

## Response of *Caragana* Seed Germination to Different Temperatures and Soil Water Content (Postprint)

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### Abstract

*Caragana korshinskii* is a leguminous xerophytic shrub in the sandy grasslands of desert and semi-desert regions in northwestern China, and is also one of the main shrub species widely used in soil and water conservation and windbreak and sand fixation practices in this region. This study investigated the response of *C. korshinskii* seed germination to temperature and soil water content under six constant temperature conditions (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, and 30 °C) controlled in an artificial climate chamber, with different gradient treatments of soil water content (4%, 8%, 12%, 16%, 20%, and 30%). The results showed that: (1) Temperature, soil water content, and their interaction had extremely significant effects on the germination percentage, germination rate, germination index, and vigor index of *C. korshinskii* seeds. The germination percentage reached its maximum of 87.5% at 10 °C, and was also relatively high at 15 °C, 20 °C, and 25 °C; when temperature increased to 30 °C, the germination percentage decreased substantially. The germination rate, germination index, and vigor index were all minimal at 5 °C, and all increased initially then decreased with rising temperature, reaching their maxima at 25 °C or 15 °C. (2) With increasing soil water content, the germination percentage, germination index, and vigor index all showed a trend of increasing first then decreasing under different temperatures, reaching their maxima at 30% soil water content treatment at 5 °C, and at 20% or 16% under other temperatures. The germination rate increased gradually with soil water content at 5 °C, while under other temperatures it increased initially then decreased with increasing soil water content, reaching its maximum at the 20% treatment at 10 °C, 15 °C, and 20 °C, and at the 16% treatment at 25 °C and 30 °C. The inhibition of *C. korshinskii* seed germination by higher temperature and soil water content during summer and autumn may represent a protective strategy to ensure that some seeds enter the soil seed bank and reduce the risk of seedling mortality, while seed germination

under lower temperature and soil water content conditions in early spring may be a compensation for this inhibitory effect.

## Full Text

### Responses of Seed Germination of *Caragana korshinskii* to Different Temperatures and Soil Water Content

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#### Abstract

*Caragana korshinskii* is a xeromorphic shrub of the Fabaceae family distributed in the sandy grasslands of desert and semi-desert regions in northwestern China, and represents one of the primary shrub species widely employed in water conservation, windbreak, and sand fixation practices. This study investigated the responses of *C. korshinskii* seed germination to varying temperatures and soil water contents under six constant temperature regimes (5, 10, 15, 20, 25, and 30°C) controlled by artificial climate incubators, with six soil water content treatments (4%, 8%, 12%, 16%, 20%, and 30%). The results demonstrated that temperature, soil water content, and their interaction exerted highly significant effects on germination percentage (GP), germination rate (GR), germination index (GI), and vigor index (VI). Seeds achieved the maximum germination percentage of 87.5% at 10°C, with similarly high values (>87.5%) observed at 15, 20, and 25°C, but germination declined substantially at 30°C. The GR, GI, and VI were all minimized at 5°C, increased initially then decreased with rising temperature, peaking at either 25°C or 15°C. Across all temperature regimes, GP, GI, and VI exhibited initial increases followed by decreases with increasing soil water content. Under 5°C, these parameters reached their maxima at 30% soil water content, whereas at other temperatures, maxima occurred at 20% or 16% soil water content. The GR of seeds at 5°C increased gradually with soil water content, while at other temperatures it showed a pattern of initial increase followed by decrease, maximizing at 20% soil water content for 10, 15, and 20°C, and at 16% for 25 and 30°C. The inhibition of seed germination by elevated temperature and soil water content during summer and autumn may represent a protective strategy ensuring partial seed incorporation into the soil seed bank and reducing seedling mortality risk. Conversely, germination at relatively low temperatures and soil water contents in early spring may compensate for this inhibitory effect.

**Keywords:** *Caragana korshinskii*; temperature; soil water content; seed germination; seedling regeneration

## Introduction

Seed germination strategy constitutes a crucial aspect of plant life history in arid desert regions, significantly influencing population regeneration and vegetation restoration. Through long-term adaptation to desert habitats, plants have evolved specialized germination strategies to ensure appropriate spatiotemporal conditions for seed germination and seedling establishment, thereby avoiding unfavorable environments and dispersing germination risks. Temperature and water represent critical environmental factors affecting seed germination. Temperature can directly influence germination or indirectly affect it by altering seed dormancy status, consequently impacting seedling growth, population recruitment, and dynamics. Within certain ranges, optimal temperatures promote seed germination, while both excessively high and low temperatures are detrimental. Water availability is a primary constraint on seed germination and seedling establishment in desert regions, with water stress potentially reducing germination rates, delaying germination processes, and impairing seedling growth by disrupting cellular osmoregulation and protective enzyme systems. Different plant species exhibit substantial variation in their temperature and water requirements for germination. For instance, the optimal germination temperature for *Sinia rhodoleuca* is relatively high (25°C), whereas *Anthriscus sylvestris* prefers lower temperatures (15°C). Similarly, the suitable soil water content for germination varies considerably among desert species: *Ammopiptanthus mongolicus*, *Hippophae rhamnoides*, and *Tetraena mongolica* require relatively high water contents (15-20%), while *Pugionium cornutum* and *P. dolabratum* need less (6-12%). Rapid germination under low soil moisture can increase seedling establishment opportunities, though precipitation uncertainty and frequent water stress may cause substantial seedling mortality later. Consequently, seed germination strategies reflect long-term adaptation to regional climate and habitat conditions.

*Caragana korshinskii*, a xeromorphic shrub of the genus *Caragana*, is predominantly distributed in sandy grasslands, deserts, and semi-deserts, serving as a key species for ecological restoration in northwestern China. Previous laboratory studies have confirmed that *C. korshinskii* seeds achieve maximum germination at 20-25°C under water supply conditions, with germination rates remaining above 80% even at 5% soil water content. Simulation studies further revealed that 2.5 mm precipitation yields 59% emergence, though this declines to less than 10% at 10 mm precipitation. While PEG-6000 simulated drought stress has demonstrated the species' strong adaptability to moisture conditions, such solutions cannot accurately replicate the stress effects of low soil water content, particularly regarding interactive effects between temperature and soil water content. This study systematically examined the effects of different temperatures and soil water contents, as well as their interactions, on *C. korshinskii* seed germination under controlled conditions, providing essential insights for artificial assisted seedling regeneration and degraded vegetation restoration in arid desert regions of northwestern China.

## Materials and Methods

### 1.1 Seed Collection and Processing

Experimental seeds were collected in late July 2019 from mature *C. korshinskii* plants (>10 years old) in the Longkeng Forest Area of Lingwu Baijitan National Nature Reserve, Ningxia (37°53 N, 106°30 E). Fully developed yellow-brown pods were harvested and air-dried naturally in the laboratory. After manual removal of pods and insect-damaged seeds, intact seeds were stored in breathable cloth bags at room temperature for two months before germination experiments.

### 1.2 Experimental Design and Sowing Method

Based on previous research documenting temperature effects (5-30°C) and soil water content effects (1-30%) on seed germination, this study employed six constant temperature treatments (5, 10, 15, 20, 25, and 30°C) in intelligent artificial climate incubators. At each temperature, six soil water content levels were established (4%, 8%, 12%, 16%, 20%, and 30%). Surface sandy soil (1-5 cm depth) collected from the Longkeng Forest Area was used as the germination substrate. For each treatment, 50 uniformly sized seeds were sown in 90 mm diameter Petri dishes containing the prepared soil. Seeds were placed on the moistened soil surface, and dishes were weighed and recorded before placement in incubators with a 14 h light/10 h dark photoperiod (light intensity ~11,500 LUX). To prevent soil water content fluctuations from seed absorption and evaporation, all dishes were weighed every 2 days using a 1/100 electronic balance, and evaporative water loss was replenished accordingly. Each treatment was replicated four times.

### 1.3 Data Recording and Germination Parameter Calculation

Germination was recorded daily with radicle protrusion ( $\geq 1$  mm) as the criterion. Germinated seed numbers were counted every 24 h, and radicle lengths of germinated seeds were measured before removal. Observations continued until no further germination occurred for seven consecutive days. The following parameters were calculated:

- **Germination Percentage (GP):**  $GP = (n/N) \times 100\%$ , where  $n$  is the number of germinated seeds and  $N$  is the total number of seeds.
- **Germination Rate (GR):**  $GR = \Sigma(G_i \times 100 / (n \times t_i))$ , where  $G_i$  is the number of seeds germinated on day  $t_i$ ; higher GR indicates faster germination.
- **Germination Index (GI):**  $GI = \Sigma(G_t / D_t)$ , where  $G_t$  is the number of seeds germinated on day  $t$  and  $D_t$  is the corresponding germination day.
- **Vigor Index (VI):**  $VI = GI \times \text{seedling length (cm)}$ . Only radicle length was measured in this study.

## 1.4 Data Analysis

Two-way ANOVA was used to analyze the effects of temperature, soil water content, and their interactions on germination parameters. One-way ANOVA with least significant difference (LSD) tests were employed to compare differences among temperatures and soil water contents within each temperature. To meet homogeneity of variance requirements, all data were square-root transformed before statistical analysis. All analyses were performed using SPSS 25.0 software.

## Results

### 2.1 Temperature Effects on Seed Germination

As shown in , temperature, soil water content, and their interaction all significantly affected GP, GR, GI, and VI ( $P < 0.01$ ). Regarding temperature effects, GP was lowest at 5°C, increased with temperature up to 10°C, then gradually declined, with all treatments above 10°C showing significantly higher GP than 5°C. GR and GI were also minimized at 5°C, maximized at 25°C, and significantly greater than all lower temperature treatments. VI was significantly smaller at 5°C than other temperatures, increasing initially then decreasing with temperature, peaking at 25°C and significantly exceeding all other treatments except 15°C.

### 2.2 Germination Percentage Under Different Temperature and Soil Water Content Treatments

Soil water content significantly affected GP ( $P < 0.01$ ), with a significant interactive effect with temperature ( $P < 0.01$ ). At 5°C, GP increased gradually with soil water content, with all treatments \$ \$12% showing significantly higher GP than 4% and 8% treatments. At 10°C, GP was significantly lower at 4% soil water content compared to other treatments, increasing as water content rose from 8% to 16%, then declining when water content increased to 30%. The maximum GP (87.5%) occurred at 20% soil water content. The trend at 15°C was similar to 10°C, with maxima at 20% soil water content and all treatments \$ \$12% significantly exceeding 4% and 8% treatments. At 20°C, GP showed a comparable pattern, peaking at 20% soil water content, with the minimum at 4% significantly lower than all other treatments. At 25°C, GP followed a similar trend, maximizing at 20% soil water content, with the 4% treatment minimum significantly lower than all others. At 30°C, GP also peaked at 20% soil water content.

### 2.3 Germination Rate Under Different Temperature and Soil Water Content Treatments

Soil water content and its interaction with temperature significantly affected GR ( $P < 0.01$ ). At 5°C, GR increased gradually with soil water content, with

all treatments \$ \$12% significantly exceeding 4% and 8% treatments. At 10°C, GR showed a significant increasing trend with soil water content, with all treatments \$ \$12% significantly greater than 4% and 8% treatments. At 15°C, GR increased initially then decreased, peaking at 20% soil water content, which was significantly higher than all treatments except 16%. At 20°C, GR followed a similar pattern, maximizing at 20% soil water content, significantly exceeding all other treatments. At 25°C, GR also peaked at 20% soil water content, significantly greater than all other treatments. At 30°C, GR maximized at 16% soil water content, significantly higher than all other treatments.

#### 2.4 Germination Index Under Different Temperature and Soil Water Content Treatments

Soil water content and its interaction with temperature significantly affected GI ( $P < 0.01$ ). At 5°C, GI increased gradually with soil water content, with all treatments \$ \$12% significantly exceeding 4% and 8% treatments. At 10°C, GI showed significant differences among soil water content treatments, with maxima at 20% and 16% significantly greater than all other treatments. At 15°C, GI increased initially then decreased, peaking at 20% soil water content, significantly higher than all treatments except 16%. At 20°C, GI followed a similar pattern, maximizing at 20% soil water content, significantly exceeding all other treatments. At 25°C, GI also peaked at 20% soil water content, significantly greater than all other treatments. At 30°C, GI maximized at 16% soil water content, significantly higher than all other treatments.

#### 2.5 Vigor Index Under Different Temperature and Soil Water Content Treatments

Soil water content significantly affected VI ( $P < 0.05$ ), with a significant interactive effect with temperature ( $P < 0.01$ ). At 5°C, VI increased with soil water content, with all treatments \$ \$12% significantly exceeding 4% and 8% treatments. At 10°C, VI increased initially then decreased, peaking at 20% soil water content, significantly higher than all other treatments. At 15°C, VI showed a similar trend, maximizing at 20% soil water content, significantly exceeding all other treatments. At 20°C, VI also peaked at 20% soil water content, significantly greater than all other treatments. At 25°C, VI maximized at 16% soil water content, significantly higher than all other treatments. At 30°C, VI peaked at 16% soil water content, significantly exceeding all other treatments.

Two-way ANOVA of effects of temperature and soil water content (SWC) on the germination of *Caragana korshinskii* seeds

[Figure 1: see original paper] Germination percentage of *Caragana korshinskii* seeds under different temperatures and soil water contents

[Figure 2: see original paper] Germination rate of *Caragana korshinskii* seeds under different temperatures and soil water contents

[Figure 3: see original paper] Germination index of *Caragana korshinskii* seeds under different temperatures and soil water contents

[Figure 4: see original paper] Vigor index of *Caragana korshinskii* seeds under different temperatures and soil water contents

## Discussion

Seed germination strategies play a crucial role in seedling regeneration, population distribution patterns, and quantitative dynamics, significantly influencing community succession and degraded vegetation restoration. In desert regions, seed germination faces greater uncertainty due to variable environmental factors. Temperature is a primary environmental factor affecting seed germination, with most species showing optimal germination between 20-25°C. While lower temperatures can inhibit germination by reducing rates and delaying processes, germination at lower temperatures may allow seedlings to develop before drought seasons, ensuring adequate root development and enhanced drought tolerance.

Our findings indicate that temperature significantly affects *C. korshinskii* seed germination, with maximum GP (87.5%) at 10°C and high GP (>87.5%) also observed at 15, 20, and 25°C. However, GP declined substantially at 30°C. Although GR, GI, and VI were maximized at 25°C or 15°C, GP and VI decreased significantly at these temperatures. Comprehensive analysis of these parameters suggests the suitable temperature range for *C. korshinskii* germination is 10-25°C, slightly broader than previously reported (20-25°C). This broader range may reflect the use of soil substrate, which better approximates early spring conditions in arid desert regions with large temperature fluctuations, potentially offsetting negative impacts of water stress. Seed germination behavior represents long-term adaptation to natural habitats; seeds that have imbibed water but fail to germinate under high summer temperatures may enter dormancy, as radicle and hypocotyl growth are more sensitive to water deficit than germination initiation.

Desert plants generally tolerate soil water deficits, requiring relatively low water content for germination. Previous studies show *C. korshinskii* can germinate and emerge under low water supply, while excessive water may cause hypoxia and reduce germination. Our results confirm that moderate drought may promote germination of some species. Soil water content significantly affected all germination parameters, which initially increased then decreased with rising water content, declining sharply when exceeding 20%. At 10-25°C, GP exceeded 80% at 16-20% soil water content, with maximum GP (87.5%) and other parameters at 20% water content. The suitable soil water content range (16-20%) aligns closely with other desert xeromorphic shrubs such as *Ammopiptanthus mongolicus*, *Hippophae rhamnoides* (15-20%), and *Tetraena mongolica* (20%), but is higher than for *Pugionium cornutum* and *P. dolabratum* (6-12%). In desert environments, rapid germination after minimal precipitation can effectively utilize fleeting opportunities while allowing adequate time for seedling development.

However, some seeds remain ungerminated even when water conditions are favorable, entering the soil seed bank to adapt to unstable environmental changes and avoid mass seedling mortality from subsequent drought.

The response patterns of *C. korshinskii* seed germination to different soil water contents may reflect recruitment limitations in natural habitats. Inhibition at high water content helps maintain continuous germination and emergence, thereby avoiding germination risks. The interactive effects of temperature and soil water content were highly significant. At low temperatures (5°C), all parameters except GR increased gradually with water content, while at moderate temperatures (10-20°C), maxima occurred at 20% water content. At high temperatures (25-30°C), maxima shifted to 16% water content. This demonstrates that adequate soil moisture can mitigate negative temperature effects, while appropriate temperatures can alleviate drought stress, and that mild drought under suitable temperatures may promote germination. These results parallel findings for *Metasequoia glyptostroboides*, where moderate drought promoted germination at lower temperatures.

The inhibition of germination by relatively high temperature and soil water content may serve as a protective strategy for summer-matured seeds to enter the soil seed bank and escape mortality, while spring germination at lower temperatures and water contents compensates for this inhibition. *C. korshinskii* seeds can germinate at relatively low temperatures and water contents, but are inhibited by higher levels—a strategy facilitating seed bank incorporation and reducing seedling death risk. Seeds protected by the soil seed bank can germinate rapidly in early spring when temperatures and water availability are lower, allowing sufficient time for root system development. This compensatory mechanism requires further investigation combining field monitoring and controlled experiments.

## Conclusion

*Caragana korshinskii* seed germination responds significantly to temperature, soil water content, and their interactions. The suitable temperature range is 10-25°C, and the optimal soil water content is 16-20%. The inhibition of germination by high temperature and soil water content during summer and autumn may represent a protective strategy ensuring seed incorporation into the soil seed bank and reducing seedling mortality risk. Germination at lower temperatures and soil water contents in early spring may compensate for this inhibitory effect, representing an adaptive strategy for population persistence in arid desert environments.

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