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Research Article

Temperature and Medium Affected Ecological Niche Breadth Study of some Leguminous Herbs from the Sultanate of Oman

Pankaj Sah, Fatma M. Al-Azri, Hafsa A. Al-Saidi and Abdullah A. Al-Sanjoor

Applied Sciences Department (APS), Applied Biology Section, Higher College of Technology (HCT), P.O. Box 74, PC 133, Al-Khuwair, Muscat, Sultanate of Oman

Abstract

Background: Conservation and restoration of Arabian arable lands warrants immediate attention from the world scientific community due to rising mean global temperature and augmented anthropogenic impact on this part of the world. Understanding the environmental factors that influence and regulate seed germination is very important for the establishment and regeneration of various plant communities in various ecosystems. **Materials and Methods:** The effects of temperature and medium were investigated in the germination behaviors of three different leguminous herbs in the hot and arid zone of Muscat. The experiment was designed to study seed germination behaviors with a temperature gradient (mean treatment: 25, 30, 35 and 40°C) and different media treatments. The data was statistically analyzed for Mean±Standard Error, Pearson bivariate correlation, one way analysis of variance (ANOVA) and *post hoc* analysis (Holm-Sidak method) by SPSS ver. 20 and Sigma Plot ver. 12. **Results:** *Trigonella foenum-graecum* L., showed the best germination trend (100%) over other studied legumes (*Pisum sativum* L. and *Glycine max* L.). Levin's standardized niche breadth analysis (B_A) also confirmed the broad temperature and media tolerance ability of *Trigonella foenum-graecum* L. It was observed that *Trigonella foenum-graecum* L., is a 'Generalist species' with broad niche breadth (B_A = 0.80). While, *Glycine max* (B_A = 0.59) was found to be a 'Moderately generalist species'. Whereas *Pisum sativum* was found to be a 'Specialist species' with narrow niche breadth (B_A = 0.33). **Conclusion:** Here, it is showed that *Trigonella foenum-graecum* L., can survive very easily in the hot and nutrient poor environmental conditions of Sultanate of Oman. Hence, it can be used as potent nitrogen sequester species in selected areas of interest to mobilize significant amounts of nitrogen in arid agricultural ecosystems. This type of study will not only help in reclaiming the degraded arable lands but also in sustainable agriculture and future agro-forestry practice in Oman and Arabia. It is finally concluded that if the farmers are encouraged to grow this crop in various seasons then it could fix significant amount of atmospheric nitrogen in soil. This will further facilitate the farmers and a possible reduction in the use of chemical fertilizers in agricultural lands will eventually help saving environment. The conservation of soil can be ensured with increased use of this species in these parts of the world. Promotion of this plant will also help in restoration of degraded arable lands of Arabia.

Key words: Seed germination, legumes, temperature regimes, nitrogen fixation, niche breadth, arid zone

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Corresponding Author: Pankaj Sah, Applied Sciences Department (APS), Applied Biology Section, Higher College of Technology (HCT), P.O. Box 74, PC 133, Al-Khuwair, Muscat, Sultanate of Oman

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Conserving natural ecosystems and their restoration is one of the most significant topics in contemporary ecology. Scientists are witnessing global land surface warming; many such extreme temperature events are expected to occur more often and more intensely in the near future. This will affect the growth and development of world's major cereal crops in several ways, thus finally affecting the production component of food security[1]. Arabian Peninsula also suffers from extreme temperatures and poor soil quality that render the plants under immense ecological stress. Arabian arid zones thus possess ecologically hostile environmental conditions for the growth and development of many plant species. Studies have shown that much of the Omani and Arabian lands are badly degraded and inability of plant species to colonize these areas is also one of the prime reasons of desertification[2].

Apart from natural limitations, augmented anthropogenic activities are also posing a direct threat to various ecosystems and their functioning at regional and global scales for past many decades[3]. The knowledge of seed biology is crucial for understanding the process and patterns within a given plant community, such as the establishment of plants, succession and natural regeneration in various environments[4]. Natural regeneration of species through seeds would depend primarily upon production and germination capacity of seeds and successful establishment of seedlings[5]. Each species has its own characteristic set of germination requirements. Whether or not viable seeds of a given species will germinate and the time at which they do so depend on a number of factors, including those present in the seed's environment[6]. Like many other physiological processes, seed germination is also regulated by temperature and every species has its own range of optimum temperature[7]. So, basically the seed germination is a complicated process including many reactions with different phases affected by temperature[8]. All growth processes within the seeds are biochemical reactions activated by the addition of water, subjecting to a decent temperature and oxygen presence. The higher the temperature is raised the faster will be the rate of chemical reactions. But there are biological limitations to how the temperature can be raised. The upper limit of rising temperature varies with plant species[8]. Water is imbibed and activates growth processes, the rate of which is temperature dependent[9]. For past many decades, a number of studies have dealt with general aspects of the environmental control of germination and dormancy[10-13]. In addition there have been several contributions that have specifically addressed ecological aspects of germination behavior[14-19]. Many researchers have described seed germination in desert plants[20,21]. Therefore, a proper understanding of the potential for natural regeneration requires knowledge of the influence of these factors on germination of species involved and the suitable period during which suitable condition exist in nature[22]. Seed germination depends on both internal and external conditions. The most important external factors include temperature, water, oxygen and sometimes light or darkness[23].

Present study deals with the effects of temperature and medium on germination behaviors of some leguminous species. Experiments with the seeds of three leguminous species would enable us to understand the relationship between temperature and medium on their germination and regeneration pattern. The Sultanate of Oman is located in the arid zone, where mean summer temperature is around 40-45 °C, hence the temperature plays a vital role in the seed germination. The accumulation of carbon dioxide and other greenhouse gases in the atmosphere is expected to further raise mean global temperature and cause observable climatic changes in the coming century[24]. In a rangeland study conducted at Saiq plateau, Oman, it was found that most of the sites are badly degraded and at risk to increasing loss of soil and vegetation[25]. Recovery of such sites thus require suitable species that not only hasten the process of recovery but could also serve significantly in the overall nitrogen economy of region by replacing nitrogen-poor habitats. The biological Nitrogen fixation in some species e.g., *Coraria* meets the relatively heavy demand for nitrogen in nutrient-poor degraded soils and thus makes it useful in afforestation and reclamation of degraded and nutrient-poor lands[5]. This not only reflects the argument previously produced for the importance of nitrogen fixing species in land reclamation, but also extends it to the subtleties of what may be involved in practice[26].

The aim of present study was to understand that which leguminous herb species had better germination rate at higher temperatures and poor media conditions in hot and arid climate of Oman. The hypothesis of this study was that leguminous species that survives and grows well in these extreme environmental conditions will also mobilize the sequestration of nitrogen in soil and thus making soil richer in nitrogenous compounds. Similar long term and field based studies should be undertaken for some years to study the restoration of Arabian arable lands. Here, we also recommend that long term studies with quantification of nitrogen sequestration in soil can also be carried out with native nitrogen fixing shrubs and trees species in a larger perspective to study the enrichment of soil nitrogen profile of Arabian arid zones.

MATERIALS AND METHODS

Arid zone and climate of Oman: The Sultanate of Oman has a hot climate with very slight rainfall. Annual rainfall in Muscat averages 100 mm, occurring mostly in the month of January. However, Dhofar region is subject to the Southwest monsoon and rainfall up to 640 mm has been recorded in the rainy season from late June-October. The climate of country usually is very hot, with temperatures reaching up to 49°C at some places in the hot months, from May-September.

Species grown with moistened filter paper in petri dishes: Seeds of three common leguminous herb (nitrogen fixing) species were chosen. We selected fenugreek (*Trigonella foenum-graecum* L.), pea (*Pisum sativum* L.) and soybean (*Glycine max* L.) for present seed germination study. Germination tests of each species were done by taking 3 replicates of 20 healthy seeds. All studied seeds were subjected to seed viability test by immersing them separately in beakers of 1000 mL distilled water. The viable seeds were heavier so they sank to the bottom of beaker. Whereas, the non-viable seeds with damaged embryos being lighter floated on the surface of water. Non-viable seeds were segregated and discarded from the beakers and only viable seeds were used for the present study[2,27]. Only seeds that sank to the bottom when immersed in water were used in our experiments. Seeds were then surface sterilized in 0.1% $HgCl_2$ solution and kept under different tests conditions. Petri dishes and filter paper were sterilized in an autoclave at 120°C for half an hour[26]. The seeds were placed in sterilized petri dishes between moistened filter papers and kept at room temperature and in four different temperatures in incubators (25, 30, 35 and 40°C constant temperatures). Prior to keeping petri dishes, the incubators were sterilized by keeping the temperature on maximum for 2 h and then wiping thoroughly with alcohol. The papers were regularly moistened with distilled water (10 mL day^{-1}) and observed daily up to 2 weeks for seed germination.

Species grown in soil medium: For growing seeds in soil medium, sand and compost mixture (1:3) sterilized at 110°C in oven[26] were filled in plastic pots. Seeds were placed in soil and watered regularly with distilled water. Seeds were observed daily for 2 weeks. For petri plates, the seeds were considered germinated if the radical exceeded 3 mm in length and in soil these were considered germinated when the shoot emerged above the soil surface.

Data collection and statistical analyses: The final seed germination percentages (GP) of all three species was calculated by using the formula:

$$GP = \frac{ni}{N} \times 100$$

where, GP is germination percentage, ni is number of seeds germinated and N is total number of seeds studied[1,26]. In order to understand the temperature and media tolerance abilities of various seeds, we calculated their ecological niche breadth following Levin's equation:

$$B = \frac{1}{\sum_{i=1}^{s} Pi^2}$$

where, B is Levin's niche breadth, Pi is proportion response of species P in the i^2 temperature regime. The data for all three species was further standardized following Levin's standardized measure of niche breadth[28]:

$$B_A = \frac{B-1}{N-1}$$

where, B_A is Levin's standardized niche breadth, B is Levin's niche breadth and N is number of temperature regimes. The standardized niche breadth measure ranges from 0-1, with 1 being a perfectly even distribution of response across the studied environmental gradients. The data was statistically analyzed for Mean±Standard Error, Pearson bivariate correlation, one way analysis of variance (ANOVA) and *post hoc* analysis all pairwise multiple comparisons (Holm-Sidak method). Values of $p<0.05$ were considered as significant. All statistical analyses were performed using Sigma Plot (Systat Software, San Jose, California USA) and IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

RESULTS

***Pisum sativum* L.: Mean seed germination:** It was found that *Pisum sativum* L., had narrow niche breadth as it germinated at 25 and 30°C only. At higher temperatures (35 and 40°C) pea seeds did not germinate at all, this shows its low tolerance capacity towards higher temperature regime. This was observed for both treatment i.e., with filter paper and with soil medium. Pearson's correlations were significantly positively correlated between days and percent seed germinations in low temperatures (r = 0.842, p<0.001, 25°C), (r = 0.693, p<0.01, 30°C) with filter paper (Fig. 1). Similar trends were also observed with soil medium treatment (r = 0.857, p<0.001, 25°C), (r = 0.838, p<0.001, 30°C) (Fig. 2). However, no germination was recorded at 35 and 40°C temperatures (Fig. 1, 2). Similar findings have also been reported by earlier workers on pea seed germination.

***Glycine max* L.: Mean seed germination:** On analyzing the germination patterns in different media conditions i.e., with moistened filter paper and with soil treatments *Glycine max* L., showed a wide range of temperature tolerance than *Pisum sativum* L., seeds. *Glycine* seeds did well at 25, 30 and 35°C temperature range. However, temperature beyond 35°C, arrested its germination totally.

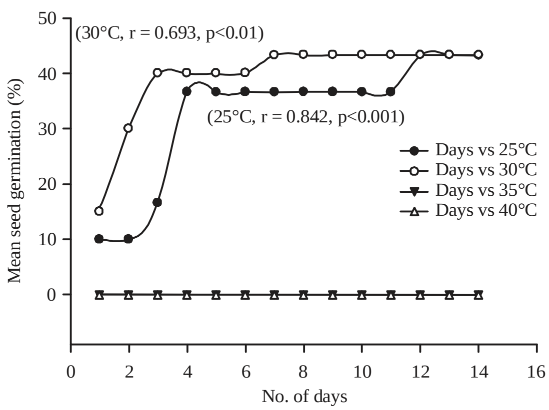

Fig. 1: *Pisum sativum* L., mean seed germination (%) with filter paper at constant temperatures

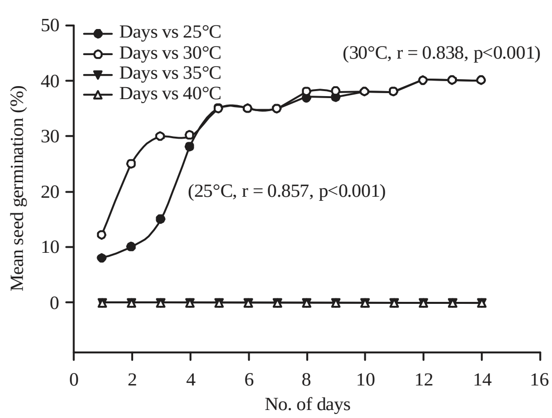

Fig. 2: *Pisum sativum* L., seed germination (%) with soil medium at alternate day/night temperature

Pearson's correlations were significantly positively correlated between days and percent seed germinations in different temperatures (r = 0.973, p<0.001, 25°C), (r = 0.957, p<0.001, 30°C) and (r = 0.953, p<0.001, 35°C) with filter paper (Fig. 3). Similar trends were also observed with soil treatment (r = 0.983, p<0.001, 25°C), (r = 0.952, p<0.001, 30°C) and (r = 0.8968, p<0.001, 35°C) (Fig. 4). However, no germination was recorded at 40°C temperatures (Fig. 3, 4). The results showed a better temperature tolerance capacity of *Glycine max* L., with medium and temperature regime.

***Trigonella foenum-graecum* L.: Mean seed germination:** It was found that the seeds of *Trigonella foenum-graecum* L., exhibited the widest range of medium and temperature tolerance ability in their germination patterns, among the studied legumes. The seeds germinated with moistened filter paper and with soil treatments in 25, 30, 35 and even 40°C. Pearson's correlations were significantly positively correlated between days and percent seed germinations in all

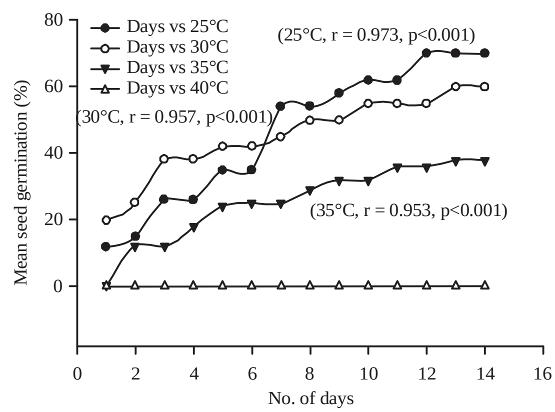

Fig. 3: *Glycine max* L., seed germination (%) with filter paper at constant temperatures

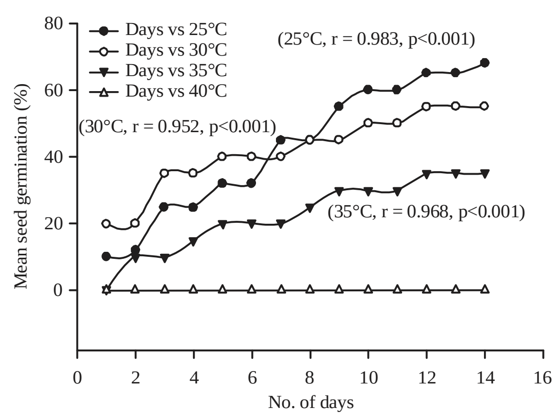

Fig. 4: *Glycine max* L., seed percent germination with soil medium at alternate day/night temperature

temperature regimes ($r = 0.679$, $p<0.01$, 25°C) ($r = 0.737$, $p<0.01$, 30°C), ($r = 0.980$, $p<0.001$, 35°C), ($r = 0.985$, $p<0.001$, 40°C) with filter paper (Fig. 5). Similar trends were also observed with soil medium treatment ($r = 0.764$, $p<0.001$, 25°C) ($r = 0.769$, $p<0.001$, 30°C), ($r = 0.990$, $p<0.001$, 35°C), ($r = 0.980$, $p<0.001$, 40°C) (Fig. 6).

Box and whisker plots for seed germination at 25°C temperature and different media conditions: Box and whisker plots show that *Trigonella foenum-graecum* L., had the highest germination percentage at 25°C in both types of media followed by *Glycine* and *Pisum* species (Fig. 7). The median and interquartile ranges differed among species and media treatments. However, in all the species the results were somewhat better in filter paper than soil media (Fig. 7). It was observed that 75% portion of the interquartile range is towards higher seed germination percentage in *Trigonella foenum-graecum* L., that clearly exhibits its tolerance to various media conditions (Fig. 7).

It was found that in all species, percent germination range was better in moistened filter paper than soil medium. Pea seeds had a wider distribution of percent germination range with filter paper at 25°C, which slightly decreased with soil medium. With filter paper, the lower quartile was 31.7% whereas it decreased to 24.6% with soil medium (Fig. 7). Earlier researchers have also found that seeds germinated well in moistened filter paper than soil medium. The interquartile range of percent germination was 93-100% and 80-98% at 25°C, with filter paper and soil, respectively (Fig. 7).

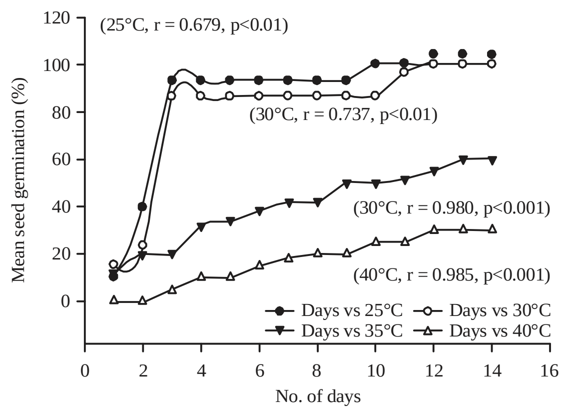

Fig. 5: *Trigonella foenum-graecum* L., seed percent germination with filter paper at constant temperature

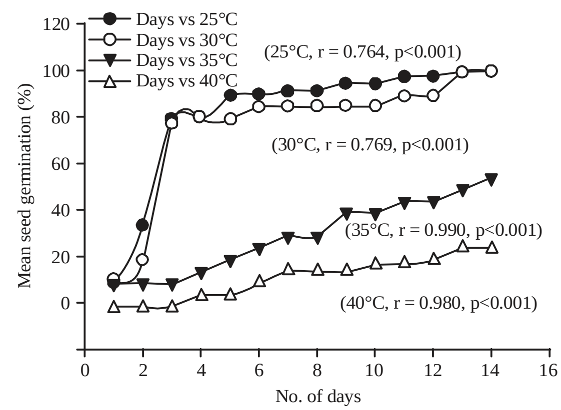

Fig. 6: *Trigonella foenum-graecum* L., seed percent germination with soil medium at alternate day/night temperature

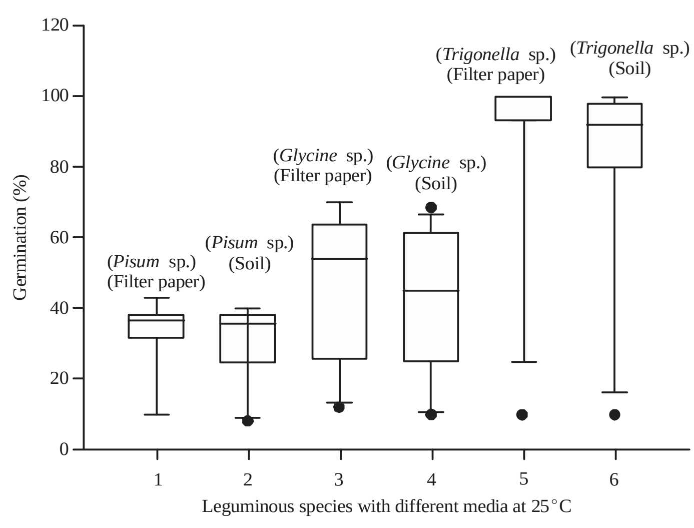

Fig. 7: Box and whisker plots displaying median, interquartile range (box) and range (whiskers) of percent seed germination for three species in two different medium at 25°C

ANOVA and *post hoc* analysis for plants seed germination at 25°C: One way analysis of variance (ANOVA one way) for percent seed germination across all three plants for different media conditions at 25°C exhibited statistically significant difference ($F = 19.862$, $df = 5$, $\alpha = 0.05$, $p<0.001$). A species by species analysis of seed germination values at 25°C temperature revealed the supremacy of *Trigonella foenum-graecum* L., in petri dishes and moistened filter paper (85.936±7.127%) followed by same species in soil medium (82.500±7.119%). *Glycine max* showed a moderate level of seed germination trend in both media. It showed 46.357±5.581% in petri dishes and moistened filter paper and 42.786±5.377% in soil medium. Whereas, *Pisum sativum* showed the least values in seed germination with 32.807±3.099% in petri dishes and moistened filter paper and 31.143±3.052% in soil.

In order to explore the data further we employed *post hoc* analysis for all pairwise multiple comparisons (Holm-Sidak method) (Table 1).

Box and whisker plots for seed germination at 30°C temperature and different media conditions: Box and whisker plots show that *Trigonella foenum-graecum* L., again had the highest germination percentage at an increased temperature of 30°C in both types of media followed by *Glycine* and *Pisum* species (Fig. 8). The median and interquartile ranges differed among species and media treatments. However, in all the species the results were somewhat better in filter paper than soil media (Fig. 8). It was observed that 75% portion of the interquartile range is towards higher seed germination percentage in *Trigonella foenum-graecum* L., that again

Table 1: *Post hoc* analysis: Holm-Sidak method (germination differences at 25°C)

Comparisons for factors	Difference of mean values	t-value	p-value	Significant (p<0.05*)
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	54.793	7.065	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Petri plate)	53.129	6.850	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Soil)	51.357	6.622	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	49.693	6.407	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	43.150	5.564	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Soil)	39.714	5.121	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Petri dishes and moistened filter paper)	39.579	5.103	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Petri dishes and moistened filter paper)	36.143	4.660	<0.001**	Yes
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	15.214	1.962	0.319	No
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	13.550	1.747	0.411	No
Glycine (Soil) vs., *Pisum* (Soil)	11.643	1.501	0.522	No
Glycine (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	9.979	1.287	0.595	No
Glycine (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	3.571	0.460	0.956	No
Trigonella (Petri dishes and moistened filter paper) vs., *Trigonella* (Soil)	3.436	0.443	0.884	No
Pisum (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	1.664	0.215	0.831	No

*Significant, **Highly significant

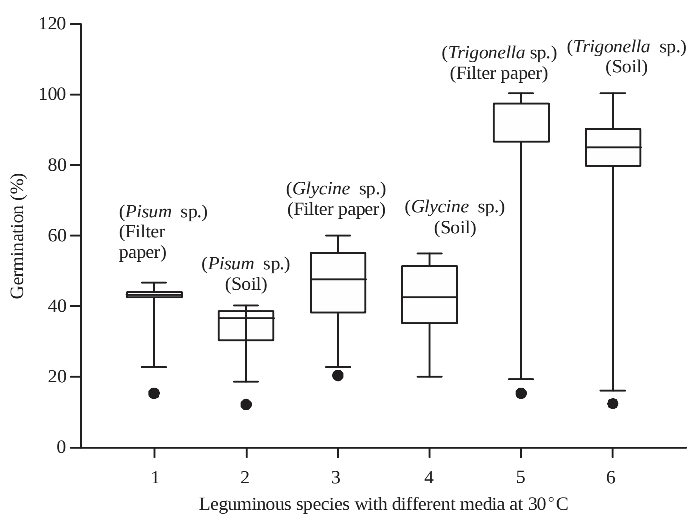

Fig. 8: Box and whisker plots displaying median, interquartile range (box) and range (whiskers) of percent seed germination for three species in two different medium at 30°C

clearly establishes its tolerance to various media and temperature conditions (Fig. 8).

Similar trends were also observed for increased temperature regimes, where upper quartile was 97.8 and 90.34% at 30°C, for filter paper and soil, respectively (Fig. 8). Although increased temperature slightly affected the percent germination of *Trigonella foenum-graecum* L., yet it performed well.

ANOVA and *post hoc* for plants seed germination at 30°C: Analysis of variance (ANOVA one way) for percent seed germination across all three plants for different media conditions at 30°C exhibited statistically significant difference ($F = 18.540$, $df = 5$, $\alpha = 0.05$, $p<0.001$). A species by species analysis of seed germination trends at 30°C temperature revealed the supremacy of *Trigonella foenum-graecum* L., in petri dishes and moistened filter paper (80.550±7.133%) followed by same species in soil medium (76.786±7.113%). *Glycine max* showed a moderate level of seed germination trend in both media. It showed 45.357±3.264% in petri dishes and moistened filter paper and 41.786±3.083% in soil medium. Whereas, *Pisum sativum* showed the least values in seed germination with 40.800±2.265% in petri dishes and moistened filter paper and 33.857±2.059% in soil.

In order to explore the data further we employed *post hoc* analysis for all pairwise multiple comparisons (Holm-Sidak method) (Table 2).

Box and whisker plots for seed germination at 35°C temperature and different media conditions: The impact of higher temperature of 35°C in germination behaviors of all seeds was noticed (Fig. 9). Box and whisker plots show that

Table 2: *Post hoc* analysis: Holm-Sidak method (germination differences at 30°C)

Comparisons for factors	Difference of mean values	t-value	p-value	Significant (p<0.05*)
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	46.693	7.066	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Soil)	42.929	6.496	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	39.750	6.015	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	38.764	5.866	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	35.986	5.446	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Petri dishes and moistened filter paper)	35.193	5.326	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Soil)	35.000	5.296	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Petri dishes and moistened filter paper)	31.429	4.756	<0.001**	Yes
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	11.500	1.740	0.466	No
Glycine (Soil) vs., *Pisum* (Soil)	7.929	1.200	0.798	No
Pisum (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	6.943	1.051	0.828	No
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	4.557	0.690	0.934	No
Trigonella (Petri dishes and moistened filter paper) vs., *Trigonella* (Soil)	3.764	0.570	0.921	No
Glycine (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	3.571	0.540	0.832	No
Glycine (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	0.986	0.149	0.882	No

*Significant, **Highly significant

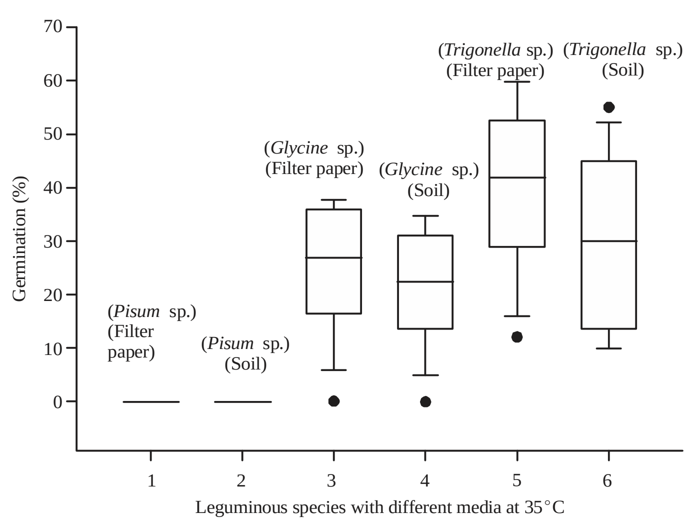

Fig. 9: Box and whisker plots displaying median, interquartile range (box) and range (whiskers) of percent seed germination for three species in two different medium at 35°C

Pisum sativum could not sustain itself at 35°C and collapsed. The other species i.e., *Glycine max* showed some tolerance at this extended temperature but its interquartile range decreased by 20% than the previous temperature treatment. The impact was also observed on the third species i.e., *Trigonella foenum-graecum* L. However, it was also found that this species successfully resisted the temperature and media effect and showed commendable data for seed germination (Fig. 9).

At 35°C, the upper whisker range was found to be 60.1 and 52.1%, for filter paper and soil, respectively. However, the outlier was found to be 55.4% for soil medium at 35°C (Fig. 9).

ANOVA and *post hoc* for plants seed germination at 35°C: Analysis of variance (ANOVA one way) for per cent seed germination across all three plants for different media conditions at 35°C exhibited statistically significant difference (F = 31.268, df = 5, α = 0.05, p<0.001). A species by species analysis of seed germination trends at 35°C temperature revealed the supremacy of *Trigonella foenum-graecum* L., in petri dishes and moistened filter paper (40.500±4.101%) followed by same species in soil medium (30.357±4.208%). *Glycine max* showed a moderate level of seed germination trend in both media. It showed 25.500±3.059% in petri dishes and moistened filter paper and 22.500±2.907% in soil medium. Whereas, *Pisum sativum* showed the least values in seed germination with 0.0±0.0% in petri dishes and moistened filter paper and 0.0±0.0% in soil.

In order to explore the data further we employed *post hoc* analysis for all pairwise multiple comparisons (Holm-Sidak method) (Table 3).

Box and whisker plots for seed germination at 40°C temperature and different media conditions: It was found that at the increased temperature of 40°C in germination trends of all seeds was clearly noticed (Fig. 10). Box and whisker plots show that two of the species i.e., *Pisum sativum* and *Glycine max* could not sustain themselves at 40°C and did not germinate at all. The only species that could sustain itself even at the extreme temperature of 40°C was *Trigonella foenum-graecum* L. (Fig. 10). Although the interquartile ranges of seed germination in both media was reduced yet the species showed good germination range. The results clearly establish that only *Trigonella foenum-graecum* L., has the greatest range of temperature and media tolerance abilities among all three studied leguminous herbs.

When we observed the effect of 40°C, on the germination percentage of *Trigonella foenum-graecum* L., box and whisker plots showed that upper whiskers ranges were at 30.0% and 25.0%, with moistened filter paper and soil medium, respectively (Fig. 10).

ANOVA and *post hoc* for plants seed germination at 40°C: Analysis of variance (ANOVA one way) for per cent seed germination across all three plants for different media conditions at 40°C exhibited statistically significant difference (F = 25.726, df = 5, α = 0.05, p<0.001). A species by species analysis of seed germination trends at 40°C temperature revealed the supremacy of *Trigonella foenum-graecum* L., in petri dishes and moistened filter paper (17.00±2.850%) followed by same species in soil medium (12.214±2.384%). *Glycine max* showed a moderate level of seed germination trend in both media. It showed 0.0±0.0% in petri dishes and moistened filter paper and 0.0±0.0% in soil medium. Whereas, *Pisum sativum* showed the least values in seed germination with 0.0±0.0% in petri dishes and moistened filter paper and 0.0±0.0% in soil.

Table 3: *Post hoc* analysis: Holm-Sidak method (germination differences at 35°C)

Comparisons for factors	Difference of mean values	t-value	p-value	Significant (p<0.05*)
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	40.500	9.696	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	40.500	9.696	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	30.357	7.268	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Soil)	30.357	7.268	<0.001**	Yes
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	25.500	6.105	<0.001**	Yes
Glycine (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	25.500	6.105	<0.001**	Yes
Glycine (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	22.500	5.387	<0.001**	Yes
Glycine (Soil) vs., *Pisum* (Soil)	22.500	5.387	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	18.000	4.309	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Petri dishes and moistened filter paper)	15.000	3.591	0.003**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Trigonella* (Soil)	10.143	2.428	0.084	No
Trigonella (Soil) vs., *Glycine* (Soil)	7.857	1.881	0.231	No
Trigonella (Soil) vs., *Glycine* (Petri dishes and moistened filter paper)	4.857	1.163	0.575	No
Glycine (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	3.000	0.718	0.724	No
Pisum (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	0.000	0.000	1.000	No

*Significant, **Highly significant

In order to explore the data further we employed *post hoc* analysis for all pairwise multiple comparisons (Holm-Sidak method) (Table 4).

Levin's standardized niche breadth (B_A) analysis across temperature gradients and media conditions: We observed the combine effects of various temperatures and nutrient media on the seed germination behaviors of all three species by calculating their niche breadth following Levin equation. Levin's niche breadth analysis is of prime significance in ecological and environmental studies and is a useful tool for quantifying species niche parameters over various environmental gradients[29]. It is a very effective measure in understanding a species behavior and capacity to tolerate various gradients of its environment[2,26]. Present study is important as it identifies the temperature and low-nutrient resistant property of *Trigonella foenum-graecum* L., from the region through various statistical analyses (Fig. 11). It was found that *Trigonella foenum-graecum* L., is basically a *generalist species* that can tolerate a wide range of temperature and nutrient stress with a significant broad niche breadth (B_A = 0.80). While, *Glycine max* (B_A = 0.59) was found to be a 'Moderately generalist species' that could not tolerate the higher temperature and nutrient poor environments. Whereas *Pisum sativum* was found to be absolutely 'specialist species' that was highly sensitive with even a slight increase in temperate and nutrient poor environments, with extremely narrow niche breadth (B_A = 0.33) (Fig. 11). These results clearly signify the ecological

Table 4: *Post hoc* analysis: Holm-Sidak method (germination differences at 40°C)

Comparisons for factors	Difference of mean values	t-value	p-value	Significant (p<0.05*)
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Petri dishes and moistened filter paper)	17.000	7.925	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	17.000	7.925	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Pisum* (Petri dishes and moistened filter paper)	17.000	7.925	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	17.000	7.925	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Petri dishes and moistened filter paper)	12.214	5.694	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Petri dishes and moistened filter paper)	12.214	5.694	<0.001**	Yes
Trigonella (Soil) vs., *Pisum* (Soil)	12.214	5.694	<0.001**	Yes
Trigonella (Soil) vs., *Glycine* (Soil)	12.214	5.694	<0.001**	Yes
Trigonella (Petri dishes and moistened filter paper) vs., *Trigonella* (Soil)	4.786	2.231	0.184	No
Pisum (Petri dishes and moistened filter paper) vs., *Glycine* (Petri dishes and moistened filter paper)	0.000	0.000	1.000	No
Pisum (Soil) vs., *Glycine* (Petri dishes and moistened filter paper)	0.000	0.000	1.000	No
Pisum (Soil) vs., *Glycine* (Soil)	0.000	0.000	1.000	No
Glycine (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	0.000	0.000	1.000	No
Pisum (Petri dishes and moistened filter paper) vs., *Pisum* (Soil)	0.000	0.000	1.000	No
Pisum (Petri dishes and moistened filter paper) vs., *Glycine* (Soil)	0.000	0.000	1.000	No

*Significant, **Highly significant

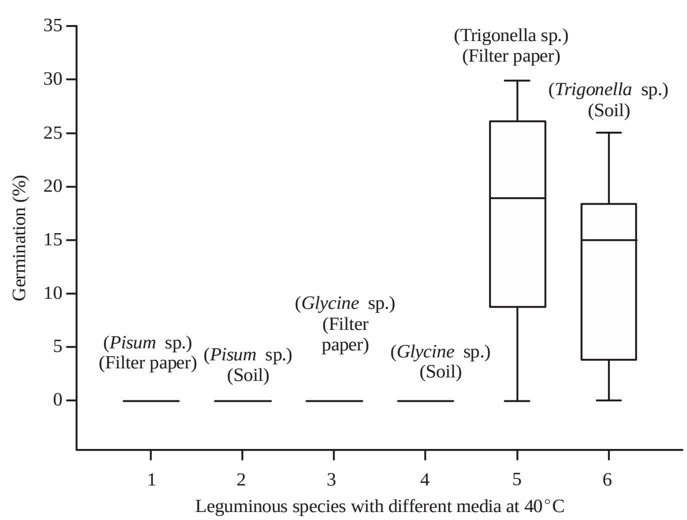

Fig. 10: Box and whisker plots displaying median, interquartile range (box) and range (whiskers) of percent seed germination for three species in two different medium at 40°C

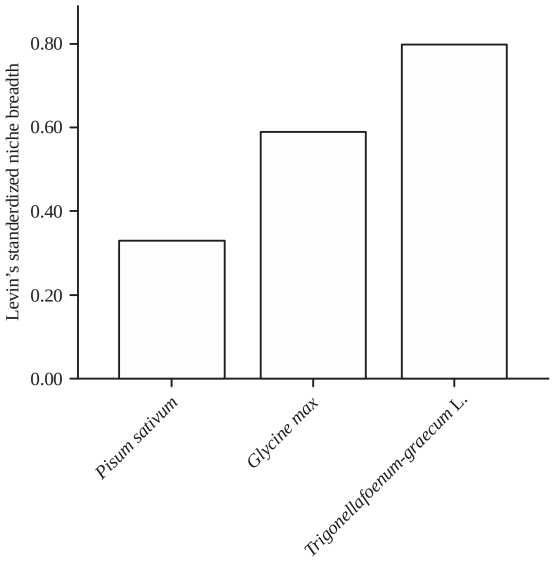

Fig. 11: Levin's standardized niche breadth analysis (B_A) for all three leguminous species in varying temperatures and media regimes

importance of *Trigonella foenum-graecum* L., for better functional, more productive and sustainable arable ecosystems.

DISCUSSION

Restoration of various ecosystems has become a core issue in contemporary ecological studies. Within the varied contexts of environmental policy many important factors such as the conservation of imperilled species populations and restoration of damaged habitats, an emphasis on idealized optimal conditions has guided to increasingly specific targets for management[30]. Seed germination is an important life-cycle transition because it determines subsequent plant survival and reproductive success. To detect optimal spatiotemporal conditions for germination, seeds act as sophisticated environmental sensors integrating information such as ambient temperature[31].

Scientists have found that plant reproduction and growth is highly vulnerable to global climate change components such as surging of atmospheric carbon dioxide, temperature and ultra violet-B (UV-B) radiation[32]. It has been also clearly found that temperature regimes regulate seed germination by affecting enzymatic activities, reaction rates and changes in the physical state of cellular components. On one hand, low temperature may inhibit the catabolic activity and on the other hand temperatures higher than the optimum tend to bring about the disruption of metabolic processes by inactivating some enzymes and denaturing proteins[33]. However, if some leguminous plants can exhibit resistance to any of these factors then it can help in conservation and restoration of degraded lands by fixing significant amount of atmospheric nitrogen in soil. The poor temperature and media tolerance properties of *Pisum sativum* L., in the present study can be justified by many earlier studies from various parts of the world. While studying the effect of long heat stress on pea seed germination, it was found that temperature of 28°C was optimum for protrusion and forthcoming root growth; both functions were slowed down after 30°C and completely stopped thereafter[34]. Similar trends were also observed for 30°C temperature range (Fig. 8). Other workers have also found that the growth of *Pisum sativum* L., was severely affected by the increased[35] temperatures of 27 and 32°C.

On studying the effects of different combinations of day and night temperatures during reproductive growth on soybean seed quality, it was reported that a combination of 35°C day temperature and 30°C night temperature during seed fill and maturation or 35°C day temperature from flowering to maturity decreased germination[36].

Our results have shown that fenugreek showed the best germination rates over other legumes in the present study. Although fenugreek is a native of the Mediterranean region of Europe, it extends to central Asia and North Africa as well. It is also grown very satisfactorily in Central Europe, UK and USA. This wide distribution of its cultivation in the world is characteristics of its adaptation to variable climatic conditions and growing environments[37]. It is drought resistant and can survive in varied temperatures. Earlier workers have also shown that for Mediterranean legumes, base temperatures ranged from 0-5°C, optimum temperature ranged from 16-22°C, whereas maximum temperature ranged from 30-40°C for edible legumes[38] and for forage legumes[39].

In this study we found that all the species showed better results with filter paper than other media conditions. This result can be explained with other relevant studies. While, working with seed germination ecology of some leguminous and actinorhizal species, it was found that in all species, seeds on filter paper had higher ($p<0.05$) germination than seeds in soil[26]. It was also found that found that the higher the temperature in which, the seeds are placed to germinate, the smaller is the quantity of oxygen available to the embryo[40]. This resulted in poor germination as obtained at 35 and 40°C for most of the studied species, except *Trigonella foenum-graecum* L., which showed a wide variety of medium and temperature tolerance. Earlier researchers suggested that the species that germinate readily over a relatively wide range of temperatures should be easier to establish in the field than those with highly specific temperature requirements[41,42]. On degraded sites where original soil has deteriorated markedly, these nitrogen-fixing species can be regenerated through seeds. Once their crops become sufficiently large the return of nutrients through nutrient-rich leaf litter of nitrogen-fixing species will hasten the process of soil nutrient enrichment[26]. Soils rich in carbon and nitrogen will facilitate the process of autogenic succession and thus many different species will colonize the degraded lands to transform it into a more diverse, productive and sustainable ecosystem. Long term productivity is measured in terms of sustainable ecosystem processes that maintain fertility and the overall ecosystem health as well as recovery after adverse disturbances[43]. Demographic recruitment processes, such as seed germination and seedling emergence, are critical transitional phases to the re-establishment of degraded plant populations[44].

Direct seeding has long been a modus operandi for forest regeneration in many parts of the world, advantages of which include control over stocking and genetic material, it being simpler and less expensive than planting especially when nursery facilities are limiting or non-existent[45]. In nitrogen-fixing species the biological nitrogen fixation meets the relatively heavy demand for nitrogen in nutrient-poor degraded soils and thus makes them useful in afforestation and reclamation of degraded and nutrient-poor lands[26]. This indicates that temperature of the seed bed is a major deciding factor in determining the germination and regeneration capacity of seeds. Therefore, a preliminary study on seed germination behavior of species over a temperature range could be useful in selection of site for establishment of a particular species. From this study we have shown the difference among three leguminous species in their ecological niche to tolerate different temperature and media regimes. This study establishes the supremacy of *Trigonella foenum-graecum* L., over *Glycine max* L., and *Pisum sativum* L., in their seed germination ecology. This is a model study performed with annual leguminous herbs; we recommend that similar long term studies can be conducted with native leguminous shrubs and trees species. Once identified, Arabian countries can promote the establishment of suitable shrubs or trees species to reclaim degraded lands. This will pave way towards a greener Arabia.

CONCLUSION

Present study was focused on the nitrogen fixing crops used for arable lands. It is successfully shown that *Trigonella foenum-graecum* L., can resist high temperatures and nutrient poor conditions of Arabian arid lands. We finally recommend that long term field based experiments with phenological studies of leguminous trees should be performed. The quantification of soil nitrogen sequestration rates should also be studied in order to understand the modified nitrogen economy of Arabian arid zones. The result could lead to the facilitation of autogenic succession in these areas. We believe that these types of studies will eventually help in conservation and restoration of arable and general land ecosystems of Arabia.

SIGNIFICANT STATEMENTS

The present study is very relevant to the current problem of 'Arable land-loss' and 'Increasing desertification' in Arabian Peninsula. Present study focused on the temperature and media tolerance abilities of nitrogen fixing crops used for arable lands. These results indicate the significance of leguminous herb 'fenugreek' or *Trigonella foenum-graecum* L., that can resist experimental temperature and nutrient stress conditions. This species can germinate readily over a relatively wide range of temperatures and media conditions and would be to easier to establish in the field than those with highly specific temperature requirements. Increased use of this species will help in significant sequestration of atmospheric nitrogen in poor soils. The present study is thus an attempt to find the solution for restoration of Arabian arable lands.

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