

Germination and Recovery of the Seed of Three *Nepeta* species under Drought and Temperature Stresses

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Article History: Received 20 July 2025/Accepted in revised form 21 November 2025

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ABSTRACT

Propagation through seeds is an effective method for renewing plant populations and increasing their genetic diversity, but the process of seed germination is complex and relatively difficult due to the limitations of environmental conditions. Wild plants that reproduce through seed may be affected by changes in humidity and temperature. The present study aimed to investigate the ecological adaptation strategies of seed germination of three species of *Nepeta haussknechtii*, *N. pogonosperma* and *N. glomerulosa* subsp. *staffina* under different hydrothermal conditions. Controlled experiments were conducted to investigate the germination performance of *Nepeta* seeds at different temperatures (10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C) and simulated drought stress conditions using polyethylene glycol 6000 (PEG) concentrations (0, -0.3, -0.6, -0.9, and -1.2 MPa); since no germination was observed at 40°C and -1.5 MPa, the results of these values were excluded from the experiment. After 14 days, fresh ungerminated seeds from the hydrothermal treatments were transferred to distilled water (under 25°C conditions) to study the recovery of germination, which was also recorded at 2-day intervals for 14 days. The results showed that temperature, drought stress, and their interaction significantly affected the germination percentage and germination rate of seeds ($p < 0.01$). The germination percentage and germination rate of the seed were significantly higher at 25°C compared to other temperatures ($p < 0.01$). Drought stress inhibition on seed germination was increased by PEG-6000 solution concentrations. The percentage of ungerminated seeds that recovered after transfer to distilled water varied with temperature. Rehydration germination results showed that extremely high temperatures and drought stress conditions prevented seed germination of *Nepeta* seeds by destroying the seed structure. The seeds that fail to germinate under drought stress recover germination more quickly at lower temperatures than at higher temperatures. These results may indicate that a small percentage of *Nepeta* seeds can survive drought conditions and extreme higher temperatures will be able to germinate after drought levels are reduced by rainfall. Therefore, the predicted warmer and drier climate will inhibit *Nepeta* seed germination, indicating that temperature changes appear to play an important role in the recovery of *Nepeta* seed germination from drought stress.

Keywords: Germination percentage, Germination rate, Polyethylene glycol, Temperature

INTRODUCTION

Nepeta L. is the largest genus of Lamiaceae in Iran, with 75 species [1] of which 39 species are endemic to Iran. The *Nepeta* is a multiregional genus of the "Lamiaceae" (Labiatae or Mint) family. Species of *Nepeta* are a valuable part of traditional medicine and are used extensively, particularly in the Himalayan region of India (Uttarakhand, Himachal Pradesh, Jammu and Kashmir, Leh-Ladakh), Pakistan (Khyber Pakhtunkhwa and Pakistan Kashmir), Nepal (Baglung district), also in China and hilly regions of Turkey and Iran. *Nepeta* species are extensively used as a remedy against a variety of ailments and conditions like chicken pox, tuberculosis, malaria, pneumonia, influenza, measles, stomach disorders, eye complaints, respiratory disorders, asthma, colds, coughs, etc. [2]. It is an Irano-Turanian element and Iran is one of the centers of diversity for the genus. Iran is an arid and semi-arid country, and water scarcity and global warming are major challenges for the country. It is predicted that the temperature and the runoff changes in all basins in Iran will increase by 2030 [3].

Wild Plants are exposed to various levels of moisture stress during their life cycle [4]. Seed germination is a crucial stage in the life history of flowering plants, which is influenced by both seed characteristics and external environmental conditions. This stage is particularly critical for the renewal of vulnerable plant populations [5, 6]. Due to differences in plant species and their habitats, the germination characteristics of plant seeds and their response to environmental factors vary [7]. Therefore, the process of seed germination of flowering plants and the factors affecting it have become one of the important topics of research in the fields of ecology and botany.

Humidity and temperature typically influence the regulation of seed germination. Extremely high or low temperatures and low soil water potential can all affect seed germination, potentially limiting seedling regeneration and ultimately affecting plant population dynamics [8]. In studies investigating the interaction effects of temperature and drought stress on seed germination, Guedes *et al.* [9] research shows that *Apeiba tibourbou* seed germination is significantly influenced by the interaction of temperature and drought stress. The inhibition of seed germination under PEG-6000 stress is mainly attributed to the low water potential caused by osmotic effects. Under a water potential of -0.2 MPa, the seed germination percentage is significantly reduced, with a germination percentage of 51% at 25 °C and the most pronounced reduction to 37% at 30 °C [9]. Silva *et al.* [10] found that the germination percentage of *Barbarea verna* seeds decreased with decreasing water potential and that higher temperature (35 °C) showed a stronger inhibitory effect on germination compared to lower temperatures (20 °C and 25 °C). Most seeds that fail to germinate under simulated drought conditions with PEG survive and can germinate rapidly and relatively uniformly with reduced drought stress [11, 12]. This adaptive behavior represents a survival strategy for plants in adverse environments. For example, Elnagar *et al.* [13] found that *Salsola imbricata* seed germination can tolerate relatively low drought

stress, and seeds that fail to germinate under drought stress recover germination more quickly at lower temperatures than at higher temperatures.

Currently, many arid and semi-arid regions around the world are facing drought and degradation, which affects species diversity and succession of plant communities [14]. Many species are susceptible to changes in ecological factors caused by habitat changes. Therefore, it is essential to investigate the impact of sensitive ecological factors such as water and temperature on the reproductive processes of these plants. This study aims to investigate the effects of temperature treatments and PEG-6000-simulated drought stress on the germination characteristics of *Nepeta haussknechtii*, *N. pogonosperma* and *N. glomerulosa* subsp. *staffina* seeds, revealing the ecological adaptation mechanisms of seed germination to moisture and temperature conditions. The results may provide a theoretical basis for predicting the impact of future climate change on seed germination and for identifying optimal temperature and moisture conditions for *Nepeta* seed germination.

MATERIALS AND METHODS

The seeds of *Nepeta haussknechtii* (accession no. 30216; collecting year 2009; origin 38° 39' N, 48° 07' E), *N. pogonosperma* (accession no. 29623; collecting year 2009; origin 36° 33' N, 50° 283' E) and *N. glomerulosa* subsp. *staffina* (accession no. 27220; collecting year 2009; origin 30° 50' N, 51° 44' E) was provided by the Natural Resources Gene Bank of Iran (NRGB). According to NRGB rules, the vouchers are stored in NRGB with the same accession no.

The seeds were regenerated in 2022 in the research field of NRGB, Karaj, Iran, and stored at 4 °C for later use. Six constant temperature conditions of 10°, 15°, 20°, 25°, 30° and 35 °C established by six laboratory incubators. Polyethylene glycol 6000 (PEG-6000) solutions of 0, -0.3, -0.6, -0.9, and -1.2 MPa were used to simulate different levels of drought stress [15]. Since no germination was observed at 40°C and -1.5 MPa, the results of these values were excluded from the experiment. The germination test was continuously monitored and recorded for 14 days for seeds of all three species (without cold stratification treatment). After 14 days of germination testing, the remaining fresh ungerminated seeds [16] of the hydrothermal treatments were washed with sterile water and dried with filter paper. A rehydration test was performed with distilled water under 25 °C conditions. Daily observations were made, and the test was terminated when these ungerminated seeds had been monitored for 14 days. Germination percentage was calculated from the formula " $G_p = \frac{ng}{nt} \times 100$ " where G_p is the germination percentage, ng is the number of germinated seeds, and nt is the number of seeds planted [17]. Germination rate was obtained from the formula " $Gr = \sum \frac{ni}{di}$ " where Gr is the germination rate, ni is the number of germinated seeds on day i , and di is the number of days after the start of the experiment [18]. After testing the normality of the data, analysis of variance and comparison of means of germination percentage and rate were performed using Duncan's test using the SAS software (version 9).

RESULTS

Different concentrations of PEG-6000 solution simulating drought stress, various temperatures in drought conditions, and their interactions had a significant effect on the germination percentage and germination rate of *Nepeta* species seeds ($p < 0.001$) (Table 1). Germination percentage was significantly higher at the control than at other PEG-6000 concentrations ($p < 0.001$) (Table 2). The germination rate decreased with increasing PEG-6000 solution concentration, being significantly higher at the control ($p < 0.001$) (Table 2). In drought conditions, temperature had a significant impact on seed germination percentage and germination rate of *Nepeta* species ($p < 0.001$) (Table 2). Germination percentage and germination rate increased initially with rising temperature, peaking at 25 °C, and then decreased significantly from 25 °C to 35 °C in all three species ($p < 0.01$). *N. pogonosperma* had a higher amount of germination percentage and rate than the two other species (Table 2).

Recovery had a significant effect on the germination percentage and germination rate of *Nepeta* species seeds ($p < 0.001$) (Table 3). Recovery germination percentages substantially increased with increases in PEG concentrations (Table 4). All three species had a strong recovery response, indicating a positive effect on the germination of their seeds after exposure to drought for 14 days. Germination percentage and germination rate were significantly higher at the higher PEG solution concentrations than at other concentrations ($p < 0.001$) (Table 4). Temperature had a significant impact on recovery, germination percentage and rate of *Nepeta* species ($p < 0.001$) (Table 4). Recovery germination percentage and rate decreased and then increased with rising temperature, with a low point at 30 °C (Table 4).

In general, in the three species the interaction between temperature and drought stress showed that the germination percentage was highest in the control treatment at the six studied temperature, and decreased with increasing PEG-6000 solution concentrations (Fig. 1). The exceptions were observed in the *N. haussknechtii* and *N. pogonosperma* of that in the *N. haussknechtii* the highest germination percentage (100%) was observed at 25 °C with both control and PEG-6000 concentration -0.3 MPa. However, in the *N. pogonosperma* the highest germination percentage (100%) was observed at 25 °C with control and PEG-6000 concentrations of -0.3 MPa and -0.6 MPa (Fig. 1). This effect varies with the species. *N. glomerulosa* seeds are the most severely affected by a change in temperature. *N. glomerulosa* at lower and moderate temperatures had a substantial recovery response, but high temperatures caused irreparable injury to the seeds. *N. haussknechtii* and *N. pogonosperma* seeds had a better recovery response at the lower temperature, but higher temperature substantially prevented recovery (Fig. 1).

Temperature change had an effect on the recovery germination of *Nepeta* species in both drought and non-drought conditions (Fig. 2). The proportion of ungerminated seeds showed a wide variation, between 10° and 35 °C (Fig. 2). All three species had a poor recovery response at temperatures of 30° and 35 °C, indicating a negative effect on the germination of their seeds after exposure to drought for 14 days. In *Nepeta* species, embryos unable to germinate after stress relief were subsequently confirmed to be non-viable (ungerminated; Figure 2). The proportion of ungerminated seeds showed a wide variation; in *N. haussknechtii*, between 0% (at 10 °C under -0.6 MPa PEG concentration; at 20 °C under control and -0.6 MPa PEG concentration; at 25 °C under control and -0.3 MPa PEG concentration) and 96% (at 35 °C under -1.2 MPa PEG concentration) (Figure 2). In *N. pogonosperma*, between 0% (at 20 °C under control; at 25 °C under control, -0.3 MPa and -0.6 MPa PEG concentration) and 92% (at 10 °C under control) (Figure 2). In *N. glomerulosa*, between 9.33%

(at 10 °C under -1.2 MPa PEG concentration) and 75.66% (at 35 °C under -1.2 MPa PEG concentration). The total germination at different PEG-6000 solution concentrations varied significantly based on species and temperature. *N. glomerulosa* showed fewer fluctuations at a temperature of 10 °C; however, in *N. haussknechtii* and *N. pogonosperma* the total germination after recovery in distilled water following pretreatment at PEG-6000 solution concentrations differs significantly from the distilled water controls. In all species, total germination fluctuations among PEG-6000 solution concentrations were not high at a temperature of 35 °C. At this temperature, *N. glomerulosa* showed higher tolerance to drought (Figure 2).

The rate of recovery of germination of *Nepeta* species was affected by the pretreatment concentration of PEG. The rate of recovery of germination progressively increased with increases in the PEG pretreatment concentration (Table 5). In general, there is little difference between temperatures except at the 30 and 35 °C temperatures, where there was no recovery at a PEG concentration of -1.2 MPa in *N. haussknechtii*. At higher PEG concentrations, the warmer temperature significantly decreased the germination rate (Table 5); however, the cooler and moderate temperatures increased the germination rate. At lower PEG concentrations, the cooler and moderate temperatures had almost the same rate of germination. However, the rate of recovery from the high PEG treatment was optimal in moderate (15° to 25 °C) and minimal in the warmest (30°-35 °C) temperature (Table 5).

Table 1 Results of variance analysis of germination percentage (GP) and germination rate (GR, unit: seed.l⁻¹) of *Nepeta* seeds under different PEG concentration (MPa) and temperatures (°C)

S. O. V.	d.f.	<i>N. haussknechtii</i>		<i>N. pogonosperma</i>		<i>N. glomerulosa</i>	
		GP	GR	GP	GR	GP	GR
Temp. (T)	5	46.52 **	1.37 **	143.06 **	6.93 **	43.01 **	1.27 **
PEG con. (D)	4	100.47 **	3.43 **	80.09 **	6.27 **	80.60 **	2.71 **
T×D	20	24.55 **	0.81 **	38.43 **	2.25 **	24.55 **	0.81 **
Error		0.29	0.01	0.13	0.01	0.23	0.01
CV%		17.36	17.53	6.91	9.13	15.46	16.88

** : Significant at 1 percent.

Table 2 Mean comparison germination percentage (GP) and rate (GR, unit: seed.l⁻¹) of *Nepeta* seeds under different PEG concentration (MPa) and temperatures (°C)

PEG Con. (MPa)	<i>N. haussknechtii</i>		<i>N. pogonosperma</i>		<i>N. glomerulosa</i>	
	GP	GR	GP	GR	GP	GR
0	45.33 a	1.52 a	61.56 a	4.00 a	34.17 a	1.14 a
-0.3	29.11 b	0.88 b	56.22 b	2.96 b	21.83 b	0.66 b
-0.6	8.00 c	0.22 c	44.89 c	1.50 c	6.00 c	0.16 c
-0.9	6.22 c	0.15 c	32.22 d	0.91 d	4.67 c	0.11 c
-1.2	1.78 d	0.03 d	10.35 e	0.23 e	1.33 c	0.02 d
Temperature (°C)						
10	2.13 d	0.03 d	2.40 f	0.09 f	2.40 d	0.04 d
15	12.0 c	0.38 c	38.57 d	1.29 d	12.00 c	0.38 c
20	21.47 b	0.73 b	66.40 b	3.62 b	21.47 b	0.73 b
25	39.47 a	1.14 a	78.67 a	4.10 a	39.47 a	1.14 a
30	23.47 b	0.74 b	52.53 c	2.13 c	23.47 b	0.74 b
35	10.00 c	0.33 c	9.60 e	0.37 e	10.00 c	0.33 c

Different letters indicate statistically significant differences (at 5 percent)

Table 3 Results of variance analysis of recovery germination percentage (GP) and germination rate (GR, unit: seed.l⁻¹) of *Nepeta* seeds under recovery conditions

S. O. V.	D.f.	<i>N. haussknechtii</i>		<i>N. pogonosperma</i>		<i>N. glomerulosa</i>	
		GP	GR	GP	GR	GP	GR
Temp. (T)	5	46.93 **	5.48 **	46.83 **	4.73 **	72.49 **	5.74 **
PEG con. (D)	4	131.13 **	10.59 **	113.22 **	9.91 **	48.64 **	2.56 **
T×D	20	33.64 **	3.07 **	27.79 **	2.56 **	22.82 **	1.6199 ⁿ
Error		1.67	0.11	1.02	0.10	1.61	0.11
CV%		18.29	16.34	18.43	19.28	11.40	10.02

** : Significant at 1 percent, ⁿ : not significant.

Table 4 Mean comparison germination percentage (GP) and rate (GR, unit: seed.l⁻¹) of *Nepeta* seeds under recovery conditions

PEG Con. (MPa)	<i>N. haussknechtii</i>		<i>N. pogonosperma</i>		<i>N. glomerulosa</i>	
	GP	GR	GP	GR	GP	GR
0	2.89 e	0.19 e	2.00 e	0.15 e	20.22 d	1.05 c
-0.3	10.44 d	0.82 d	12.44 d	0.97 d	32.89 c	2.04 b
-0.6	37.33 c	3.02 c	19.11 c	1.76 c	54.00 ab	3.03 a
-0.9	51.78 b	4.27 b	26.67 b	2.39 b	49.33 b	2.81 a
-1.2	62.00 a	5.10 a	60.67 a	5.20 a	61.33 a	3.16 a
Temperature (°C)						
10	60.80 a	5.46 a	52.00 a	4.82 a	78.40 a	4.48 a
15	47.47 b	4.19 b	30.13 b	2.62 b	51.47 c	3.72 b
20	37.60 c	3.41 c	24.53 b	2.19 b	60.80 b	3.44 b
25	25.87 d	1.38 d	13.33 cd	1.10 c	35.73 d	1.44 c

30	7.73 e	0.43 e	8.53 d	0.65 c	16.80 e	0.63 d
35	17.87 d	1.21 d	16.53 c	1.19 c	18.13 e	0.79 d

Different letters indicate statistically significant differences (at 5 percent)

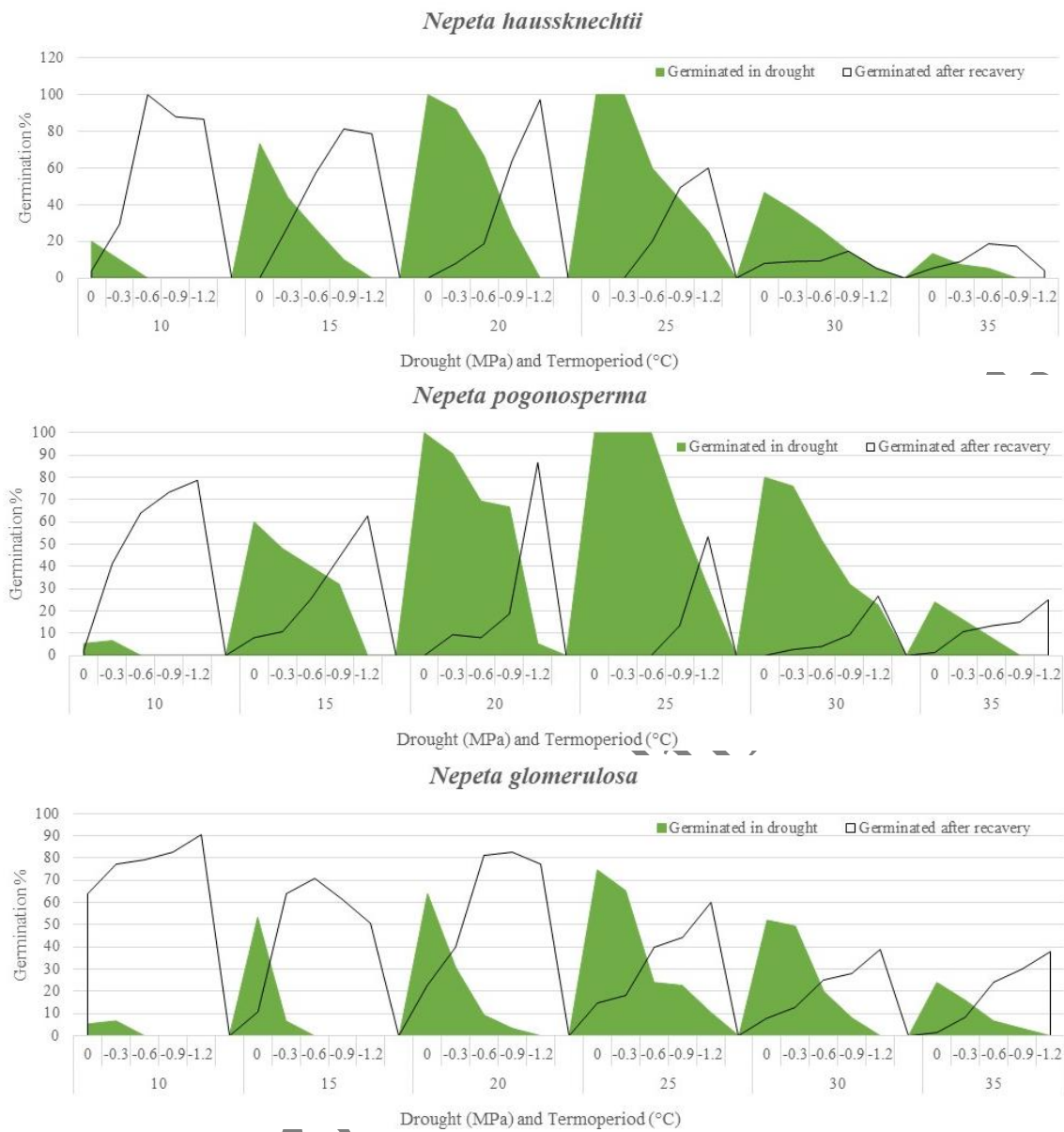


Fig. 1 Comparison of germination percentage in drought at different PEG concentrations (MPa) and temperatures (°C); and germination percentage in the recovery condition of *Nepeta* seeds

ACCEPT

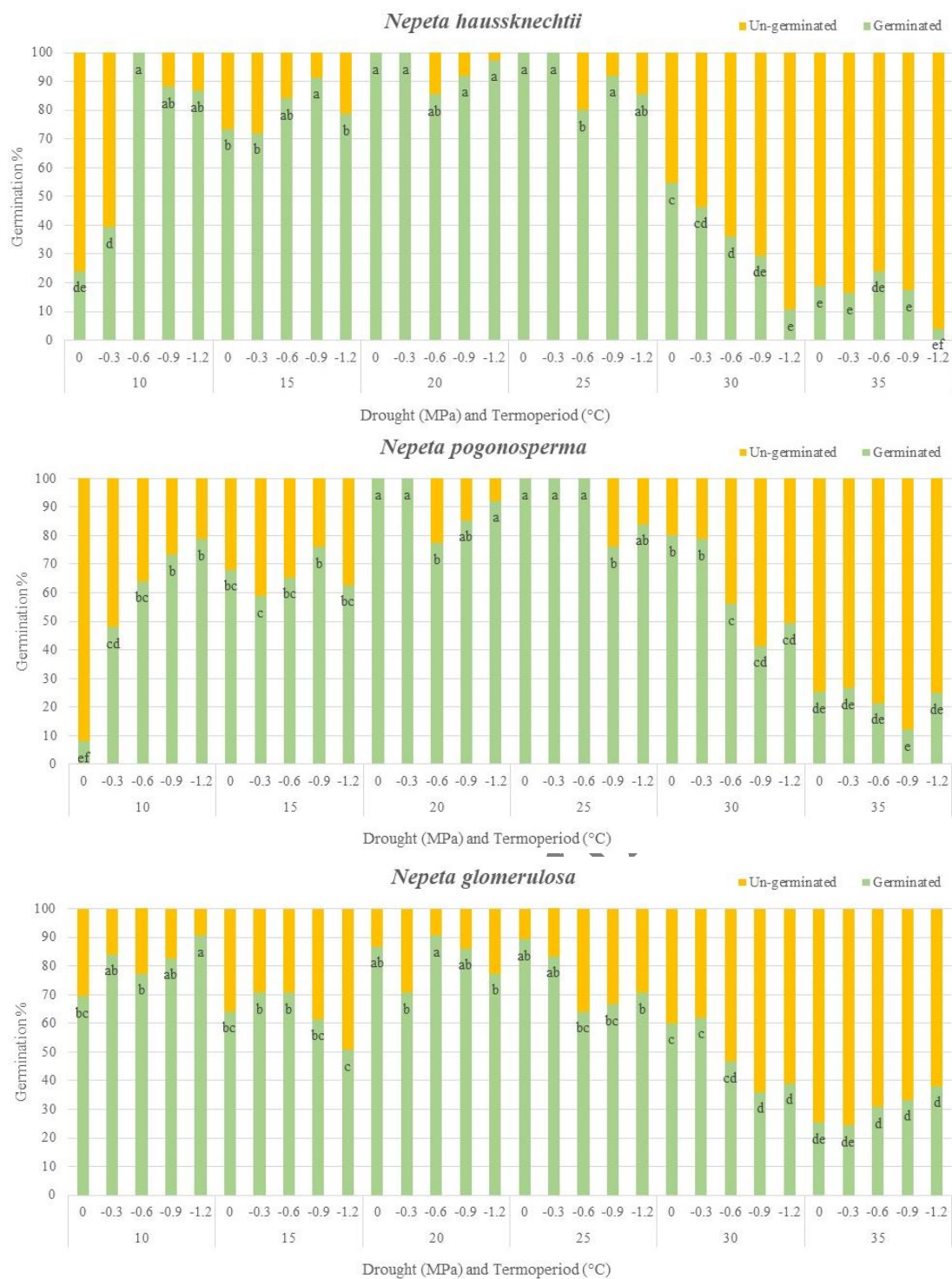


Fig. 2 Percentage of germinated (in drought and recovery conditions) and ungerminated seeds of *Nepeta*

Table 5 Mean comparison germination rate (unit: seed.1⁻¹) of *Nepeta* seeds under different PEG concentration (MPa) and temperatures (°C) and recovery condition

Temperature (°C)	PEG Con. (MPa)	<i>N. haussknechtii</i>		<i>N. pogonosperma</i>		<i>N. glomerulosa</i>	
		Drought	Recovery	Drought	Recovery	Drought	Recovery
10	0	0.56 e	0.28 f	0.17 f	0.22 f	0.08 f	3.77 d
	-0.3	0.19 f	2.56 d	0.28 f	3.6 d	0.1 f	4.21 d
	-0.6	0 f	8.44 a	0 f	6.18 b	0 f	4.62 d
	-0.9	0 f	8.48 a	0 f	6.65 b	0 f	4.57 d
	-1.2	0 f	7.55 b	0 f	7.45 b	0 f	5.25 c
15	0	3.2 d	0 f	2.17 d	0.58 e	1.7 e	0.69 e
	-0.3	1.23 e	1.47 e	2.03 d	0.86 e	0.19 f	5.37 c
	-0.6	0.56 e	4.99 c	1.05 e	2.28 d	0 f	5.26 c
	-0.9	0.18 f	6.99 b	0.76 e	4.17 c	0 f	4.42 c
	-1.2	0 f	7.5 b	0 f	5.23 c	0 f	2.88 d

20	0	8.11 a	0 f	8.04 a	0 f	2.3 d	0.94 e
	-0.3	3.71 d	0.5 e	5.02 c	0.51 e	1 e	2.02 d
	-0.6	2.04 d	1.65 e	2.72 d	0.61 e	0.28 f	4.71 c
	-0.9	0.71 e	5.82 c	2.22 d	2.11 d	0.08 f	5.07 c
	-1.2	0 f	9.06 a	0.12 f	8.06 a	0 f	4.46 c
25	0	8.35 a	0 f	8.47 a	0 f	2.42 d	0.61 e
	-0.3	4.43 c	0 f	6.66 b	0 f	1.98 e	0.33 f
	-0.6	1.46 e	1.64 e	3.21 d	0 f	0.63 e	1.44 e
	-0.9	1.2 e	2.6 d	1.54 e	2.1 d	0.47 e	1.64 e
	-1.2	0.86 e	2.64 d	0.62 e	4.49 c	0.19 f	3.15 d
30	0	2.24 d	0.56 e	4.01 c	0 f	1.82 e	0.23 f
	-0.3	1.57 e	0.08 f	3.39 d	0.06 f	1.44 e	0.05 f
	-0.6	0.93 e	0.25 f	1.72 e	0.28 f	0.2 f	1.13 e
	-0.9	0.51 e	0.88 e	0.95 e	2.01 d	0.25 f	0.65 e
	-1.2	0.23 f	0.39 f	0.58 e	2.25 d	0 f	1.09 e
35	0	0.43 e	0.29 f	1.16 e	0.11 f	0.8 e	0.04 f
	-0.3	0.18 f	0.28 f	0.41 f	0.83 e	0.56 e	0.24 f
	-0.6	0.15 f	1.14 e	0.28 f	1.22 e	0.19 f	1.02 e
	-0.9	0 f	0.87 e	0 f	2 d	0.09 f	0.51 e
	-1.2	0 f	3.46 d	0 f	3.72 d	0 f	2.16 d

Different letters indicate statistically significant differences (at 5 percent)

DISCUSSION

Seed germination of wild plants under natural conditions is regulated by changes in soil dryness and ambient temperature. Temperature is known to be a pivotal factor affecting seed germination [19, 20]. Present results indicated that germination percentage and germination rate of *Nepeta* were maximized at 25 °C, indicating that 25 °C is the optimum temperature for *Nepeta* seed germination. Very high or very low temperatures affect seed germination, reducing and even preventing seed germination [21].

Under normal conditions, drought negatively affects the germination of studied species of *Nepeta* seeds, an effect that has also been reported in many other plant species [22], leading to reduced germination and seedling survival rates and stunted growth and development of plant seedlings. The combined effect of high temperature and reduced water potential (drought) severely reduces the percentage of seed germination [20]. At the studied temperatures, the percentage of *Nepeta* seed germination and the germination rate decreased with increasing PEG-6000 solution concentration. Reports also show that the combined effect of high temperature and reduced water potential severely reduces the percentage of *Pinus yunnanensis* seed germination [23] and *Chloris virgata* [11]. Present results indicated that under optimal temperature (25 °C), *Nepeta* species showed partially tolerance to drought; in *N. haussknechtii* at the combined conditions of 25 °C temperature and -0.3 MPa PEG-6000 solution concentration, and *N. pogonosperma* at the combined conditions of 25 °C temperature and -0.3 and -0.6 MPa PEG-6000 solution concentrations, seeds exhibited optimal germination parameters. Sensitivity of seed germination to drought stress in lower temperatures, 10 °C and 15 °C, is higher than at higher temperatures (30 °C and 35 °C), indicating that seeds show a kind of tolerance to drought stress simulated by PEG-6000 solution under higher temperatures. In contrast to the present results, Toscano *et al.* [24] showed that drought stress significantly inhibits the germination of *Helianthus annuus* seeds, especially when the optimum temperature exceeds 20 °C. The present results in *N. haussknechtii* and *N. pogonosperma* indicated that seeds exhibit strong tolerance to drought stress simulated by PEG-6000 solution under optimum temperature, and moderate drought did not inhibit seed germination. In response to variable or unpredictable environments, seeds employ a variety of strategies. They may germinate rapidly to increase their competitive advantage in dry conditions by increasing the growth time of seedlings after germination. Alternatively, seeds can employ delayed germination as a buffer strategy to prevent germinating and reproducing in the same year, thereby reducing the risks of reproductive failure across their distribution area [25-27]. In the early spring, as temperatures rise, mild drought stress can aid in the germination of *Nepeta* seeds, providing sufficient time for rooting before the onset of warmer late spring and summer temperatures, thereby increasing seedling survival. However, as summer temperatures increase, these seeds become more sensitive to drought, potentially leading to their entry into dormancy and the formation of a soil seed bank. Dormancy, therefore, protects newly germinated seedlings from the challenging conditions of high temperature and drought, and also provides an opportunity to wait for more favorable conditions for subsequent germination attempts [28].

Seeds that do not germinate under specific temperature and drought stress conditions show re-germination after being transferred to non-stressed conditions [4, 20, 29, and 30]. The ability to recover from drought is known as an adaptive strategy selected by the environment to cope with drought stress [3]. Despite the observation of germination inhibition under drought, the *Nepeta* seeds were able to recover their germination after drought stress was removed and under optimal temperature. Studies reported that seeds of *Seriphidium transiliense* and *Salsola imbricata*, which did not germinate under conditions of drought and high temperature, germinated immediately after transfer to distilled water. This suggests that most of these seeds can still germinate after the effects of drought have subsided [13, 31]. Based on the rehydration germination percentages of *N. haussknechtii* seeds transferred to 25 °C after treatment with temperature and PEG-6000 solution stress, seeds treated with -0.3 MPa PEG-6000 solutions exhibited a rehydration germination percentage lower than those treated with -0.6, -0.9 and -1.2 MPa PEG-6000 solutions. This could be attributed to the fact that seeds treated with -0.3 MPa PEG-6000 solutions promoted germination of some seeds, while in others, despite not germinating, internal physiological and biochemical changes occurred that depleted the seeds' internal storage nutrients, thereby preventing germination upon rehydration. However, some of the seeds treated with -0.6, -0.9 and -1.2 MPa PEG-6000 solution exhibited mostly complete or higher inhibition, preserving internal seed nutrients intact. Upon transfer to suitable temperatures and relief from drought stress, these seeds germinated immediately. Moreover, the observation of recovery germination of some seeds even from the lowest water potential may be interpreted as the seeds entering a form of secondary

dormancy during the dry period and can germinate when water becomes available [20, 32]. On the other hand, the percentage of ungerminated seeds that recovered after transfer to distilled water varied with temperature. Rehydration germination results showed that extremely high temperatures and drought stress conditions prevented seed germination of *Nepeta* seeds by destroying the seed structure and embryo death [32, 33]. The seeds that do not germinate under drought stress are more likely to germinate at lower temperatures than at higher temperatures. These results may indicate that a small percentage of *Nepeta* seeds can survive drought conditions and extreme temperatures will be able to germinate after drought levels are reduced by rainfall. Therefore, the predicted warmer and drier climate will inhibit *Nepeta* seed germination, indicating that temperature changes appear to play an important role in the recovery of *Nepeta* seed germination from drought stress.

The recovery of germination was not similar to that obtained in distilled water controls. The pretreatments at reduced osmotic potentials at many temperatures had a stimulatory effect on germination. In confirmation of the present results, in other shorter-term tests of germination recovery [33], it was observed that several plant species had higher germination percentages after experiencing osmotic stress.

CONCLUSION

This study investigated the effects of temperature, drought stress, and their interaction on the germination of *Nepeta* seeds. The results in *N. haussknechtii* showed that conditions simulating drought stress I) at 20 °C with under control, and II) at 25 °C with under control and -0.3 MPa PEG-6000 solution were most conducive to seed germination. Seeds of *N. haussknechtii* exhibited tolerance to PEG-6000 simulated drought stress at 15 °C, more than 30 °C. In *N. pogonosperma* conditions, simulating drought stress I) at 20 °C under control, and II) at 25 °C under control, -0.3 and -0.6 MPa PEG-6000 solution were most conducive to seed germination. Seeds of *N. pogonosperma* exhibited the tolerance to PEG-6000 simulated drought stress at 30 °C, more than 15 °C. In *N. glomerulosa*, conditions simulating drought stress at 25 °C under control were most conducive to seed germination. Seeds of *N. glomerulosa* exhibited tolerance to PEG-6000 simulated drought stress at higher temperatures (30 °C and 35 °C) more than at lower temperatures (10 and 15 °C). Following the relief of stress conditions, the seeds of the studied *Nepeta* species resumed germination. The germination of the studied species of *Nepeta* seeds demonstrates a certain selective adaptability to temperature and moisture conditions, which likely evolved as an ecological adaptation strategy in long-term environments.

ACKNOWLEDGMENTS

This work was supported by the Agricultural Research, Education and Extension Organization, and Research Institute of Forests and Rangelands (RIFR), Iran; Project no. 02-09-09-056-000625.

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Accepted to Online Publishing