Seed germination and seedling emergence of *Gundelia tournefortii* L. in response to some environmental factors

Germinación de semillas y emergencia de plántulas de *Gundelia tournefortii* L. en respuesta a algunos factores ambientales

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ABSTRACT

Gundelia tournefortii has long been considered a valuable medicinal and edible plant in the Mediterranean areas. Seeds are the main means through which *G. tournefortii* can propagate in space and in time. The aim of this experiment was to evaluate the impact of environmental factors on seed germination of this wild medical and food plant. In this study, we investigated the germination and emergence responses of seeds of *G. tournefortii* collected from Kamyaran and Sonqor in western Iran to the environmental factors of light, temperature, salt stress, water potential, and sowing depth. The results showed that the optimal temperature for germination was 20°C, with a maximum germination percentage of about 70% for both populations. In general, the two populations responded to the tested factor(s) similarly. Light was not required for germination of *G. tournefortii*. The species was tolerant to water stress (germinating more than 50% under water potential up to -1.0 MPa), while sensitive to salt stress. More than 50% of the seedlings of *G. tournefortii* were able to emerge from depths more than 12 cm. According to the results, rain-fed fields located in the western parts of Iran are suitable for the planting of *G. tournefortii*.

Key words: water potential, salt stress, sowing depth, seedling emergence.

RESUMEN

Gundelia tournefortii es considerada como una valiosa planta medicinal y de alimento en las zonas mediterráneas. Las semillas son el principal medio por el cual *G. tournefortii* puede garantizar su permanencia en el espacio y en el tiempo. El objetivo de este estudio fue evaluar el impacto de los factores ambientales en la germinación de las semillas de esta planta medicinal silvestre. En este estudio, investigamos las respuestas de germinación y emergencia de semillas de *G. tournefortii* recolectadas en Kamyaran y Sonqor, ubicadas en el oeste de Irán, a los factores ambientales de temperatura, luz, estrés salino, potencial osmótico y profundidad de siembra. Los resultados mostraron una temperatura óptima de 20°C con un porcentaje máximo de germinación de aproximadamente 70% para ambas poblaciones. En general, ambas poblaciones respondieron de manera similar a los factores evaluados. La luz no se requirió para la germinación de *G. tournefortii*. Las plantas fueron tolerantes al estrés hídrico (germinando más del 50% bajo potencial hídrico hasta -1,0 MPa), mientras que fueron sensibles al estrés salino. Más del 50% de las plántulas de *G. tournefortii* pudieron emerger de profundidades de enterramiento de más de 12 cm. Acorde a los resultados, los campos de secano ubicados en la parte occidental de Irán son favorables para la plantación de *G. tournefortii*.

Palabras clave: potencial hídrico, estrés salino, profundidad de siembra, emergencia de plántulas.

Introduction

Gundelia tournefortii L., native to western Asia (Lev-Yadun & Abbo, 1999), is a well-known edible and important medicinal wild plant from the Asteraceae family (Vitek *et al*., 2017). It is a native plant that grows naturally in some regions of Iran, particularly in the rangelands of the western part (Karimzadeh *et al*., 2023).

This wild species has been used to treat a wide range of diseases (Abu-Lafi *et al*., 2019; Han *et al*., 2022; Keskin *et al*., 2022). The compounds found in *G tournefortii* have several pharmacological effects (Dastan & Yousefzadi, 2016; Hani *et al*., 2024). Furthermore, the flowers, leaves, seeds and stems of *G. tournefortii* are used as food sources (Konak *et al*., 2017; Saraç *et al*., 2019).

Nevertheless, multiplication of this medicinal plant *in vitro* and *in vivo* is challenging as its seeds are dormant and need specific conditions to break dormancy and to germinate (Mattana *et al*., 2022). Seed germination is one of the most important steps in a plant's life cycle, and the transition from seed to seedling is a crucial event (Rajjou *et al*., 2012). Temperature, light, moisture, salinity, and soil

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burial depth are the main environmental factors affecting seed germination and seedling emergence (Mattana *et al*., 2009; Nosratti *et al*., 2017).

Globally, seed germination and early growth of seedlings are negatively affected by environmental stress factors, especially drought and salinity (Llanes *et al*., 2016). These stress factors inhibit plant growth and reproductive development, leading to substantial reductions in crop production (Nosratti *et al*., 2023). Among environmental factors, temperature has a major role in determining the periodicity of seed germination and the distribution of the species (Guan *et al*., 2009).

Seed culture is a cost-effective substitute for commercial propagation methods and is now extensively used for the commercial propagation of a wide range of plant species, *e.g.*, medicinal plants (Chen *et al*., 2016). Nevertheless, the propagation of medicinal plants by seeds could be affected by numerous abiotic factors such as temperature, light, salt stress, and pH (Elhindi *et al*., 2016; Hesami *et al*., 2018; Murch *et al*.,2004).

There is little research on the effect of environmental factors on *G. tournefortii* seed germination. A better understanding of the effect of these factors on seed germination of *G. tournefortii* is required to create a better environmental situation for its cultivation as well as its propagation under controlled conditions. Hence, the aim of this research was to study the impact of temperature, light, burial depth, moisture, and salinity on the germination and seedling emergence of *G. tournefortii*.

Materials and methods

Site and seed description

Mature secondary capitula, hereafter "capitula", of two populations of *G. tournefortii* were harvested from nearly 600 plants growing in pastures in Sonqor County (34°46'58" N, 47°35'54" E), Kermanshah province, Iran, and Kamyaran County (34°47'43" N, 46°56'12" E), Kurdistan province, Iran, during June 2022 (Tab. 1). Capitula were considered mature when the plants of *G. tournefortii* had turned yellow and completely senesced. All tests in the present research were conducted using the seeds of both populations of *G. tournefortii* (Sonqor and Kamyaran populations).

Because of the low germination percentage observed in preliminary tests (lower than 20%), the fruit complex (*i.e.*, the secondary capitulum or disseminule) was manually removed, leaving only the seed coat, which is a thin, papery layer surrounding the embryo (Hind, 2013). For both populations of *G. tournefortii*, the 1,000-seed weight and 1,000-capitula weight were about 110 and 240 g, respectively.

TABLE 1. Precipitation and average daily air temperature of the study site during the growth season of *Gundelia tournefortii* in Sonqor and Kamyaran (Iran) in 2022.

	Precipitation (mm)		Temperature (ºC)	
Month	**Sonqor**	**Kamyaran**	**Sonqor**	**Kamyaran**
March	120	93.5	6.0	10.5
April	89.1	61.1	11.2	13.3
May	63.5	93.3	17.5	21.3
June	32.2	41.0	21.5	25.0

Germination test procedure

Five replicates of 20 seeds of *G. tournefortii* were put in 9 cm diameter Petri dishes containing two sheets of filter paper (Whatman No. 1, Maidstone, UK) moistened with 9 ml of distilled water or treatment solution. All Petri dishes were firmly wrapped with clear parafilm to reduce evaporation and then placed in a germination chamber (JAL TEB, model 200L) under a constant temperature of 20°C. The recording of germinated seeds was done when the radicle appeared (2 to 3 mm), and the measurement of this variable continued daily for two weeks.

Effect of light and temperature on germination

Constant temperature treatments during seed germination consisted of 10, 15, 20, 25, 30, and 35°C under light/dark or continuous darkness. To apply the light treatments, Petri dishes with *G. tournefortii* seeds were sealed with transparent polyethylene bags, while the dark treatments were wrapped in aluminum foil. The light intensity in the germination chamber was 150 μmol m^{-1} s^{-1} using fluorescent lamps.

Effect of salinity on germination

Seeds of *G. tournefortii* were exposed to six levels of salt stress using solutions containing 0 (control), 4, 8, 16, 32, 64, 128, and 256 mM of sodium chloride (NaCl) (Payamani *et al*., 2018). Treated seeds in Petri dishes were germinated under conditions described in the procedure for the germination test.

Effect of water stress on germination

The effect of different water potential on seed germination of *G. tournefortii* was assessed by incubating seeds in polyethylene glycol (6000) solutions with osmotic potentials of 0.0 (distilled water), –0.1, –0.2, –0.4, –0.6, –0.8, –1.0, or –1.2

MPa. The solutions were prepared based on the method described by Michel (1983). Seeds of both populations were then incubated in the light/temperature conditions as described in the germination test procedure.

Effect of soil burial depth on seedling emergence

The influence of different burial depths of 0, 2, 4, 6, 8, 10, and 12 cm on the appearance of *G. tournefortii* seedlings was examined. Seeds were placed at the desired depth in pots (25 cm in diameter by 15 cm in height), which were filled with a soil mixture (24% sand, 33% silt, 37% clay, 0.57% of total organic matter, and a pH 7.0). For each depth, 20 seeds were distributed on the surface of the soil and then covered with the same soil mixture to reach the desired depth. All the pots were placed in the greenhouse at 25/18 °C with a 12-h photoperiod. Pots were watered as needed to maintain sufficient soil moisture. Emergence was recorded weekly for a month. Seedlings were considered emerged when two fully developed leaves appeared on the soil surface. Seedlings were carefully separated to avoid soil problems after weekly measurement.

Statistical analyses

All trials, including those for soil burial depth, salinity, water stress, light and temperature were conducted as a completely randomized design with factorial arrangement of treatments with five replicates. The Kolmogorov–Smirnov test using the statistical software SPSS showed that the data are normal. Data were analyzed by the ANOVA procedure in MSTAT-C, and the LSD (Least significant difference) mean comparison test was applied. Regression analysis was done on data achieved from NaCl and water stress sowing depth experiments using the SigmaPlot software (v. 14.0, SyStat Software, Point Richmond, CA, USA). Germination percentages obtained from various concentrations of salt or in water were fitted to the following functional three-parameter exponential model (Nosratti *et al.*, 2019):

$$G_{max}/[1+(X/X_{50})^{Grate}] \qquad (1)$$

where G is the total germination (%) at each NaCl or osmotic potential X, G_{max} is the maximum germination (%), X_{50} is the NaCl concentration or osmotic potential required for 50% reduction of the maximum germination, and G_{rate} is the slope.

The seedling emergence percentage obtained at different soil depths was fitted to the following two-parameter exponential decay model (Nosratti *et al.*, 2019):

$$E_{max}e^{(-Erate.x)} \qquad (2)$$

In this model, E is the final seedling emergence percentage at burial depth x, E_{max} indicates the maximum seedling emergence (%), and E_{rate} is the slope.

Results and discussion

Temperature and light

Seed germination of both populations of *G. tournefortii* was not affected by light. Germination percentage increased linearly with temperature up to an optimal temperature (20°C), after which the germination rate declined (Fig. 1). Maximum seed germination percentage was achieved when seeds were incubated at 20°C (69% for both Sonqor and Kamyaran populations) (Fig. 1). In both populations, germination declined further as temperature increased to 25°C. Negligible seed germination (20%) occurred at low temperature (about 20 to 24%).

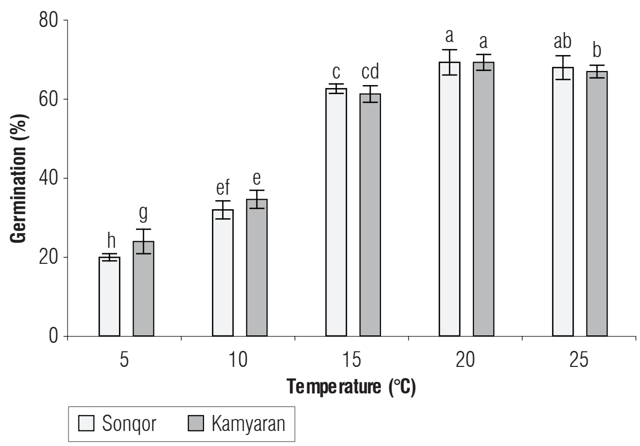

FIGURE 1. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to temperature. Vertical bars represents the standard error of mean. Means with the same letter are not significantly different according to the LSD test.

The results of this study indicate that the germination of this medicinal species is limited during winter and summer in Iran. Seedlings of *G. tournefortii* typically emerge in the early spring when temperatures range between 15 and 20°C in western parts of Iran. Similar to our results, Mattana *et al.* (2022) concluded that the best temperature for seed germination of *G. tournefortii* collected from various areas of the Mediterranean was 15°C. Hence, it is recommended to incubate seeds of *G. tournefortii* at 15 to 20°C temperature range for successful germination under controlled condition.

Salinity

Seed germination of both populations of *G. tournefortii* collected for this study decreased as salt concentrations increased, described well by a three-parameter logistic model (P<0.001) (Fig. 2). The germination percentage was 43% and 39% at 64 mM of NaCl for the Sonqor and Kamyaran populations, respectively. Less than 5% germination was observed at 256 mM salinity in both populations (Fig. 2). The mM concentration of NaCl necessary for 50% reduction of the highest germination (X_{50}) of *G. tournefortii* seeds was 95 and 92 mM for the Sonqor and Kamyaran populations, respectively.

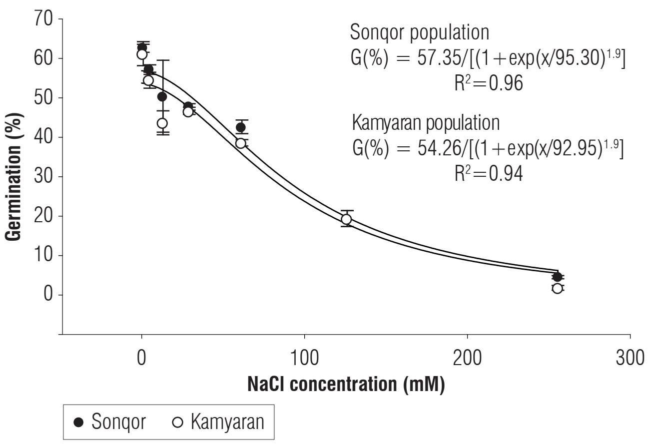

FIGURE 2. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to sodium chloride (NaCl) in the germination media. Vertical bars represents the standard error of mean (n= 5) and the lines represent a three-parameter logistic model fitted to the data.

Salinity, via increased osmotic pressure in germination media, reduces water absorption by seeds, which in turn affects metabolic and physiological processes that reduce or prevent seed germination (Gupta & Huang, 2014; Tlahig *et al*., 2021; Uçarlı, 2020).

In general, the germination of *G. tournefortii* under different salinity stresses indicates that a higher percentage of seeds can germinate in soils predominantly found in western provinces of Iran, where NaCl concentrations in the soil are less than 250 mM (Emadodin *et al*.,2012). Our results suggest that *G. tournefortii* could not tolerate saline soils like halophytes (salt-tolerant plants). Based on our results, *G. tournefortii* can be considered a salt-sensitive species (glycophyte) when compared with other species (Uçarlı, 2020).

Water potential

In both populations, seed germination decreased as water potential decreased (Fig. 3). A sigmoid model accurately described the germination of both populations in response to water stress. Non-stressed seeds germinated more than 70% for both populations, while germination decreased to about 50% at –1.2 MPa. The water potential required for 50% inhibition of the maximum germination was –1.91 and –1.85 MPa for Sonqor and Kamyaran population, respectively (Fig. 3).

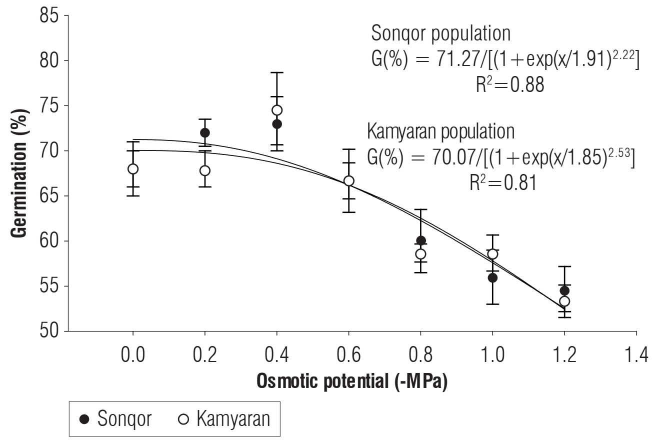

FIGURE 3. Seed germination of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to a water potential (-MPa) in germination media. Vertical bars represents the standard error of mean (n= 5) and the lines represent a three-parameter logistic model fitted to the data.

As evident from the X_{50} parameter, seed germination in both populations of *G. tournefortii* was more tolerant to water stress compared to other crops and medical plants (Khojasted & Chahouki, 2013; Sheikh-Mohamadi *et al*., 2018; Xiong & Zhu, 2002). These results show that *G. tournefortii* is well adapted to water shortage during germination. Therefore, this medical species can germinate well in rain-fed farmlands. The higher tolerance to water stress by *G. tournefortii* is consistent with its natural distribution, as this medical species frequently populates dryland rangelands (Esbati *et al*., 2021; Khojasted & Chahouki, 2013). Based on these results, it can be concluded that planting *G. tournefortii* under rain-fed conditions is feasible.

Sowing depth

Seedlings of both populations of *G. tournefortii* emerged favorably (higher than 60%) from sowing depths up to 6 cm (Fig. 4). Increasing burial depth reduced the emergence percentage similarly for both Sonqor and Kamyaran populations. The maximum germination (68% for both populations) was observed for seeds located on soil surface, while seeds buried 12 cm deep in the soil managed to emerge seedlings in both populations (Fig. 4).

It is well documented that the seedling emergence of most plant species decreases as seed burial depth increases in the soil (Grundy *et al*., 2003). In general, the burial depth of

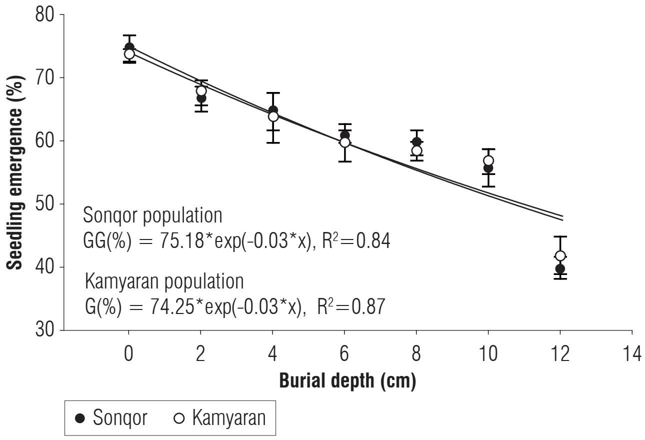

FIGURE 4. Seedling emergence from seeds of two populations (Sonqor and Kamyaran) of *Gundelia tournefortii* in response to burial depth. Vertical bar represents the standard error of mean (n= 5) and the lines represent a two-parameter exponential decay model fitted to the data.

most crop species is less than 15 cm (Kluyver *et al*., 2013). Therefore, *G. tournefortii* is well suited for cultivation in farmlands as a medicinal plant. The hypogeal germination characteristic of *G. tournefortii*, where cotyledons remain below the soil surface during seedling development, is the main reason for its ability to emerge from deeper layers of soil. Therefore, it doesn't require extra force to penetrate soil when compared with epigeal emergence in which the cotyledons extend above the soil surface (Gardarin *et al*.,2016). In addition, seeds of *G. tournefortii* are large enough to support seedling emergence from greater depth.

Conclusions

There was only a slight and non-significant difference in seed germination between the Kamyaran and Sonqor populations in response to various environmental factors evaluated in this study. Seed germination of *G. tournefortii* at the very high and low temperature conditions examined in our study indicates its potential for cultivation in a broad range of temperature conditions. Seeds of *G. tournefortii* still germinated under high levels of osmotic stress, while they were sensitive to salt stress. This suggests that *G. tournefortii* has a capacity to be planted in a broad range of farmlands, especially in rain-fed areas in western provinces of Iran and other parts of the world affected by drought stress and low concentration of NaCl in the soil. The highest soil depth from which seedling of *G. tournefortii* can emerge is higher than the typical depth of regular tillage in Iran. Therefore, such information can be valuable in developing its cultivating methods such as tillage systems and best soil conditions. Furthermore, the data obtained from this study may be helpful for development of species-specific propagation protocols and *ex situ* conservation of *G. tournefortii*.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

IN: conceptualization, research. MEGH: conceptualization, writing, and editing supervision. HF: conceptualization, visualization, writing, and editing. IN: conceptualization, writing, and editing supervision. All authors have read and approved the final version of the manuscript.

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