# Effects of Different Concentrations of Sodium Selenite Solution on Seed Germination of Metasequoia glyptostroboides (Postprint)

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## Abstract

Metasequoia glyptostroboides is recognized as a 'living fossil' in the plant kingdom, with natural native communities distributed only in localized areas of Lichuan in Enshi Prefecture, Shizhu in Chongqing, and Longshan in Hunan. Selenium is a trace element required for plant growth. In Enshi, the primary habitat of Metasequoia mother trees, a three-dimensional selenium resource environment has formed, yet natural regeneration of Metasequoia communities in this region is difficult, with regeneration seedlings or saplings rarely observed in the understory. Therefore, in light of selenium resources, investigating the relationship between selenium and Metasequoia seed germination is of great significance for the natural regeneration and propagation of Metasequoia. This study determined the germination rates of sown seeds of native Metasequoia glyptostroboides under different environmental conditions (temperature: 20°C, 25°C, 30°C; light: 12 h light/12 h dark, 24 h total darkness; with or without seed soaking) to screen for optimal germination conditions. Under these conditions, seeds were treated with different concentrations of sodium selenite (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 $mg \cdot L^{-1}$), and changes in germination were observed to reveal the influence of selenium on Metasequoia seed germination. The results showed that when Metasequoia seeds were treated with sodium selenite solution at a selenium concentration of 0.25 $mg \cdot L^{-1}$, the germination rate, germination potential, and germination index all reached their maximum values at 34.0%, 29.0%, and 13.9, respectively. When selenium concentration exceeded 0.25 $mg \cdot L^{-1}$, the germination rate, germination potential, and germination index of Metasequoia seeds began to decrease with increasing concentration, reaching their minimum values at a selenium concentration of 16.0 $mg \cdot L^{-1}$ at 0.5%, 0%, and 0.025, respectively. Thus, low-concentration selenium treatment (0–0.25 $mg \cdot L^{-1}$) exerted a certain promotional effect on Metasequoia seed germination, whereas high-concentration selenium treatment (>0.25 $mg \cdot L^{-1}$) demonstrated a certain inhibitory effect. The research findings may provide theoretical support for field seedling cultivation of Metasequoia seeds and studies on natural regeneration mechanisms.

## Full Text

# Effects of Different Sodium Selenite Concentrations on Seed Germination of *Metasequoia glyptostroboides*

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### Abstract

*Metasequoia glyptostroboides* is revered as a "living fossil" in the plant kingdom. Native communities of this species are restricted to limited areas in Lichuan (Enshi, Hubei), Shizhu (Chongqing), and Longshan (Hunan) counties. Selenium is an essential trace element for plant growth. Enshi, the primary habitat of *M. glyptostroboides* mother trees, features a unique three-dimensional selenium resource environment. However, natural regeneration of *M. glyptostroboides* communities in this region is extremely difficult, with few seedlings or saplings observed in the understory. Therefore, investigating the relationship between selenium and *M. glyptostroboides* seed germination holds significant importance for understanding the natural regeneration mechanisms of this species.

This study first determined the germination rates of native *M. glyptostroboides* seeds under various environmental conditions (temperature: 20°C, 25°C, 30°C; light: 12 h light/12 h dark, 24 h continuous darkness; with or without seed soaking) to identify optimal germination conditions. Subsequently, under these selected conditions, seeds were treated with different concentrations of sodium selenite (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 $mg \cdot L^{-1}$) to examine selenium' s effects on germination.

The results demonstrated that seed germination rate, germination potential, and germination index all peaked at a selenium concentration of 0.25 $mg \cdot L^{-1}$, reaching 34.0%, 29.0%, and 13.9, respectively. When selenium concentrations exceeded 0.25 $mg \cdot L^{-1}$, these three parameters decreased progressively with increasing concentration, reaching their minimum values of 0.5%, 0%, and 0.025 at 16.0 $mg \cdot L^{-1}$. These findings indicate that low selenium concentrations (0–0.25 $mg \cdot L^{-1}$) promote *M. glyptostroboides* seed germination, whereas high concentrations (>0.25 $mg \cdot L^{-1}$) exert inhibitory effects. This research provides theoretical support for field nursery practices and studies on natural regeneration mechanisms of *M. glyptostroboides*.

**Keywords:** *Metasequoia glyptostroboides* seed; selenium concentration; germination

## Introduction

Natural *Metasequoia glyptostroboides*, a monotypic genus in the Taxodiaceae family, is a nationally protected first-class plant in China (Zong et al., 2012). Due to its rapid growth, tall and straight form, and lightweight timber, it is widely used as an ornamental and timber species. Additionally, *M. glyptostroboides* contains various flavonoid compounds that protect myocardial cells, and its seeds contain multiple active components (Yang et al., 2010), offering broad prospects for pharmaceutical, chemical, and agricultural applications (Tian et al., 2006). As an ideal representative species for studying the genetic structure of relict plants, its ecological, economic, and socio-cultural values are recognized worldwide. However, natural regeneration is extremely difficult (You, 2008), characterized by low germination rates and absence of seedling/sapling recruitment beneath mother trees, resulting in declining natural populations.

Selenium plays a vital role in plant physiological processes and can promote seed germination. Sodium selenate solutions have been shown to enhance germination in buckwheat (*Fagopyrum esculentum*), soybean (*Glycine max*), peanut (*Arachis hypogaea*), and kidney bean (*Phaseolus vulgaris*), though this promotion is closely concentration-dependent (Yu, 2012). Enshi's soil selenium content reaches a maximum of 178.8 $g \cdot g^{-1}$, with an average of 19.11 $g \cdot g^{-1}$—significantly higher than many regions in China and worldwide—representing abundant selenium geological resources (Tang, 2004). The world' s only existing native *M. glyptostroboides* populations inhabit the mountainous areas centered around Xiaohe in this region, yet whether this distribution correlates with selenium resource distribution remains unreported.

Seeds are the reproductive units of seed plants and the material foundation for vegetation restoration and reconstruction. Current research on *M. glyptostroboides* seeds primarily focuses on storage conditions (Fu, 1981), germination conditions (Liao and Zhou, 1989), ecophysiological characteristics of germination (Xin et al., 2004), and litter effects on germination (You and Ma, 2008). However, no studies have reported on selenium' s effects on *M. glyptostroboides* seed germination. Therefore, investigating the influence of selenium—given its prominence in the native habitat—on *M. glyptostroboides* seed germination holds practical significance for the species' natural regeneration and breeding.

## 1. Materials and Methods

**1.1 Experimental Materials** **Plant material:** Seeds were collected in October 2017 from native *M. glyptostroboides* mother tree No. 4541 in Hexin Village, Zhonglu Town, Lichuan City, Enshi Prefecture (elevation: 1,153 m; longitude: 108°38' 53" ; latitude: 30°07' 20" ; age: 85 years; height: 30 m; DBH: 105.6 cm). The 1,000-seed weight was 2.47 g. After collection, seeds were sealed in plastic bags and stored at 4°C (Jing et al., 2011) until use.

**Reagents:** 75% ethanol, sodium selenite reagent, distilled water.

**Equipment:** Water bath, electronic balance, beakers, petri dishes, burettes, filter paper, gauze, glass rods, etc.

**1.2 Experimental Methods** We employed three primary factors affecting seed germination—temperature, light, and soaking—to identify optimal conditions for *M. glyptostroboides* seed germination.

**(1) Seed soaking:** We manually selected 2,400 uniform, relatively plump seeds and surface-sterilized them in 75% ethanol for 1 minute, followed by five rinses with distilled water and blotting with filter paper. Half of the seeds (1,200) were soaked in 45°C distilled water for 48 hours (Li et al., 2006) to ensure full water absorption, then filtered. The remaining 1,200 seeds were not soaked.

**(2) Light conditions:** We simulated natural conditions with 12 h light/12 h dark cycles at constant temperature, and 24 h continuous darkness. For continuous darkness treatments, petri dishes containing seeds were wrapped in aluminum foil and multiple black plastic bags, then placed in the same incubator under 12 h light/12 h dark conditions. Seed observations were conducted under green light.

**(3) Temperature:** Treated seeds (with various soaking and light combinations) were placed in incubators at 20°C, 25°C, or 30°C for constant-temperature germination (these temperature gradients were based on unpublished preliminary experiments). Twelve treatment combinations were established, with 50 seeds per treatment and four replicates. To maintain moisture, seeds were observed every 24 hours for germination detection and counting, with distilled water added as needed to keep filter paper moist.

**(4) Exogenous selenium concentrations:** We manually selected 1,600 uniform, relatively plump seeds. Sodium selenite solutions were prepared at concentrations of 0 (control), 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 $mg \cdot L^{-1}$. Seeds were soaked in these solutions for 12 hours to ensure full water absorption, then filtered. Fifty seeds were selected per treatment with four replicates. Seeds were placed in 9 cm petri dishes with filter paper, moistened with appropriate distilled water, and observed every 24 hours for germination counting, with water added as needed to maintain moisture.

**1.3 Measurement Indicators and Calculations** Beginning when seeds were placed in incubators, germination was observed and recorded every 24 hours. Germination was defined as visible emergence of white radicles. Observations for each group were completed within a 10-minute window over four weeks. Based on recorded data, germination rate, germination potential, and germination index were calculated for each treatment.

(1) **Germination rate** G = (Number of germinated seeds Ga / Total number of tested seeds Gn) × 100%

(2) **Germination potential** = (Number of germinated seeds on day 7 G7 / Total number of tested seeds Gn) × 100%

(3) **Germination index** GI = Σ(Gt / Dt), where Gt is the number of seeds germinated on day t, and Dt is the day of germination.

**1.4 Data Processing** Data were recorded and transformed using Excel software. Statistical analysis was performed using SPSS 18.0, and graphs were generated using GraphPad Prism 5. One-way ANOVA and Duncan' s multiple comparison tests were applied to analyze the effects of different sodium selenite concentrations on germination indicators, with significance level set at $\alpha = 0.05$.

## 2. Results

**2.1 Effects of Initial Conditions on *M. glyptostroboides* Seed Germination** The effects of temperature, light, and soaking on seed germination are presented in [Figure 1: see original paper].

[**Figure 1: see original paper**] The average germination percentage under temperature + light + (no)soaking treatments

As shown in [Figure 1: see original paper], germination rates varied under different combinations of temperature, light, and soaking treatments. At 20°C, the highest average germination rate (46.5%) occurred with soaked seeds under continuous darkness, which was 16% and 17% higher than soaked seeds under 12 h light/dark and unsoaked seeds under 12 h light/dark, respectively. The lowest germination rate (19.5%) occurred with unsoaked seeds under continuous darkness. At 25°C, the minimum germination rate was 17.5% for unsoaked seeds under continuous darkness, while the maximum (44.0%) occurred with soaked seeds under 12 h light/dark. No significant difference was observed between soaked seeds under continuous darkness and unsoaked seeds under 12 h light/dark. At 30°C, soaked seeds under continuous darkness achieved the highest germination rate (42.5%), which was 1.5% higher than soaked seeds under 12 h light/dark and 21% higher than both unsoaked treatments. These results indicate that the highest germination rate occurs at 20°C with soaked seeds under continuous darkness.

ANOVA revealed that temperature ($P = 0.821$) and light ($P = 0.583$) had no significant effects on germination rate, while soaking ($P = 0.000$) had a highly significant effect. The interactions of temperature × light ($P = 0.020$) and light × soaking ($P = 0.013$) were significant, whereas temperature × soaking ($P = 0.685$) was not. The combined effects of all three factors were significant ($P = 0.015$). Based on these findings, we selected the conditions of 20°C, seed soaking, and continuous darkness as the baseline for subsequent selenium concentration treatments.

**2.2.1 Effects of Different Exogenous Selenium Concentrations on Germination Rate** [**Figure 2: see original paper**] The average germination percentage under different concentrations of exogenous sodium selenite

Germination rate critically influences seedling development. As shown in [Figure 2: see original paper], selenium concentration significantly affected *M. glyptostroboides* seed germination (P = 0.000). The highest average germination rate (34.0%) occurred at 0.25 mg · $L^{-1}$ selenium, which was 8.5% higher than the control (0 mg · $L^{-1}$). Beyond 0.5 mg · $L^{-1}$, germination rates declined progressively with increasing concentration, dropping sharply to 1.5% at 4.0 mg · $L^{-1}$ and further to 1.0% and 0.5% at 8.0 mg · $L^{-1}$ and 16.0 mg · $L^{-1}$, respectively. These results demonstrate that selenium concentrations of 0–0.25 mg · $L^{-1}$ promote germination, with the most pronounced effect at 0.25 mg · $L^{-1}$, while concentrations exceeding 0.25 mg · $L^{-1}$ exhibit inhibitory effects that intensify with concentration, becoming most severe at 16.0 mg · $L^{-1}$.

Germination rates at 0, 0.5, 1.0, and 2.0 mg · $L^{-1}$ differed significantly from those at 4.0, 8.0, and 16.0 mg · $L^{-1}$. No significant differences were observed among 0, 0.5, 1.0, and 2.0 mg · $L^{-1}$ treatments. The 0.25 mg · $L^{-1}$ treatment differed significantly from all other concentrations and yielded the highest germination rate.

**2.2.2 Effects of Different Exogenous Selenium Concentrations on Germination Potential [Figure 3: see original paper]** The average germination energy under different concentrations of exogenous sodium selenite

Selenium concentration had a highly significant effect on germination potential (P = 0.000) ([Figure 3: see original paper]). The 0.25 mg · $L^{-1}$ treatment differed significantly from all other concentrations. The highest average germination potential (29.0%) occurred at 0.25 mg · $L^{-1}$, which was 7.5%, 10.0%, 12.5%, and 18.5% higher than the control (0 mg · $L^{-1}$), 0.5 mg · $L^{-1}$, 1.0 mg · $L^{-1}$, and 2.0 mg · $L^{-1}$ treatments, respectively. Germination potential dropped to 0.5% at 4.0 mg · $L^{-1}$ and to 0% at both 8.0 mg · $L^{-1}$ and 16.0 mg · $L^{-1}$. These findings indicate that selenium concentrations of 0–0.25 mg · $L^{-1}$ promote germination potential, with optimal promotion at 0.25 mg · $L^{-1}$, while concentrations exceeding 0.25 mg · $L^{-1}$ exhibit increasingly severe inhibitory effects, most pronounced at 16.0 mg · $L^{-1}$.

**2.2.3 Effects of Different Exogenous Selenium Concentrations on Germination Index [Figure 4: see original paper]** The average germination index under different concentrations of exogenous sodium selenite

Germination index is a crucial indicator of seed vigor. Our results show that appropriate sodium selenite concentrations promote the germination index of *M. glyptostroboides* seeds. As shown in [Figure 4: see original paper], the average germination index exhibited a unimodal pattern across selenium concentrations, reaching its maximum (13.9) at 0.25 mg · $L^{-1}$, followed by the control (9.1). The index declined progressively with increasing concentration, dropping sharply to 0.35 at 4.0 mg · $L^{-1}$ and below 0.1 at both 8.0 mg · $L^{-1}$ and 16.0 mg · $L^{-1}$. These results demonstrate that selenium concentrations of 0–0.25 mg $\cdot$ L$^{-1}$ promote the germination index, with optimal promotion at 0.25 mg $\cdot$ L$^{-1}$, while concentrations exceeding 0.25 mg $\cdot$ L$^{-1}$ exhibit increasingly severe inhibitory effects, most pronounced at 16.0 mg $\cdot$ L$^{-1}$.

## Discussion

Germination rate plays a vital role in forest regeneration (Yilmaz, 2008). *Metasequoia glyptostroboides* faces extreme difficulties in natural regeneration, primarily due to two factors: thick understory litter prevents the lightweight seeds from contacting soil directly, and poor seed quality results in extremely low germination rates. Field germination rates are only 18% (You et al., 2008). In contrast, our study achieved an average germination rate of 35% across different environmental conditions, with a maximum of 47%.

The highest germination rate occurred at 20°C with soaked seeds under continuous darkness, while the lowest occurred at 25°C with unsoaked seeds under continuous darkness. Among the three factors examined, seed soaking had the greatest influence, consistent with findings by Liao and Zhou (1989). Temperature variations within a certain range had minimal effects, and no significant differences were observed between light conditions, though continuous darkness yielded higher rates, suggesting that *M. glyptostroboides* seeds are light-sensitive (Xin et al., 2004; Jing et al., 2011).

Selenium participates in plant biological antioxidation and metabolism (Han et al., 2010) and modifies enzyme activities. Studies have reported that selenium significantly promotes germination in soybean (He et al., 2011; He et al., 2012) and wheat (*Triticum aestivum*) (Miao et al., 2013). Our results show that exogenous selenium concentrations had highly significant effects on *M. glyptostroboides* seed germination rate, potential, and index. All three parameters peaked at 0.25 mg $\cdot$ L$^{-1}$ and reached minima at 16.0 mg $\cdot$ L$^{-1}$, exhibiting a clear trend of initial increase followed by decrease with rising concentration. Thus, low selenium concentrations (0–0.25 mg $\cdot$ L$^{-1}$) promote germination, with optimal effects at 0.25 mg $\cdot$ L$^{-1}$, while high concentrations (>0.25 mg $\cdot$ L$^{-1}$) inhibit germination, with inhibition intensifying at concentrations exceeding 4.0 mg $\cdot$ L$^{-1}$.

Li et al. (2006) reported that selenium concentrations around 0.05 mg $\cdot$ L$^{-1}$ enhanced soybean germination rates, potential, and index. As a trace element, selenium at appropriate concentrations can increase osmotic adjustment substance content and improve corn seed germination capacity (Xu et al., 2017), but exhibits clear inhibition at excessive concentrations (>0.05 mg $\cdot$ L$^{-1}$). These findings align with our study and other selenium research (Mao and Wang, 2011; He et al., 2012), demonstrating that low-concentration selenium soaking promotes seed germination and seedling growth, while exceeding a critical concentration inhibits these processes. This may occur because selenium ion stress affects amylase activity critical for germination potential, leading to reduced germination and slow growth (Lin et al., 2004). These effects vary among species and soaking durations, with optimal selenium concentrations differing due to interspecific genetic properties (Zhou et al., 2007; Miao et al., 2013) and enzyme activity expression during germination (Zhang et al., 2003; Han et al., 2010).

The selenium concentration gradients in our study were based on previous research (Zhao, 2011; Yang et al., 2012; Li et al., 2012), which presents certain limitations. In reality, selenium often co-occurs with cadmium, and different selenium valence states may have varying effects on seed germination and seedling growth. Therefore, further research is needed to investigate the effects of different selenium valence states and concentrations on *M. glyptostroboides* seed germination and development.

## Conclusion

By determining germination rates under different environmental conditions (temperature, light, and soaking), we identified that *M. glyptostroboides* seeds achieve highest germination at 20°C with soaking under continuous darkness. Selenium exerts highly significant effects on germination rate, potential, and index. Low selenium concentrations (0–0.25 $mg \cdot L^{-1}$) promote germination, with optimal promotion at 0.25 $mg \cdot L^{-1}$ where all three parameters reach maxima. High selenium concentrations (>0.25 $mg \cdot L^{-1}$) inhibit germination, with inhibition intensifying at higher concentrations and becoming most severe at 16.0 $mg \cdot L^{-1}$. Seed vigor and uniformity directly affect seedling establishment. Our findings demonstrate that appropriate exogenous selenium concentrations can promote *M. glyptostroboides* seed germination while ensuring vigor and uniformity. Therefore, in future production practices, pre-soaking with appropriate exogenous selenium during suitable seasons could artificially promote natural regeneration of *M. glyptostroboides*.

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*Note: Figure translations are in progress. See original paper for figures.*