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EFFECT OF TEMPERATURES AND SALINITY ON SEED GERMINATION RATE OF ROSARY PEA FROM MOUNT VIFA OF SAUDI ARABIA

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Abstract

Abrus precatorius L. is a valuable herbaceous medicinal plant with a wide range of therapeutic properties. The present study aims to investigate some of the biological characteristics of *Abrus precatorius* in Saudi Arabia by studying its germination potential and shortening of its dormancy period at constant and varied temperature regimes under control and salt conditions. Seeds were collected from a wide thermal range of continuous and changing temperature systems and tested for their response to heat and salinity. Preliminary experiments have revealed that seeds could not germinate unless they were treated with H_2SO_4 to break dormancy. According to the findings, cock-eye seeds germinated much better in variable and constant temperature systems (25, 25/15, 30/20, 35/25°C). The germination rate was 98% at 35°C. It dropped to 26% at 5°C, MDG was zero seed/day, and T_{50} was not obtained. After 20 days at 15°C. However, seedlings did not develop at low variable and constant temperatures (5, 15/5°C). The seeds of *Abrus precatorius* were observed to be resistant up to 400 mM NaCl. Salinity substantially affected the germination of *A. precatorius* seeds compared to the control (0 mM NaCl). With 200, 400 and 600 mM NaCl, the germination rate reduced by approximately 60 to 90 and 100%, respectively, compared to the control. This study will provide baseline data and help revive endangered species like *A. precatorius* plants and their critical adaptation for preserving Saudi Arabia's biodiversity and survival in highly salinized environments.

Key words: Abrus precatorius, Seed germination, Dormancy, Salt stress, Temperature.

Introduction

In dry and arid situations, germination is essential,the first and most fundamental stage of the plant life cycle. The germination process is impacted by various environmental conditions, including temperatures, salinity, light, and humidity (Al-Gharaibeh *et al.,* 2017). Temperature and salinity severely limit the germination of several species in arid settings. In addition, salinity stress is a critical global issue that has a detrimental impact on crop development and yield (Alatar, 2011; Khan *et al.,* 2022). The optimal temperatures for germination differed depending on the plant species (Song *et al.,* 2006; El Sabagh *et al.,* 2019). Seed germination is one of the main critical growth stages affecting the initial plant population. The mechanism of the effect of temperature on the germination process was described elsewhere (Delgado *et al.,* 2016; Waraich *et al.,* 2021), and heat stress at any growth phase can cause substantial yield losses. It has been demonstrated that the seed germination rate and seedling establishment were significantly higher at fluctuating temperatures than at constant temperatures (Al-Khateeb, 2006; Delgado *et al.,* 2015; Ahmad *et al.,* 2021). Based on meteorological knowledge from observational sites, a wide thermal range of reciprocal and constant temperature systems was chosen for the study of the effect of temperature on seed germination to determine the response of seeds to temperature and the relationship between their geographical distribution (Baskin & Baskin, 1998; Amini *et al.,* 2016; Al-Gharaibeh *et al.,* 2017). Salinity is a significant abiotic factor influencing seed germination (El-Keblawy, 2004; Al-Khateeb, 2006; Delgado *et al.,* 2015, 2016). It has also been revealed that the germination process gradually reduces with increasing salinity and that the negative impact of salinity stress on seed germination varies depending on plant species. When the salt is reduced or eradicated, many endemic desert plants and halophyte seeds that did not develop in saline circumstances show better growth (Al-Khateeb, 2006; Singhal *et al.,* 2021). This phenomenon can occur even if the seeds have been exposed to salinity concentrations that inhibit germination (Melendo & Giménez, 2018).

The cock-eye (*Abrus precatorius*) is a plant in the Leguminosae family, sub-family Papilionoideae (Bhati *et al.,* 2013). It is a herbaceous perennial plant. In countries like India, Sri Lanka, Thailand, the Philippines, South China, tropical Africa, the West Indies, and some of Saudi Arabia, it flourishes under tropical or subtropical climates (Al-Hammad & Al-Ammari, 2017). *Abrus precatorius* is a poisonous plant with one of the most lethal toxins, Abrin, a toxalbumin. Its seeds contain aspirin, a highly toxic chemical. *Abrus precatorius* has traditionally been used to treat tetanus and prevent rabies. This plant's leaves, roots, and seeds are used in traditional medicine (Bhatia *et al.,* 2013). *Abrus precatorius* is a widely growing medicinal plant used to treat wounds, sores and scratches caused by dogs, cats, and mice, and is also used in several purposes of traditional medicine (Vyas, 2017). It is known that the plant can have a wide range of therapeutic effects, including antibacterial, antifungal, antitumor, analgesic, antispasmodic, antidiabetic, antiserotonergic, antimigraine, and anti-inflammatory effects, which can be used to treat ulcers, wounds, scratches of the throat, and sores. It is also regarded as a great source of natural ingredients for creating industrial products and pharmaceuticals to treat various illnesses Bhatia *et al*., (2013). Due to insufficient evidence and literature available regarding germination performances of *Abrus precatorius* to temperature and salinity. So, this experiment aimed to assess the influence of temperature and salinity on the germination of *Abrus precatorius* to understand further the conditions needed for germination in arid areas. The stiff seed coat of *A. precatorius* makes seed propagation difficult, which

explains the species' widespread distribution. Therefore, to prevent it from declining further and to satisfy the needs of the traditional medicine sector, it is crucial to develop this medicinally significant taxon through proper propagation (Baskin *et al.*, 2007).

Materials and Methods

Plant material and growth conditions: The seeds of A.s precatorius were collected from Mount Vifa in the northeastern provenance of Jazan, located in the southwest of the Kingdom of Saudi Arabia at latitude 17.41 north and longitude 43.05 east. After being packed, the seeds were transported to the lab. Abrus precatorius seeds were treated with concentrated sulfuric acid for one hour to break dormancy, as no seed was discarded without treatment during early trials.

Germination test and estimation of germination rate: The percentage of control and germination seed samples were used to compute the germination rate, which was collected for assays every 24 h for 30 days. Mean daily germination (MDG) was defined as the number of seeds germinated per day in relation to the maximum number of germinated seeds. The average time to germinate 50% of the seeds was used to estimate germination speed.

Thermal systems: Seeds were incubated in five alternating thermal regimes (12 h light: 12 h dark) (15/5, 20/10, 25/15, 30/20, and 35/25 m) and four fixed thermal systems (5, 15, 25, and 35°C). Temperature ranges were chosen based on climatic data collected in the study areas. Ten replications of each type were assigned, and seeds were germinated in a plastic Petri dish (9 cm). Then seeds were imbibed between two sheets of paper for each replicate (Whatman N.01). Distilled water was added every four days, as needed.

Salinity stress treatments: The same number of seeds (10 seeds per Petri dish) were germinated for 30 days at 15-25°C in the dark/light (12 h light: 12 h dark) on two sheets of filter paper soaked in 10mL distilled water or 200 mM, 400 mM, or 600 mM of NaCl. The Petri dishes were replaced with fresh sodium chloride (NaCl) solutions every five days.

Statistical analysis

The germination data were collected and transformed prior to statistical analysis (Ahmed & Khan, 2010) to ensure the homogeneity of variance. The effects of temperature, salinity, and their interactions on seed germination and germination rate were determined using analysis of variance (ANOVA) in the SPSS version 23 for Windows. If the ANOVA revealed a significant effect (p<0.05), the treatment means were compared using posthoc Bonferroni tests (SPSS Inc., 2015).

Results

Temperatures effect on germination of *A. precatorius*: According to the current study, there was no germination at the temperature regime of 15/5°C (Fig. 1). From 15/5°C to 25/15°C, the germination rate rose, then slightly fell, but this trend was not statistically significant. In terms of constant temperature, the temperature of 25°C produced the superior germination rate (Fig. 2). The temperatures regime 25/15°C had the highest mean daily germination, but the germination speed was fastest for the temperature regime 20/10°C followed by 35/25°C (Table 1).

Effect of constant temperature on the germination success of *A. precatorius*: The findings evidence that there were statistically significant differences (p<0.05) between different temperature regimes at 5 and 15°C, where the germination rate was approximately 0% and 20%, respectively (Fig. 2). At 25°C, the germination rate was 98%, at 35°C, it dropped to 26%, and at 5°C, MDG was zero seed/day, and T_{50} was not obtained. After 20 days at 15°C, we observed an increase in MDG (0.06 seed/day and T_{50}. When T_{50} was obtained after only 4 days, the highest MDG was obtained at 25°C; it was approximately 0.32 seed/day. At 35°C, MDG was 0.11 seed/day, and T_{50} was 16 days (Table 2).

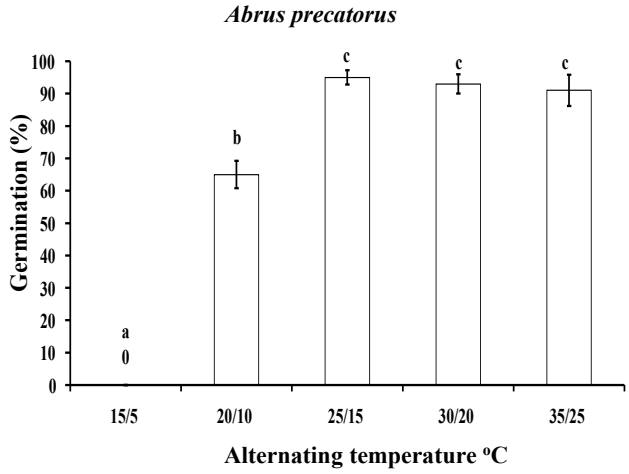

Fig. 1. Effect of temperature on the germination rate of *A. precatorius* imbibed with water under 12/12 light-dark conditions in response to variable temperature regimes.

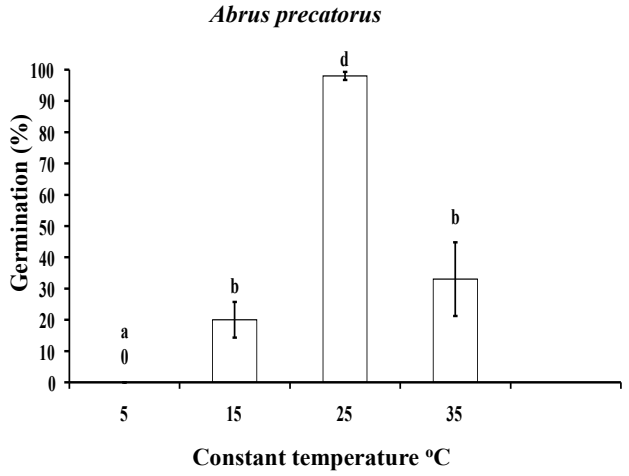

Fig. 2. Effect of temperature on the germination rate of *A. precatorius* imbibed with water under 12/12 light-dark conditions in response to constant temperatures.

Table 1. Main daily germination (MDG) and germination speed (T_{50}) of *A. precatorius* in response to variable temperature regimes (15/5°C, 20/10°C, 25/15°C, 30/20°C and 35/25°C). ND, undefined.

Temperature regime °C	Main daily germination (MDG) %	Germination speed (T50) number/Day
15/5	0	**ND**
20/10	0.21	22
25/15	0.32	9
30/20	0.31	6
35/25	0.30	19

Data were the value of means (±SE). Different letters indicate significant differences (p<0.05) among different temperatures

Table 2. Main daily germination (MDG) and germination speed (T_{50}) of *A. precatorius* in response to constant temperatures (5, 15, 25, and 35°C). ND, undefined.

Temperature regime °C	Main daily germination (MDG) %	Germination speed (T50) number/Day
5°C	**ND**	0
15°C	20	0.06
25°C	4	0.32
35°C	16	0.11

Data were the value of means (±SE). Different letters indicate significant differences (p<0.05) among different temperatures

Table 3. Effect of salinity on the germination rate of *A. precatorius* imbibed with distilled water or 200, 400 and 600 mM NaCl and germinated in 12/12 light-dark conditions at temperatures of 25/15°C.

Temperature regime	Treatments (mM NaCl)	Germination rate (%)
25/15°C	0	95 ± 2.2^{b}
	200	40 ± 6.3^{c}
	400	9 ± 4.3^{d}
	600	0^{a}

Data were the value of means (±SE). Different letters indicate significant differences at (p<0.05)

Effect of salinity on germination success of *A. precatorius*: The results of a one-way ANOVA of traits by salinity (S) and their interactions on the seed germination rate of A. precatorius in a 12 h photoperiod condition for 30 days at a temperature regime of 25/15°C under 0-600 mM NaCl are provided in Table 3. Salinity significantly affected the germination of A. precatorius seeds compared to the control (0 mM NaCl); with 200, 400 and 600 mM NaCl, the germination rate was reduced by approximately 60 to 90 and 100%, respectively, when compared to the control.

When ungerminated control seeds were transferred to distilled water, 100% of seeds remained dormant. At 200 mM NaCl, only 62% of the seeds were germinated, 25% remained dormant (Table 4). Similarly, when treated ungerminated seeds (600 mM NaCl) were transferred to distilled water, 49% germinated, 24% remained dormant.

Salinity appeared to affect the germination of *A. precatorius*, according to the findings shown in Table 5. MDG was approximately 0.32 seed/day at 0 mM NaCl, and T_{50} was obtained after 4 days at the temperature regime (25/15°C). After 19 days of treatment with 200 mM NaCl, we observed a decrease in MDG (0.13 seed/day) and a reduction in T_{50}. The MDG of 400 mM-treated seeds was approximately 0.03 seed/day, and T_{50} was obtained after 22 days. T_{50} was not determined (ND) for seeds treated with 600 mM, and MDG approached zero seeds per day.

Discussion

The germination potential and dormancy duration of *A. precatorius* seeds were evaluated in this investigation. Acid scarification was applied to the seeds of *A. precatorius* seeds. No seed was discarded after being treated with acids for 60 min, indicating that the seeds were 96% more likely to germinate. Numerous scarification methods have been discovered to result in a variety of *A. precatorius* germination rates (90% after seven days and 95% after 12 days, respectively) (Qadir *et al.,* 2012; Prakash *et al.,* 2013). It can be viewed as a potential method for encouraging germination and removing some plant species from dormancy (Nasir *et al.,* 2001; Alderete-Chavez *et al.,* 2011). It was fascinating to explore the acid scarification treatment processes and the time required to break the dormancy in *A. precatorius*, which failed seeds to germinate (Prakash *et al.,* 2013). It was once proposed that the seed coat could only prevent germination by restricting gas and water flow (Nautiyal *et al.,* 2014). The percentage of seed germination in *A. precatorius* L. seeds treated with sulphuric acid for different temperatures has been determined in various studies (Figs. 1 and 2; Tables 1 and 2). *A. precatorius* seeds were germinated under various temperature regimes (15/5°C, 20/10°C, 25/15°C, 30/20°C and 35/25°C) and constant temperature regimes (5, 15, 25, and 35 °C). Various species of seeds and seeds from the same plant can germinate at various temperatures. The present findings revealed that a constant temperature of 25°C is the optimal temperature for the germination of *A. precatorius* seeds and that a temperature regime of 25/15°C is the most effective. Many seeds germinate slightly above room temperature, 16-24°C, whereas others germinate only in reaction to temperature fluctuations between warm and cold (Ghosh & Maiti, 2014). In other studies, seeds were kept at 30°C throughout all the pretreatment (Sharma & Sharma, 2010). A recent investigation on how temperature affects this plant's germination found that maintaining a temperature of 30°C for 4 days considerably boosted the germination rate (Ghosh & Maiti, 2014). It has been reported that treatment at higher temperatures and for more extended periods decreased germination in most of the examined species (Elliot, 2000).

Seeds' ability from the *A. precatorius* plant to withstand high salt stress under different light/dark regimes was also investigated in this study. Salinity reduced *A. precatorius* seed germination until 400 mM NaCl, after which no seed germination occurred (Tables 3-5). In non-saline settings, higher germination rates were attained. An increase in salt concentration decreased the ultimate percentage of germination and

slowed germination., which is stopped at 600 mM NaCl. However, seeds placed in distilled water germinated after the salinity stress was removed (Table 4). The tolerance and recovery from salinity vary by species (Song *et al.,* 2006), with 500 mM NaCl for *Limonium stocksii* (Zia & Khan, 2004) and *Medicago ruthenica* and *Salsola affinis* (Guan *et al.,* 2009; Wei *et al.,* 2008) and *Halostachys capsica* (Zheng *et al.,* 2005) and 600 mM NaCl for *Limonium supinum* (Melendoa & Giménez, 2018). The final recovery percentages in high salt treatments (600 mM NaCl) were near 50% (Table 4), indicating that exposure to high concentrations of NaCl did not permanently inhibit germination. Antecedently, NaCl at different concentrations (0, 20, 40, 60, 80 and 100 mM) induced better callus proliferation in *A. precatorius* (Lafna & Deepa, 2021). Nevertheless, when subjected to significant salinity stress, some halophyte seeds either did not survive or recovered just partially (Khan & Gul, 2006). The pharmaceutical industry uses different methods to isolate and detect secondary metabolites from plant extracts. GC-MS analysis is one of the advanced techniques to identify other organic metabolites from the extracts of medicinal plants (Safaei-Ghomi *et al.,* 2009). To adjust to challenging stress situations, salt-tolerant plants produce different secondary metabolites to serve cellular functions essential for physiological processes. Secondary metabolites have no role in the growth and development of plants, but they are required to survive in the environment (Yang *et al.,* 2018).

Table 4. Germination rate, dormancy and death of seeds of A. precatorius imbibed with 200, 400 and 600 mM NaCl, transferred to distilled water under 12/12 light-dark conditions at temperatures regime of 25/15°C.

Temperature regime °C	Treatments (mM NaCl)	Number of germinating seeds	Dormant seeds	Dead seeds
25/15 °C	0	0	4	0
	200	37	15	8
	400	57	22	12
	600	49	24	27

Data were the value of means (±SE). Different letters indicate significant differences at (p<0.05)

Table 5. Main daily germination (MDG) and germination speed (T_{50}) of A. precatorius imbibed with distilled water or 200, 400 and 600 mM NaCl under 12/12 light-dark conditions at temperatures regime of 25/15°C.

Temperature regime °C	Treatments (mM NaCl)	Main daily germinating (MDG) %	Germination speed (T50) number/Day
25/15 °C	0	0	4
	200	37	15
	400	57	22
	600	49	24

Data were the value of means (±SE). ND, undefined; Different letters indicate significant differences (p<0.05)

Conclusion

In our study *A. precatorius* seeds were evaluated for their ability to germinate and whether their dormancy period would reduce following acid scarification under both control and salt stress conditions. These results showed that *A. precatorius* seeds were not dormant and exhibited nearly 100% germination capacity under non-saline conditions for the temperature regimes tested, with maximum germination occurring at 25/15°C. The optimum temperature for *A. precatorius* germination is 25°C. Seed germination of *A. precatorius* reduced with an increase in salinity (200-400 mM), reaching a complete inhibition at 600 mM NaCl. These observations might increase understanding of how seeds germinate in plants under stress mediated by climate change.

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