol)	Germination %	Root Length (cm)	Length shoot (cm)	Vigor index
0	20.97±5.39 a	0.58±0.21 a	0.51±0.16 a	0.52±0.17 a
50	21.04±7.36 a	0.82±0.22 a	0.57±0.19 a	0.59±0.24 a
100	25.21±6.13 a	0.93±0.25 a	0.60±0.11 a	0.71±0.20 a
200	34.37±9.25 a	0.96±0.21 a	0.62±0.17 a	0.96±0.29 a

Means of column followed with the same letters are mot significant based on Duncan method P< 0.05

Table 3. Influence of drought stress by seed priming interactions on germination rate and mean germination time in *T. cuneifolia* in laboratory conditions

Drought stress (bar)	Ascorbic acid (mMol)	Germination rate	Mean time germination
0	0	3.71±0.91 a	25.21±2.73 b
	50	4.87±1.01 a	24.47±1.00 b
	100	5.68±0.71 a	23.20±3.06 b
	200	8.78±0.34 a	23.20±3.06 b
-4	0	1.78±0.32 b	29.53±2.99 a
	50	2.38±0.44 ab	28.50±4.58 a
	100	2.73±0.24 ab	28.32±0.75 a
	200	3.34±0.26 a	27.66±2.76 a
-8	0	0.72±0.63 b	32.34±1.87 a
	50	1.00±0.26 b	31.57±1.39 a
	100	1.33±0.55 b	31.42±2.54 a
	200	3.21±0.32 a	30.49±1.05 a

Means of column followed with the same letters are mot significant based on Duncan method P< 0.05

Results of factorial experiment analysis for greenhouse are presented in Table 4. Results showed the significant effects of priming on SLA, root length, shoot and root fresh and dry weight. There were significant effects of drought stress on leaf area, and shoot and root fresh weigh. There was no significant priming × drought stress interaction for none of traits (Table 4).

Seed priming with acid ascorbic (AsA) significantly improved the seedling dry weight, shoot dry weight, and SLA (Table 4). However, drought stress significantly decreased seedling dry weight, leaf area, and SLA. The plant height, seedling emergence rate and seedling emergence value were considered as morphological parameters and the highest value for the plant height, seedling emergence rate and seedling

emergence value belonged to 200mMol seed priming treatment with ascorbic acid (Table 5). In contrast, root length was increased under drought conditions (Table 6). The plant height, shoot weight, root weight, leaf area, and specific leaf area were considered as morphological parameters and the highest value for shoot and root biomass belonged to 3-day treatment and for root length, 12-day treatment showed the highest value (Table 7).

Table 4. Analysis of variance for morphological traits of *T. cuneifolia* species under priming treatments with ascorbic acid and drought levels in greenhouse condition

Source of variation	DF	MS								
		Plant height	Leaf area	Specific Leaf area	Leaf number	Root length weigh	Shoot Fresh weigh	Shoot dry weight	Root fresh weigh	Root dry weight
Priming(P)	3	15.94^{ns}	3.20^{ns}	591.8^{**}	345.91^{ns}	66.24^{**}	5.20^{**}	0.20^{**}	0.28^{**}	0.017^{*}
Drought(D)	3	29.12^{ns}	7.60^{**}	152.4^{ns}	338.53^{ns}	3.62^{ns}	6.00^{**}	0.05^{ns}	0.16^{**}	0.003^{ns}
P×D	9	16.85^{ns}	0.87^{ns}	189.6^{ns}	50.79^{ns}	4.09^{ns}	1.32^{ns}	0.02^{ns}	0.04^{ns}	0.005^{ns}
Error	64	24.13	1.58	106.40	201.06	10.98	1.46	0.05	0.02	0.005

* and ** significant at 5 and 1%, respectively and ns indicates not significant

Table 5. Effect of seed priming with ascorbic acid on seedling emergence rate and seedling emergence germination percentage in *T. cuneifolia* species

Ascorbic acid (mMol)	Seedling emergence rate (number / day)	Seedling emergence Value (%)	Plant height (cm)
0	3.04±0.24 b	13.15±1.09 b	7.04±0.39 b
50	3.16±0.35 b	13.66±1.44 b	7.12±0.56 b
100	3.27±0.37 b	14.00±1.27 b	7.17±0.68 b
200	3.98±0.41 a	14.83±1.55 a	7.32±0.60 a

Means of column followed with the same letters are mot significant based on Duncan method P< 0.05

Table 6. Influence of seed priming with ascorbic acid on root and shoot length seedling, leaf area and specific leaf area of *T. cuneifolia* in greenhouse condition

Ascorbic acid (mMol)	Plant height (cm)	Leaf area cm^2	Specific leaf area (cm^2/g)	Leaf number	Root length (cm)	Shoot Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry Weight (g)
0	19.3±1.0a	2.9±0.27b	18.3±2.4b	20.4±2.1a	7.8±0.70a	0.96±0.15b	0.15±0.02b	0.06±0.01b	0.01±0.01c
50	19.8±1.0a	3.5±0.30ab	20.2±2.4b	20.4±2.1a	5.5±0.54b	1.93±0.39a	0.31±0.04a	0.13±0.03b	0.02±0.01bc
100	20.2±1.3a	3.7±0.32ab	21.2±2.2b	26.9±2.7a	5.4±0.79b	1.96±0.26a	0.32±0.06a	0.26±0.05a	0.06±0.01ab
200	21.4±0.8a	3.8±0.26a	30.5±2.5a	29.4±3.8a	3.3±0.73b	2.05±0.26a	0.39±0.05a	0.32±0.05a	0.07±0.02a

Means of column followed with the same letters are mot significant based on Duncan method P< 0.05

Table 7. Effect of drought stress on morphological traits of *T. cuneifolia* in greenhouse condition

Watering period (days)	Plant height (cm)	Leaf area cm^2	Specific leaf area (cm^2/g)	Leaf number	Root Length (cm)	Shoot Fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry Weight (g)
3	21.1±1.1a	4.35±0.28a	26.4±2.6a	32.6±3.3a	4.95±0.71a	2.52±0.32a	0.37±0.05a	0.32±0.05a	0.05±0.01a
6	20.9±0.98a	3.70±0.29ab	22.4±3.1a	24.7±3.2a	5.69±0.77a	1.59±0.29b	0.28±0.06a	0.17±0.04b	0.05±0.02a
9	20.3±1.2a	3.07±0.29b	21.6±2.4a	24.6±2.9a	5.70±0.80a	1.50±0.29b	0.27±0.05a	0.17±0.04b	0.03±0.01a
12	18.5±0.86a	3.05±0.23b	19.8±1.9a	23.9±2.6a	5.93±0.83a	1.28±0.19b	0.25±0.05a	0.11±0.02b	0.03±0.01a

Means of column followed with the same letters are mot significant based on Duncan method P< 0.05

Discussion

This study investigated whether seed priming with AsA can improve drought resistance germination rate in *T. cuneifolia*. Drought caused the decreased seedling dry weight, shoot length and germination percent in *T. cuneifolia* in limited water availability during the imbibition phase of germination and it is the primary reason for delayed and erratic stand establishment (Murillo-Amador *et al.*, 2002). Our findings of maximum germination rate in seeds primed with AsA are supported by the findings of Akhondi *et al*. (2004), Mukherjee *et al*. (2010), Dianati Tilaki *et al*. (2010), and Dianati Tilaki *et al*. (2009) who also observed that priming of plant seeds

improved vigor more than control. Seed germination was reduced under salt and drought stress conditions due to physiological injuries under such conditions and these stressed seeds are desiccation sensitive (Demir Kaya *et al.*, 2006; Wiebe and Tiesses, 1979). Results showed that mean germination time was increased with increasing the drought stress. Masoumi Zavariyan *et al.* (2015) stated that increasing drought stress reduced water absorption by seeds, the time required for water absorption increases and the resulting delayed germination process occurs. Sadat Noori *et al*. (2015) and Dianati Tilaki *et al*. (2009) also observed that drought stress increased mean germination time more than control. Results showed that seed priming with AsA improved the Leaf area, and shoot fresh and dry weight. Bahreininejad *et al.* (2013) obtained similar results to our results.

In many rain fed areas, germination and subsequent seedling growth can be inhibited by adverse conditions in the field. Priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. Our findings revealed that seed priming with acid ascorbic (AsA) is a simple and useful technique for enhancing seedling emergence rate. These effects can improve seedling establishment and field performance of this important food legume. In this study, priming with AsA improved the seed germination rate and some morphological traits in seedling of *T. cuneifolia*.

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اثر پرایمینگ بذر با اسید اسکوربیک بر روی جوانه زنی بذر و صفات مورفولوژیکی گیاهچه *Taverniera cuneifolia* در شرایط تنش خشکی

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چکیده. گونه *Taverniera cuneifolia* به خانواده لگومینوزه تعلق دارد و یکی از گیاهان علوفه‌ای چند ساله است که در نواحی جنوبی ایران می‌روید. هدف از انجام این تحقیق بررسی اثر خشکی و پرایمینگ با اسیدآسکوربیک بر صفات جوانه‌زنی بذر گونه‌ی *Taverniera cuneifolia* در شرایط آزمایشگاهی و صفات مورفولوژیکی در شرایط گلخانه‌ای بود. این مطالعه در سال ۱۳۹۴ در ایران انجام شد. در شرایط آزمایشگاه، این تیمارها شامل تنش خشکی با پلی اتیلن گلایکول ۶۰۰۰ در چهار سطح (۰، ۴-، ۸- و ۱۶- بار) و پرایمینگ با اسیدآسکوربیک در چهار سطح (۰، ۵۰، ۱۰۰ و ۲۰۰ میلی‌مولار) بمدت ۲۴ ساعت در چهار تکرار و ۳۰ عدد بذر در هر تکرار بود. آزمایش فاکتوریل در قالب طرح پایه به صورت کاملا تصادفی بود. در آزمایش گلخانه بذرهای پرایم شده گونه *T. cuneifolia* در چهار سطح (۰، ۵۰، ۱۰۰ و ۲۰۰ میلی‌مولار) در چهار تکرار و ۳۰ عدد بذر در هر تکرار در داخل گلدان‌های پلاستیکی در شرایط گلخانه قرار داده شدند. آبیاری تمامی گلدان‌ها تا مرحله جوانه‌زنی با آب مقطر صورت گرفت. برای ایجاد تنش خشکی بر نهال‌ها از چهار دوره آبیاری (۳، ۶، ۹ و ۱۲ روزه) استفاده شد. همچنین برای مدت ۶۴ روز گیاهان با استفاده از حد ظرفیت مزرعه آبیاری شدند و در نهایت صفات مورفولوژیکی و فیزیولوژیکی اندازه گیری شد. نتایج تجزیه و تحلیل داده‌ها نشان داد که افزایش تنش خشکی سبب کاهش معنی‌داری در میانگین صفات جوانه‌زنی، موفولوژیکی و فیزیولوژیکی گونه *T. cuneifolia* شد. کمترین جوانه‌زنی بذر، طول ساقه‌چه، طول ریشه‌چه و شاخص بنیه در سطح خشکی ۱۶- (بار) مشاهده شد. بالاترین سرعت جوانه‌زنی بذر در اسیدآسکوربیک ۲۰۰ میلی‌مولار و بدون تنش خشکی (شاهد) مشاهده شد. در گلخانه، بالاترین سطح ویژه برگ (cm^2/g)، سطح برگ (cm^2)، بیوماس وزن خشک در تیمار پرایمینگ بذر غلظت ۲۰۰ میلی مولار اسید اسکوربیک مشاهده شد. نتیجه کلی نشان داد که پرایمینگ بذر با اسید اسکوربیک (AsA) سبب بهبودی در سرعت جوانه‌زنی بذر و صفات مرفولوژیک و نهال‌های *T. cuneifolia* شده است.

کلمات کلیدی: *Taverniera cuneifolia*، خشکی، جوانه زنی بذر، پزایمینگ بذر، اسید اسکوربیک، صفات مرفولوژیکی