



## The Effect of Silicon Seed Priming on Germination and Biochemical Indices of Maize (SC 704) Across Different pH Ranges

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### ABSTRACT

**Objective:** This study aimed to investigate the effects of seed priming with silicon on germination and biochemical responses of maize (*Zea mays* L. SC 704) under different pH levels of the growth medium.

**Method:** The experiment was conducted as a factorial arrangement within a completely randomized design (CRD) with three replications. Treatments consisted of four priming levels: control (distilled water) and silicon (as sodium silicate) at concentrations of 50, 100, and 150 mg L<sup>-1</sup>, and three pH levels of the growth medium (5, 6.5, and 8). The measured traits included germination indices (percentage and rate), seedling growth (fresh weight, vigor weight, and length), ion leakage (relative electrolyte leakage, REL), and biochemical indices (activities of  $\alpha$ -amylase and protease, soluble sugar content, total protein, and silicon uptake)

**Results:** Deviation from the optimal pH (6.5) toward acidic (pH 5) or alkaline (pH 8) conditions significantly reduced germination and growth indices while increasing ion leakage. Seed priming with silicon, particularly at 150 mg L<sup>-1</sup>, markedly mitigated the adverse effects of pH changes. Under acidic conditions (pH 5), this treatment increased germination percentage by 63.19% compared to the non-silicon control at the same pH, reduced relative electrolyte leakage (REL), and significantly enhanced  $\alpha$ -amylase and protease activities, soluble sugar content, total protein, and silicon uptake. The interaction between silicon and pH was significant only for germination rate, highlighting the specific role of silicon in accelerating germination under unfavorable pH conditions.

**Conclusions:** Seed priming with silicon at 150 mg L<sup>-1</sup> effectively enhances germination capability and reinforces the physiological and biochemical status of maize seeds under non-optimal pH conditions, particularly acidic stress. This approach holds significant promise for improving field establishment in soils with suboptimal pH.

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## Highlights

- Silicon priming improved germination, rate and vigor at all pH levels, especially pH 5.
- Silicon (150 mg L<sup>-1</sup>) reduced electrolyte leakage and membrane damage under pH stress.
- Silicon enhanced  $\alpha$ -amylase, protease, soluble sugars and total protein in seedlings.
- Silicon benefits were pH-independent, ensuring robust stress alleviation during germination.

## 1. Introduction

Maize (*Zea mays* L.) is one of the world's most crucial cereal crops, playing a fundamental role in the food and economic security of numerous nations. Successful germination and early establishment are critical prerequisites for realizing the crop's final yield potential (Hernandez-Apaolaza, 2022). However, this sensitive stage is often compromised by various environmental stresses, among which soil pH variation is one of the most widespread and challenging.

Deviation of the growth medium pH from the optimal range (neutral to slightly acidic), particularly toward strongly acidic conditions, exerts multiple detrimental effects on plants (Barrow & Hartemink, 2023). These effects include: (i) toxicity of aluminum and manganese ions; (ii) impaired uptake, translocation, and utilization of essential nutrients (e.g., phosphorus, calcium, magnesium); (iii) disruption of cellular osmotic and ionic balance; and (iv) induction of secondary oxidative stress due to overproduction of reactive oxygen species (ROS). Collectively, these disturbances ultimately lead to reduced germination percentage and rate, weakened seedling growth, and diminished seed vigor (Shi et al., 2014; Gharbi et al., 2025).

In this context, developing cost-effective and environmentally friendly strategies to enhance stress tolerance during early growth stages is a research priority. Seed priming is a simple yet

effective technique that increases a seed's ability to withstand unfavorable conditions by inducing controlled physiological and biochemical pre-germinative changes (Younas et al., 2022). Among various priming agents, the use of silicon (Si) has recently garnered significant attention.

Although not considered an essential element, silicon is recognized as a "beneficial element" due to its numerous advantageous roles. Evidence indicates that silicon application (particularly as seed priming) can enhance plant resistance to a wide array of biotic and abiotic stresses. Its mechanisms of action include deposition in cell walls to increase mechanical tissue strength, reduction of electrolyte leakage and maintenance of membrane integrity, regulation of ionic and osmotic balance, and, most importantly, strengthening of the plant's antioxidant defense system. By boosting the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as elevating levels of non-enzymatic compounds, silicon aids in neutralizing ROS and prevents oxidative damage to vital macromolecules like lipids, proteins, and DNA (Hernandez-Apaolaza, 2022; Jiang et al., 2022; Ajay et al., 2025).

Specifically under acidic stress, silicon plays a dual protective role. It can chelate toxic aluminum ions (Al<sup>3+</sup>) in the soil or rhizosphere, preventing their uptake and translocation into the plant (Kopittke et al., 2017). Furthermore, reports suggest that

silicon priming increases the activity of germination-related enzymes such as  $\alpha$ -amylase and protease, elevates the content of compatible metabolites like soluble sugars and proline, and enhances energy reserves in seeds—all of which contribute to more rapid and uniform germination (Nafarrate-ramos et al., 2022; Weisany et al., 2023).

Despite growing evidence of silicon's benefits, most existing studies have focused on its effects under drought or salinity stress. Systematic research investigating the efficacy of silicon seed priming in maize across a broad spectrum of growth medium pH (including acidic, neutral, and alkaline conditions) remains scarce. Moreover, a comprehensive understanding of the simultaneous effects of this treatment on an integrated set of physiological indices (e.g., germination percentage and rate, seed vigor, ion leakage) and key biochemical indices ( $\alpha$ -amylase and protease activities, soluble sugar content, total protein, and silicon uptake) in maize is lacking.

Therefore, the primary objective of this research was to investigate the effect of priming maize seeds with different silicon levels on germination and related biochemical indices under three pH levels of the growth medium (5, 6.5, and 8). This study sought to answer the following questions: (1) Can silicon priming alleviate the adverse effects of pH deviation from the optimum on maize germination? (2) Which silicon level is most effective in improving the studied indices? and (3) How are the measured changes in biochemical indices correlated with the observed improvement in germination traits? The findings of this research could provide a scientific basis for proposing a practical and low-cost strategy to improve maize field establishment in soils with non-optimal pH.

## 2. Materials and Methods

### 2.1. Site, plant material, and experimental design

This study was conducted in 2024 at the seed physiology laboratory of the university of Mohaghegh Ardabili, Iran. The plant material consisted of maize (*Zea mays* L.) seeds, hybrid single cross 704, from the 2023 production batch of Moghan Agro-Industry Company. Seeds were selected for uniformity in size and appearance and were free from disease. A preliminary germination test on unprimed seeds using the rolled paper towel method with distilled water at 25°C for 7 days indicated a germination percentage of 89.6% based on radicle emergence ( $\geq 2$  mm). The experiment was arranged as a factorial in a completely randomized design (CRD) with three replications. The first factor comprised four seed priming levels, and the second factor comprised three pH levels of the growth medium, resulting in a total of 12 experimental treatments. In each replication (experimental unit), 25 seeds were placed on filter paper in a Petri dish.

#### • Priming level (Si):

1. Control (Hydro-primed): distilled water.
2. Primed with Silicon 50 mg L<sup>-1</sup> (Si50).
3. Primed with Silicon 100 mg L<sup>-1</sup> (Si100).
4. Primed with Silicon 150 mg L<sup>-1</sup> (Si150).

#### • Growth medium pH level:

1. pH = 5 (Acidic condition).
2. pH = 6.5 (Neutral/optimal condition, as the pH control).
3. pH = 8 (Alkaline condition).

## 2.2. Experimental procedures

### 2.2.1. Seed sterilization and silicon priming

Seeds were surface-sterilized in 1% sodium hypochlorite for 5 minutes and rinsed thoroughly with sterile deionized water. Silicon priming solutions (50, 100, and 150 mg L<sup>-1</sup>) were prepared using sodium silicate (Na<sub>2</sub>SiO<sub>3</sub>, analytical reagent grade, Sigma-Aldrich, CAS: 338443, St. Louis, MO, USA) in deionized water. Seeds were soaked in the assigned solution (seed-to-solution ratio 1:5, w/v) for 12 hours at 20 ± 2°C. Hydro-primed control seeds were soaked under identical conditions in deionized water. After priming, seeds were blotted on filter paper and dried at room temperature until they returned to their original moisture content (approximately 10–12%). Moisture content was determined according to the ISTA standard oven-drying method (103°C for 17 h) using three subsamples of 10 seeds per treatment.

### 2.2.2. pH adjustment and preparation of the germination medium

To examine the isolated effect of pH on seed germination, the germination medium was prepared using deionized water adjusted with 0.1 M HCl or 0.1 M NaOH to achieve pH levels of 5, 6.5, and 8. Non-buffered, pH-adjusted water was used to avoid confounding effects from buffer compounds. After stabilization, 10 mL of the pH-adjusted water was added to sterile Petri dishes (9 cm) lined with two layers of Whatman No.1 filter paper. Deionized water without pH modification (natural pH ≈ 6.5) was included as an additional control. All solutions were refreshed every 48 hours, and pH was verified before and 24 hours after renewal. All other environmental conditions, including temperature (25 ± 1°C), complete darkness, and uniform moisture, were kept

constant using a germinator (IKH 680, Iran).

## 2.3. Measurement of physiological and biochemical indices

### 2.3.1. Assessment of germination and growth traits

Germinated seeds (radicle length ≥ 2 mm) were counted daily. Final germination percentage (GP%) was determined after 7 days. Germination rate (GR) was calculated using Maguire's formula (1962):  $GR = \sum(N_i/D_i)$ , where  $N_i$  is the number of germinated seeds on day  $i$ , and  $D_i$  is day  $i$ .

After the germination period, healthy seedlings were collected. Seedling fresh weight (SFW) was measured using a digital balance (accuracy 0.0001 g). Seedling dry weight (SDW) was measured after oven-drying at 70°C for 48 hours. Seedling vigor weight (SVW) was calculated as:  $SVW = (GP\% \times SDW) / 100$ .

### 2.3.2. Assessment of membrane integrity via relative electrolyte leakage

Relative electrolyte leakage (REL) was measured according to Lutts et al. (1996) and ISTA (2023) guidelines. After the 7-day germination period, twenty uniform seedlings from each treatment were gently rinsed with deionized water and placed in sealed test tubes containing 20 mL deionized water. Tubes were incubated at 25°C for 24 hours, and initial electrical conductivity (EC<sub>1</sub>) was measured. Tubes were then autoclaved at 120°C for 15 minutes, and final conductivity (EC<sub>2</sub>) was measured after cooling. REL was calculated as:  $REL (\%) = (EC_1 / EC_2) \times 100$ .

A higher REL percentage indicates greater membrane damage and loss of semi-permeability in the seedling tissues. This calculated REL value is reported in

the results as a key physiological index of stress-induced cellular damage.

### 2.3.3. Sampling strategy and timeline for biochemical analyses

To accurately capture the dynamic biochemical changes associated with seed reserve mobilization and early seedling establishment under different treatments, a time-dependent sampling strategy was employed. Measurements were categorized based on the physiological process they represented:

**Enzymes of reserve mobilization:** Activities of  $\alpha$ -amylase and protease were assayed at the peak of their activity during germination. Based on preliminary tests, samples were collected on day 4 after sowing. At this stage, seeds had imbibed and showed radicle protrusion (2-5 mm), ensuring that the enzymatic machinery for breaking down starch and protein reserves was highly active. Whole germinated seeds (including the endosperm and embryonic axis) were used for extraction.

**Stress metabolites and nutritional status:** The contents of total soluble sugars and total soluble protein were measured in 7-day-old seedlings at the conclusion of the experiment. This timing reflects the cumulative metabolic status and osmotic adjustment of the established seedling in response to the prolonged pH and priming treatments.

**Silicon uptake:** The concentration of silicon was also determined in the whole 7-day-old seedlings to quantify the total amount of the element absorbed and translocated during the early growth phase.

### 2.3.4. Biochemical assays

All biochemical measurements were performed on fresh tissue. Extraction was carried out using chilled 50 mM phosphate buffer (pH 7) at 4°C. After homogenization and centrifugation, the

supernatant was used for the following analyses:

**$\alpha$ -Amylase activity:** Determined by the Bernfeld (1955) method using soluble starch as substrate. Reducing sugars were measured at 540 nm. Enzyme activity was expressed as micromoles of maltose released per minute per gram fresh weight.

**Protease activity:** Assessed by the Anson (1938) method using acid-denatured hemoglobin. Absorbance change (due to tyrosine/tryptophan) measured at 280 nm. Activity was reported as the change in absorbance per minute per gram fresh weight.

**Total soluble sugar content:** Quantified by phenol-sulfuric acid method at 485 nm (Moretti et al., 2020) using glucose standard.

**Total soluble protein content:** Determined by Bradford (1976) method at 595 nm using Coomassie Brilliant Blue G-250 and BSA standard.

### 2.3.5. Silicon content measurement

Silicon content was measured in dried biomass of 7-day-old seedlings. Plant material was dry-ashed at 550°C for 4 hours, dissolved in 2M HCl, and determined colorimetrically using the silicon-molybdate blue method at 810 nm (Yoshida et al., 1976).

## 2.4. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) after confirming normal distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test). Mean comparisons were performed using Duncan's Multiple Range Test at  $P < 0.05$ . All statistical computations were conducted using SAS version 9.4 and Excel 2019.

### 3. Results and Discussion

#### 3.1. Germination and growth traits

The results of the analysis of variance (Table 1) revealed that the main effects of both pH and silicon on all measured germination and growth traits (germination percentage and rate, seedling vigor weight and length, and REL) were highly significant ( $P < 0.01$ ). However, the interaction effect between these two factors was significant ( $P < 0.05$ ) only for germination rate. The absence of significant pH  $\times$  silicon interactions in most traits can be explained by the soil-less nature of the experimental system, where typical pH-related stress mechanisms such as aluminum toxicity at low pH or micronutrient unavailability at high pH are largely absent. Therefore, pH acted mainly as a mild physiological factor rather than a strong stressor, and the responses to pH and silicon were largely additive rather than synergistic (Rizwan et al., 2015).

Lowering the growth medium pH to 5 (acidic condition) significantly reduced final germination percentage, germination rate, seedling vigor, and fresh weight, while simultaneously increasing relative

electrolyte leakage (REL) – an indicator of membrane damage (Table 2). The observed growth inhibition under low pH can be primarily attributed to proton ( $H^+$ ) toxicity, which disrupts cellular ion homeostasis and pH-sensitive metabolic processes, leading to induced oxidative stress (Meng et al., 2024). This acidic environment can also directly compromise the activity of key germination enzymes, such as  $\alpha$ -amylase, whose optimal pH is typically near neutral (pH 6.5-7.0) (Cherrate et al., 2023; Vallabhaneni et al., 2025). Conversely, raising the pH to 8 (alkaline condition) also caused a significant decline in these indices compared to the optimal pH (6.5). This reduction is likely due to impaired availability and uptake of essential micronutrients (e.g., iron, zinc, manganese), whose solubility decreases in alkaline conditions.

The application of silicon at all tested levels significantly improved germination and growth traits while reducing REL (Table 2). The most pronounced effect was observed at 150 mg L<sup>-1</sup> silicon (Si150). This amelioration can be ascribed to silicon's well-documented roles in stabilizing cell walls and membranes, reducing electrolyte leakage, improving

**Table 1.** Analysis of variance (mean squares) for the effects of pH, silicon (Si) priming, and their interaction on germination, growth, and physiological traits of maize.

جدول ۱. تجزیه واریانس (میانگین مربعات) اثرات pH، پرایمینگ سیلیسیم (Si) و برهمکنش آنها بر صفات جوانه‌زنی، رشد و فیزیولوژیکی ذرت

Source of variation منابع تغییر	Degrees of freedom درجه آزادی	Final germination percentage درصد جوانه زنی نهایی	Germination rate سرعت جوانه زنی	Seedling weight vigor قدرت وزنی گیاهچه	Seedling length vigor قدرت طولی گیاهچه	Relative electrolyte leakage نشت نسبی الکترولیت
pH	2	341.42**	9.208**	11.9**	269792.7**	402.5**
Silicon (Si) سیلیکون	3	89.75**	30.38**	379.39**	2758293.5**	685.3**
pH $\times$ Si برهمکنش	6	9.29 ns	2.21*	0.68 ns	18904.7 ns	18.7 ns
Error خطا	24	8.65	0.82	0.804	9082.7	12.4
CV (%) ضریب تغییرات (%)		3.48	3.64	6.85	5.56	8.5

\*\* and \* indicate significance at the 1% and 5% probability levels, respectively; ns, indicates non-significant.

\*\* و \* به ترتیب نشان‌دهنده معنی‌داری در سطح احتمال ۱٪ و ۵٪ هستند ns. بیانگر عدم معنی‌داری است.

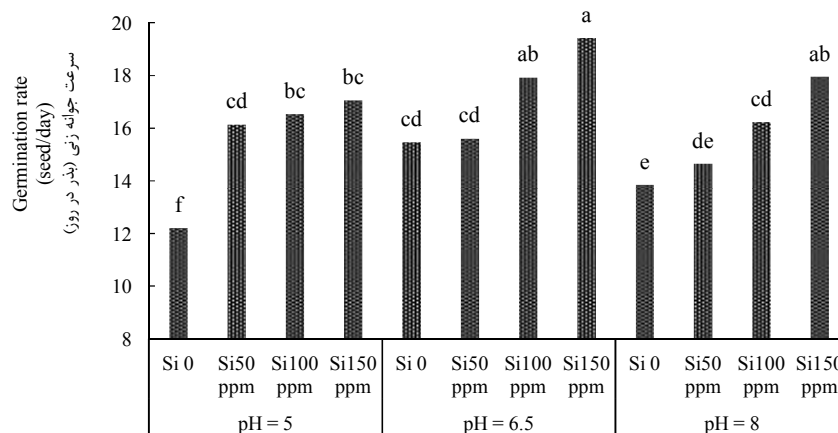
**Table 2.** Mean comparison of the main effects of pH and silicon priming levels on germination, growth, and physiological traits of maize.

جدول ۲. مقایسه میانگین اثرات اصلی سطوح pH و پرایمینگ سیلیسیم بر صفات جوانه‌زنی، رشد و فیزیولوژیکی ذرت.

Treatment تیمار	Germination (%) درصد جوانه‌زنی	Germination rate (seeds/day) سرعت جوانه‌زنی (بذر در روز)	Seedling weight vigor قدرت وزنی گیاهچه	Seedling length vigor قدرت طولی گیاهچه	Relative electrolyte leakage (%) نشت نسبی الکتروولیت (%)
pH Level سطوح pH					
5 (Acidic) اسیدی	81.815 b	12.2665 b	12.2665 b	1653.69 b	52.3 a
6.5 (Neutral) خنثی	90.514 a	14.1964 a	14.1964 a	1884.44 a	24.8 c
8 (Alkaline) قلیایی	80.817 b	12.8046 b	12.8046 b	1603.19 b	35.1 b
Silicon Level (mg L <sup>-1</sup> ) سیلیکون (میلی گرم در لیتر)					
0 (Control) شاهد	80.810 c	13.890 d	5.4722 d	1099.67 d	48.7 a
50	83.209 bc	15.4987 c	10.9189 c	1456.34 c	35.5 b
100	85.250 b	16.9078 b	15.1683 b	1930.19 b	29.4 c
150	88.259 a	18.1511 a	20.7973 a	2368.91 a	22.6 d

Different letters within a column and for each treatment factor (pH or Si) indicate significant differences according to Duncan's multiple range test ( $P < 0.05$ ).

در هر ستون و برای هر فاکتور تیماری (pH یا Si)، میانگین‌های فاقد حرف مشترک، بر اساس آزمون چنددامنه‌ای دانکن در سطح احتمال ۵ درصد دارای اختلاف معنی‌دار هستند.



**Fig. 1.** Interaction of seed priming with silicon and growth medium pH on the germination rate of maize. Different lowercase letters above the bars indicate significant differences among treatment combinations according to Duncan's multiple range test ( $P < 0.05$ ). The synergistic effect of silicon priming (particularly at 150 mg L<sup>-1</sup>) in mitigating the inhibitory effect of acidic pH (5) on germination speed is clearly demonstrated.

شکل ۱. برهمکنش پرایمینگ بذر با سیلیسیم و pH محیط کشت بر سرعت جوانه‌زنی ذرت. حروف کوچک متفاوت بر روی نمودار میله‌ای، نشان‌دهنده اختلاف معنی‌دار بین تیمارهای ترکیبی بر اساس آزمون چنددامنه‌ای دانکن در سطح احتمال ۵ درصد می‌باشد. اثر هم‌افزایی پرایمینگ با سیلیسیم (به‌ویژه در غلظت ۱۵۰ میلی‌گرم بر لیتر) در کاهش اثر بازدارندگی pH اسیدی (۵) بر سرعت جوانه‌زنی به وضوح نشان داده شده است.

osmotic adjustment, and enhancing the plant's antioxidant defense system (Hasan et al., 2024).

The significant interaction for germination rate (Fig. 1) indicates that silicon specifically and more effectively enhanced the rate of germination under

acidic stress (pH 5). This finding aligns with Hasanaklou et al. (2023), who reported that silicon accelerates germination rate under acidic conditions by stimulating hydrolytic enzyme activity and improving water uptake.

### 3.2. Biochemical traits

Based on the results presented in Table 3, the main effect of silicon was highly significant ( $P < 0.01$ ) for all measured biochemical traits ( $\alpha$ -amylase and protease activity, soluble sugar content, total protein, and silicon uptake). In contrast, the main effect of pH was significant only for total protein and silicon uptake, and the pH  $\times$  silicon interaction was not significant for any of these traits.

#### 3.2.1. Activity of hydrolytic enzymes

The activities of  $\alpha$ -amylase and protease enzymes increased linearly and significantly with rising silicon concentrations (Table 4). This enhancement likely occurred due to the stimulation of hormonal signaling pathways (e.g., gibberellins) and a reduction in oxidative stress mediated by silicon (Liang et al., 2023). Higher activity of these enzymes facilitates the efficient breakdown of internal seed reserves (starch and proteins), providing the necessary precursors for energy and cellular structure in the developing seedling, which is directly linked to improved germination rate and percentage (Nafarrate-ramos et al., 2022).

#### 3.2.2. Metabolic reprogramming and enhanced protein turnover

The main effect of pH was significant for total protein content ( $P < 0.05$ ). Seedlings grown at pH 6.5 showed the highest total protein, which was significantly greater than that at pH 8. Protein content at pH 5 was intermediate and did not differ significantly from either pH 6.5 or pH 8 (Table 4). The reduction in total protein under alkaline conditions (pH 8) may be attributed to impaired protease activity and reduced nitrogen metabolism efficiency in an alkaline environment.

Silicon priming fundamentally reprogrammed the primary metabolism of germinating maize seeds, leading to significant accumulation of soluble sugars and total protein (Table 4). This contrasts with the typical germination pattern where protease activation primarily drives the net degradation of storage proteins to fuel growth (Li et al., 2011). Our results reveal a distinct, silicon-mediated mechanism: the coordinated increase in both protease activity and total protein pool suggests an accelerated yet anabolically biased protein turnover. Silicon likely facilitates the efficient hydrolysis of storage proteins while simultaneously channeling the liberated amino acids into the synthesis of new functional proteins—including stress-responsive enzymes—thereby enhancing the seedling's metabolic capital.

**Table 3.** Analysis of variance (mean squares) for the effects of pH, silicon (Si) priming, and their interaction on biochemical traits of maize seedlings.

جدول ۳. تحلیل واریانس (میانگین مربعات) اثرات pH، پرایمینگ سیلیسیم (Si) و برهمکنش آنها بر صفات بیوشیمیایی گیاهچه‌های ذرت.

Source of variation منابع تغییر	Degrees of freedom درجه آزادی	Soluble sugars قندهای محلول	Total protein پروتئین کل	Protease activity فعالیت پروتئاز	$\alpha$ -Amylase activity فعالیت آلفا آمیلاز	Silicon uptake جذب سیلیکون
pH	2	0.523 ns	3.097 *	0.016 ns	0.0065 ns	0.007 *
Silicon (Si) سیلیکون	3	104.536**	131.278**	9.88**	58.75**	4.714**
pH $\times$ Si اثر متقابل pH $\times$ Si	6	0.34 ns	0.495 ns	0.0397 ns	0.111 ns	0.0022 ns
Error خطا	24	0.658	0.993	0.0747	0.182	0.0021
CV (%) ضریب تغییرات (%)		5.919	5.976	7.559	5.42	5.066

\*\* and \* indicate significance at the 1% and 5% probability levels, respectively; ns, indicates non-significant.

\*\* و \* به ترتیب نشان‌دهنده معنی‌داری در سطح احتمال ۱٪ و ۵٪ هستند ns. بیانگر عدم معنی‌داری است.

**Table 4.** Mean comparison of the main effects of pH and silicon priming levels on biochemical traits of maize seedlings.

جدول ۴. مقایسه میانگین اثرات اصلی سطوح pH و پرایمینگ سیلیسیم بر صفات بیوشیمیایی گیاهچه‌های ذرت.

Treatment تیمار	$\alpha$ -Amylase Activity ( $\mu\text{mol maltose min}^{-1} \text{g}^{-1} \text{FW}$ ) فعالیت آلفا آمیلاز (میکرومول مالتوز در دقیقه در گرم وزن تر)	Soluble Sugars (mg $\text{g}^{-1} \text{FW}$ ) قندهای محلول (میلی گرم در گرم وزن تر)	Total Protein ( $\text{mg g}^{-1} \text{FW}$ ) پروتئین کل (میلی گرم در گرم وزن تر)	Protease Activity ( $\Delta\text{A}280 \text{ min}^{-1} \text{g}^{-1} \text{FW}$ ) فعالیت پروتئاز (جذب در ۲۸۰ نانومتر در دقیقه در گرم وزن تر)	Silicon Uptake (mg $\text{g}^{-1} \text{DW}$ ) جذب سیلیکون (میلی گرم در گرم وزن خشک)
<b>pH</b>					
5 (Acidic) اسیدی	7.9117 a	13.8250 a	16.6067 ab	3.5850 a	0.94333 a
6.5 (Neutral) خنثی	7.8733 a	13.4692 a	17.22 a	3.6567 a	0.91 ab
8 (Alkaline) قلیایی	7.8692 a	13.8367 a	16.2117 b	3.6050 a	0.90083 b
<b>Silicon Level (mg L<sup>-1</sup>)</b> سیلیکون (میلی گرم در لیتر)					
0 (Control) شاهد	4.8589 d	9.6633 d	11.99 d	2.3044 d	0.01667 d
50	6.9389 c	12.6433 c	15.4744 c	3.3956 c	0.7322 c
100	8.9633 b	14.8089 b	18.4133 b	3.9589 b	1.20333 b
150	10.7778 a	17.7256 a	20.84 a	4.8033 a	1.72 a

Different letters within a column and for each treatment factor (pH or Si) indicate significant differences according to Duncan's multiple range test ( $P < 0.05$ ). FW: Fresh Weight; DW: Dry Weight;  $\Delta\text{A}280$ : Change in absorbance at 280 nm.

در هر ستون و برای هر فاکتور تیماری (pH یا Si)، میانگین‌های فاقد حرف مشترک، بر اساس آزمون چنددامنه‌ای دانکن در سطح احتمال ۵ درصد دارای اختلاف معنی‌دار هستند. FW: وزن تر؛ DW: وزن خشک؛  $\Delta\text{A}280$ : تغییر در جذب نوری در طول موج ۲۸۰ نانومتر.

Concurrently, the rise in soluble sugars provides crucial osmolytes for turgor maintenance and readily available energy (Shao et al., 2019; Weisany et al., 2023).

### 3.2.3. Silicon uptake

As expected, the silicon content in seedling tissues increased dramatically with the concentration of silicon in the priming solution (Table 4). Greater silicon uptake provides the necessary physical and chemical foundation for its beneficial roles, including cell wall reinforcement and chelation of toxic ions (Kopittke et al., 2017; Nasukawa & Tajima, 2025).

### 3.3. Proposed mechanisms of silicon in mitigating pH stress

Integrating our findings with the literature, we propose the following mechanisms to elucidate how silicon priming improves maize germination under a range of pH conditions in a controlled, soil-less system. It is important to note that in our experimental setup, the direct

toxicity of metal ions like aluminum ( $\text{Al}^{3+}$ ) was absent.

#### 3.3.1. Amelioration of proton ( $\text{H}^+$ ) toxicity and cellular acidification

The primary stressor in our low-pH treatment is the high concentration of protons ( $\text{H}^+$ ). Silicon is known to enhance the structural integrity of the apoplast and plasma membrane. This reinforcement likely helps buffer against excessive  $\text{H}^+$  influx, maintaining a more stable cytoplasmic pH—a critical factor for the activity of pH-sensitive germination enzymes like  $\alpha$ -amylase and protease (Vallabhaneni et al., 2025).

#### 3.3.2. Protection of cellular membranes and reduction of oxidative stress

Our observation of significantly reduced REL in silicon-primed seedlings, especially under acidic stress, directly points to silicon's role in safeguarding membrane integrity. This protective effect is two-fold: (i) the physical deposition of silica in cell walls and membranes

enhances their mechanical strength and reduces permeability, and (ii) silicon mitigates the secondary oxidative stress induced by low pH by bolstering the antioxidant defense system, thereby minimizing lipid peroxidation (Jiang et al., 2022).

### 3.3.3. Enhancement of metabolic resource mobilization

This study provides evidence for a key mechanism: silicon-mediated upregulation of reserve mobilization. The significant increases in  $\alpha$ -amylase and protease activity, along with elevated levels of soluble sugars and total protein, indicate that silicon primes the seed's metabolic engine. This likely occurs through the modulation of hormonal signaling pathways (e.g., promoting a favorable gibberellin-to-abscisic acid ratio) or direct enhancement of enzyme stability under stress (Liang et al., 2023).

### 3.3.4. Promotion of anabolic priming and osmotic adjustment

Beyond stress protection, silicon appears to shift the seedling's physiology toward a more anabolic state (Ajay et al., 2025). The concurrent increase in both protease activity (catabolism) and total protein content (net anabolism) suggests silicon promotes a high flux of nitrogen turnover geared towards synthesizing new, stress-adaptive proteins. Simultaneously, the accumulation of soluble sugars acts as compatible osmolytes, aiding in osmotic adjustment, maintaining turgor pressure, and protecting cellular structures (Weisany et al., 2023).

In conclusion, in our hydroponic germination system, silicon's protective and growth-promoting effects are best

explained by a multi-faceted strategy: it fortifies physical barriers against  $H^+$  intrusion, minimizes oxidative membrane damage, and actively reprograms early metabolism to ensure vigorous seedling establishment under pH stress.

A limitation of this study is that germination was assessed based on radicle emergence ( $\geq 2$  mm) rather than the stricter 'normal seedling' criterion of ISTA. Therefore, while our results demonstrate significant improvements in early germination initiation under pH stress, extrapolation to field emergence under certified seed standards should be made with caution. Future studies should evaluate the effect of silicon priming on normal seedling development using standard ISTA protocols.

## 4. Conclusion

Silicon priming at  $150 \text{ mg L}^{-1}$  alleviates the negative effects of non-optimal pH on maize germination by enhancing hydrolytic enzyme activities ( $\alpha$ -amylase and protease), increasing soluble sugars and total protein, and reducing electrolyte leakage. The significant silicon  $\times$  pH interaction for germination rate underscores silicon's specific role in accelerating germination under pH stress. Although based on a 7-day ISTA-compliant germination test, these findings support silicon priming as a promising, cost-effective strategy for improving maize establishment in suboptimal pH soils. Field studies are recommended to confirm the long-term effectiveness of this approach.

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### **Author Contributions**

First Author: Conceptualization, execution of the experiments, data curation, formal analysis, and writing—original draft preparation.

Second Author: Conceptualization, project administration, supervision of the experimental execution, supervision of statistical analysis, and writing—review, editing, and finalization.

### **Data Availability Statement**

Data available on request from the authors.

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### **Ethical Considerations**

The authors avoided data fabrication, falsification, and plagiarism, and any form of misconduct.

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***Conflict of Interest***

The authors declare no conflict of interest.

## تأثیر پرایمینگ با سیلیسیم بر جوانه‌زنی و شاخص‌های بیوشیمیایی بذر ذرت (سینگل کراس ۷۰۴) در گستره‌های مختلف اسیدیته

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### چکیده

### اطلاعات مقاله

**هدف:** این پژوهش با هدف بررسی تأثیر پرایمینگ بذر با سیلیسیم بر بهبود جوانه‌زنی و پاسخ‌های بیوشیمیایی ذرت (هیبرید سینگل کراس ۷۰۴) در گستره‌های مختلف اسیدیته محیط کشت انجام شد.

**روش پژوهش:** آزمایش به صورت فاکتوریل در قالب طرح کاملاً تصادفی با سه تکرار اجرا شد. تیمارها شامل چهار سطح پرایمینگ [شاهد (آب مقطر) و محلول سیلیسیم با غلظت‌های ۵۰، ۱۰۰ و ۱۵۰ میلی‌گرم در لیتر] و سه سطح اسیدیته محیط کشت (۵، ۶/۵ و ۸) بودند. صفات اندازه‌گیری شده شامل شاخص‌های جوانه‌زنی (درصد و سرعت)، رشد گیاهچه (وزن تر، بنه وزنی و طول)، نشت یونی (نشت نسبی الکترولیت) و شاخص‌های بیوشیمیایی (فعالیت آنزیم‌های آمیلاز و پروتاز، محتوای قندهای محلول، پروتئین کل و جذب سیلیسیم) بودند.

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**یافته‌ها:** نتایج نشان داد که انحراف از اسیدیته بهینه (۶/۵) به سمت شرایط اسیدی (۵) یا قلیایی (۸) به طور معنی‌داری شاخص‌های جوانه‌زنی و رشد را کاهش و نشت یونی را افزایش داد. پرایمینگ بذر با سیلیسیم، به‌ویژه در غلظت ۱۵۰ میلی‌گرم در لیتر، اثرات نامطلوب تغییرات اسیدیته را به‌طور محسوس کاهش داد. در شرایط اسیدی (۵)  $\text{pH} =$  این تیمار درصد جوانه‌زنی را تا ۶۳/۱۹ درصد نسبت به شاهد فاقد سیلیسیم در همان اسیدیته افزایش، نشت نسبی الکترولیت را کاهش و فعالیت آنزیم‌های آمیلاز و پروتاز، محتوای قندهای محلول، پروتئین کل و جذب سیلیسیم را به‌طور معنی‌داری بهبود بخشید. اثر متقابل سیلیسیم و اسیدیته تنها برای سرعت جوانه‌زنی معنی‌دار بود که نشان‌دهنده نقش اختصاصی سیلیسیم در تسریع جوانه‌زنی در شرایط اسیدیته نامطلوب است.

کلیدواژه‌ها:

آمیلاز

بنه بذر

پروتاز

تحمل تنش

تعادل یونی

نشت نسبی الکترولیت

**نتیجه‌گیری:** پرایمینگ بذر ذرت با سیلیسیم (۱۵۰ میلی‌گرم در لیتر) می‌تواند به عنوان راهکاری مؤثر برای افزایش قابلیت جوانه‌زنی و تقویت وضعیت فیزیولوژیک و بیوشیمیایی بذرهای مواجه‌شده با شرایط اسیدیته غیربهینه محیط کشت، به‌ویژه تحت تنش اسیدی، به کار رود. این روش پتانسیل قابل‌توجهی برای بهبود استقرار مزرعه‌ای در خاک‌های دارای اسیدیته زیربهینه دارد.

### جنبه‌های نوآوری:

- پرایمینگ ذرت با سیلیسیم، جوانه‌زنی، سرعت و بنه را در اسیدیته‌های مختلف، به‌ویژه  $\text{pH}=5$ ، بهبود داد.
- سیلیسیم (۱۵۰ میلی‌گرم در لیتر) با کاهش نشت الکترولیت، آسیب غشا را در تنش اسیدیته کاهش داد.
- سیلیسیم فعالیت آلفا-آمیلاز و پروتاز، قند محلول و پروتئین کل گیاهچه را افزایش داد.
- اثرات مفید سیلیسیم مستقل از اسیدیته بود که نشان‌دهنده کاهش پایدار تنش در جوانه‌زنی است.

**استناد:** هاشم‌وند، مریم؛ و صدقی، محمد (۱۴۰۴). تأثیر پرایمینگ با سیلیسیم بر جوانه‌زنی و شاخص‌های بیوشیمیایی بذر ذرت (سینگل کراس ۷۰۴) در گستره‌های

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## ملاحظات اخلاقی

### پیروی از اصول اخلاق پژوهش

نویسندگان اصول اخلاقی را در انجام و انتشار این پژوهش علمی رعایت نموده‌اند و این موضوع مورد تأیید همه آنهاست. نویسندگان از هرگونه جعل، تحریف داده‌ها، سرقت علمی و سایر اشکال سوءرفتار علمی پرهیز کرده‌اند.

### مشارکت نویسندگان

نویسنده اول: اجرای آزمایش‌ها، مدیریت و پردازش داده‌ها، تحلیل رسمی نتایج، و نگارش نسخه اولیه مقاله.  
نویسنده دوم: مفهوم‌سازی پژوهش، مدیریت پروژه، نظارت بر اجرای آزمایش‌ها، نظارت بر تحلیل‌های آماری، بازبینی، ویرایش و نهایی‌سازی مقاله.

### دسترسی به داده‌ها

داده‌های این پژوهش در صورت درخواست، از نویسندگان در دسترس می‌باشد.

### تعارض منافع

بنا بر اظهار نویسندگان این مقاله تعارض منافع ندارد.

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