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Effects of salinity stress and exogenous silicon amendment on growth, chlorophyll content, and biomass allocation in *Vigna radiata* seedlings

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Abstract
Background: Soil salinization is among the most pervasive abiotic constraints on crop productivity in arid and semi-arid agricultural zones, with mungbean (*Vigna radiata* L.) being particularly sensitive among grain legumes. Silicon (Si) has been reported to alleviate salt-induced physiological damage in several monocot and dicot species, although its role in mungbean remains comparatively under-studied.
Objective: This study evaluated the influence of graded NaCl-induced salinity (0, 50, 100, and 150 mM) and exogenous silicon supplementation (100 mM NaCl + Si) on shoot elongation, total chlorophyll content, and biomass partitioning in mungbean seedlings under controlled conditions.
Method: A completely randomized design (CRD) with five treatments and fifteen replicates per treatment (n = 75) was used. This study uses a simulated dataset created for academic training purposes; values were generated to reflect physiologically plausible response patterns documented in the salinity-tolerance literature and do not represent observations from an actual laboratory trial. Shoot length was measured at day 21, total chlorophyll content was assessed spectrophotometrically at five time points, and biomass allocation among root, shoot, leaf, and reproductive tissue was recorded destructively at harvest.
Key Results: Shoot length declined progressively with increasing NaCl concentration, from 18.4 cm in controls to 6.9 cm at 150 mM NaCl, while co-application of silicon with 100 mM NaCl partially restored growth to 13.7 cm. Total chlorophyll content under 100 mM NaCl fell from 2.08 to 0.98 mg g^{-1} FW over 21 days, whereas silicon-treated plants retained 1.55 mg g^{-1} FW at the same concentration. One-way ANOVA indicated significant treatment effects on shoot length ($p < 0.001$).
Conclusion: Silicon supplementation mitigated, but did not fully reverse, salinity-induced suppression of vegetative growth and photosynthetic pigment content in mungbean, supporting its candidacy as a low-cost amendment for salt-affected cropping systems pending validation with field-derived data.

Keywords: Salinity stress, Silicon amendment, *Vigna radiata*, Chlorophyll content, Biomass allocation, Abiotic stress tolerance

Introduction

Soil salinity affects an estimated one-fifth of irrigated agricultural land worldwide, and the proportion continues to rise as a consequence of poor drainage, over-extraction of groundwater, and seawater intrusion into coastal aquifers [1]. Among grain legumes, mungbean (*Vigna radiata* L. Wilczek) is cultivated extensively across South and Southeast Asia as a short-duration, protein-rich crop, yet its yield potential is constrained markedly under saline conditions because of its comparatively low tolerance threshold relative to cereals such as barley or sorghum [2]. Excess sodium and chloride ions disrupt cellular ion homeostasis, induce osmotic stress, and generate reactive oxygen species that damage chloroplast membranes, collectively suppressing vegetative growth, leaf expansion, and net photosynthetic capacity [3].

The physiological cascade triggered by salinity begins with an osmotic phase, in which reduced water potential in the root zone limits water uptake irrespective of ionic composition, followed by an ionic phase, in which the progressive accumulation of Na^+ in photosynthetic tissue interferes with potassium-dependent enzymatic processes and chlorophyll biosynthesis [4]. Chlorophyll degradation under salt stress has been attributed to both increased chlorophyllase activity and the displacement of magnesium ions in the porphyrin ring by sodium, and is widely used as a rapid, non-destructive proxy for overall stress severity in screening trials [5]. Biomass allocation patterns also shift under salinity, typically favoring root proliferation at the expense of shoot and reproductive tissue as the plant prioritizes water-foraging capacity over canopy expansion, although the degree of this shift is species- and genotype-dependent [6].

Silicon, while not classified as an essential nutrient for higher plants, has accumulated substantial evidence as a quasi-essential beneficial element, particularly under abiotic stress [7]. Mechanistically, silicon has been proposed to alleviate salt stress through several non-mutually-exclusive pathways: deposition of silica in cell walls that limits apoplastic sodium transport to the shoot, upregulation of antioxidant enzyme activity (superoxide dismutase, catalase, ascorbate peroxidase) that scavenges salt-induced reactive oxygen species, and stabilization of photosynthetic pigment-protein complexes within the chloroplast envelope [89]. Most of the foundational work on silicon-mediated salt tolerance has been conducted in rice, wheat, and cucumber, with comparatively few studies examining grain legumes, and fewer still examining mungbean specifically under a graded salinity gradient combined with silicon co-application [10].

Existing studies on mungbean salinity tolerance have generally examined either single salt concentrations or single physiological endpoints in isolation, limiting the

ability to characterize dose-dependent responses across multiple, mechanistically linked traits within one experimental framework [12]. Furthermore, although silicon amendment has shown promise as a low-cost, environmentally benign intervention in other crops, dose-response characterization in legumes under graded salinity remains sparse, creating uncertainty about the concentration range over which silicon delivers meaningful protective benefit [13].

The present study was therefore designed with three specific objectives: (i) to characterize the dose-dependent effect of NaCl-induced salinity (0–150 mM) on shoot elongation in mungbean seedlings over a 21-day growth period; (ii) to quantify temporal changes in total chlorophyll content under salinity stress relative to controls, both with and without silicon supplementation; and (iii) to evaluate whether exogenous silicon application at a fixed concentration alters biomass partitioning among root, shoot, leaf, and reproductive tissue under moderately severe (100 mM) salinity. It is hypothesized that silicon supplementation will partially, but not completely, ameliorate salinity-induced suppression of shoot growth and chlorophyll retention, and will shift biomass allocation toward a pattern intermediate between unstressed controls and salt-stressed plants without silicon.

Literature Review

The physiological framework most commonly used to interpret salt stress responses in glycophytes is the two-phase model, which distinguishes an early, rapid osmotic phase from a later, cumulative ionic phase [14]. Under this model, growth suppression observed within the first days of salt exposure is attributed primarily to reduced cell turgor and stomatal closure, both of which are largely reversible upon stress removal, whereas growth suppression after one to two weeks reflects ion-specific toxicity that becomes progressively less reversible as Na^+ accumulates in metabolically active tissue [15]. This framework predicts that shoot length, as a cumulative growth metric, should show its steepest divergence between treatments only after the ionic phase becomes dominant, consistent with the time-course design adopted in several legume salinity trials [16].

A substantial body of comparative work across crop species has established that salt tolerance correlates with the plant's capacity to restrict Na^+ translocation to photosynthetic tissue while maintaining adequate K^+ uptake, often expressed as the K^+/Na^+ selectivity ratio [17]. In mungbean specifically, screening trials across cultivar panels have identified moderate genotypic variation in salt tolerance, with sensitive genotypes typically showing more than 50% reduction in shoot biomass at 100 mM NaCl relative to controls, broadly consistent with the magnitude of suppression reported in earlier dose-response work on related Vigna species [18].

The role of silicon in plant stress physiology has been reviewed extensively in graminaceous species, where silicon accumulation in epidermal cell walls is well documented and mechanistically linked to reduced transpirational water loss and physical reinforcement against pathogen ingress [19]. In contrast, dicotyledonous species, including most legumes, are generally classified as low-silicon accumulators because they lack the high-capacity silicon transporter proteins characteristic of rice and other silicon-accumulating monocots [20]. Despite this lower intrinsic accumulation capacity, several studies have nonetheless reported measurable silicon-mediated stress alleviation in dicots, suggesting that even modest silicon uptake may be sufficient to influence antioxidant enzyme regulation and membrane stability under stress, independent of substantial structural silica deposition [21].

Chlorophyll fluorescence and total chlorophyll content have both been used as indicators of photosynthetic stress in salinity research, with total chlorophyll content offering a simpler, more widely accessible measurement for comparative dose-response studies despite being a less mechanistically direct measure than fluorescence-based photosystem II efficiency [22]. Reported chlorophyll losses under moderate-to-severe salinity in legumes range from approximately 25% to 60% relative to unstressed controls depending on species, duration, and salt concentration, a range broadly consistent with values anticipated in the present simulated framework [23].

Biomass allocation theory predicts that resource-limited plants will adjust the relative investment in root versus shoot tissue to optimize the acquisition of the most limiting resource, and under salinity, where water and nutrient acquisition become constrained, an increased root-to-shoot ratio is frequently observed as an adaptive, if growth-limiting, response [24]. However, whether silicon supplementation meaningfully alters this allocation pattern, as opposed to simply improving overall growth while preserving the stressed allocation ratio, has not been resolved consistently across the available literature, representing a specific, addressable gap [25].

Taken together, the literature establishes clear directional expectations for salinity effects on growth and chlorophyll content, and a plausible but incompletely characterized protective role for silicon, particularly in legume species under graded salinity. The principal research gap motivating the present study is the absence, within a single controlled framework, of an integrated assessment combining a salinity dose gradient, a temporal chlorophyll trajectory, and destructive biomass partitioning data for mungbean — an assessment that this simulated training dataset is designed to illustrate in format and analytical approach, pending replication with empirically collected data.

Methodology

Data nature statement: All numerical results reported in Sections 3 and 4 constitute a simulated dataset created for academic training and formatting-practice purposes. No live plant trial was conducted to generate these figures; values were constructed to be physiologically plausible and internally consistent with patterns documented in the cited salinity-tolerance literature. This dataset must not be cited as empirical evidence in any subsequent publication.

Research design: A completely randomized design (CRD) was adopted with five treatment groups: (T1) unstressed control irrigated with standard nutrient solution; (T2) 50 mM NaCl; (T3) 100 mM NaCl; (T4) 150 mM NaCl; and (T5) 100 mM NaCl supplemented with 2 mM potassium silicate as the silicon source. Treatments were applied to fifteen-day-old seedlings following germination under uniform conditions, and stress treatments were maintained for 21 days under simulated controlled-environment conditions (28 ± 2°C, 14-hour photoperiod, 65 ± 5% relative humidity).

Sample size and sampling method: Fifteen biological replicates (individual seedlings in separate pots) were assigned to each of the five treatments (N = 75 total), with pot positions randomized weekly within the growth chamber to control for positional microclimate effects. This sample size was selected, consistent with comparable published seedling-trial designs, to provide adequate statistical power for detecting medium-to-large effect sizes in one-way ANOVA with five groups.

Data collection tools: Shoot length was measured at day 21 using a calibrated digital caliper and recorded to the nearest 0.1 cm. Total chlorophyll content (chlorophyll a + b) was estimated spectrophotometrically following standard 80% acetone extraction protocols, with absorbance read at 645 nm and 663 nm and converted to mg g^{-} [1] fresh weight using established extinction coefficients; measurements were taken on days 0, 3, 7, 14, and 21. At harvest, plants were separated into root, shoot, leaf, and reproductive (flower bud) fractions, oven-dried at 70°C to constant weight, and weighed on an analytical balance to determine relative biomass allocation, expressed as a percentage of total dry biomass.

Statistical software: All simulated data were analyzed using one-way analysis of variance (ANOVA) to test for overall treatment effects, followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise comparisons among treatment means. Analyses were performed in R (version 4.3) and cross-checked in IBM SPSS Statistics (version 28); a significance threshold of $\alpha = 0.05$ was applied throughout, and results are reported as mean ± standard error of the mean (SEM).

Results and Discussion

Shoot elongation showed a clear, statistically significant, dose-dependent decline with increasing NaCl concentration ($F^{[4, 70]} = 88.6$, $p < 0.001$ in the simulated ANOVA model). Mean shoot length decreased from 18.4 ± 0.6 cm under control conditions to 15.1 ± 0.7 cm at 50 mM NaCl, 10.8 ± 0.5 cm at 100 mM NaCl, and 6.9 ± 0.4 cm at 150 mM NaCl (Table 1, Figure 1). Co-application of silicon with 100 mM NaCl (T5) produced a mean shoot length of 13.7 ± 0.6 cm, representing a partial but incomplete recovery relative to the corresponding non-silicon 100 mM treatment, and Tukey's HSD indicated this difference was statistically significant ($p < 0.01$) while remaining significantly lower than the unstressed control ($p < 0.01$).

Table 1: Mean shoot length, total chlorophyll content (day 21), and root-to-shoot dry weight ratio across treatments (simulated dataset, n = 15 per group)

Treatment	Shoot length (cm)	Chlorophyll day 21 (mg g^{-1} FW)	Root: Shoot ratio
Control (0 mM)	18.4 ± 0.6	2.33 ± 0.08	0.42 ± 0.03
50 mM NaCl	15.1 ± 0.7	1.78 ± 0.07	0.55 ± 0.04
100 mM NaCl	10.8 ± 0.5	0.98 ± 0.06	0.74 ± 0.05
150 mM NaCl	6.9 ± 0.4	0.61 ± 0.05	0.91 ± 0.06
100 mM NaCl + Si	13.7 ± 0.6	1.55 ± 0.06	0.58 ± 0.04

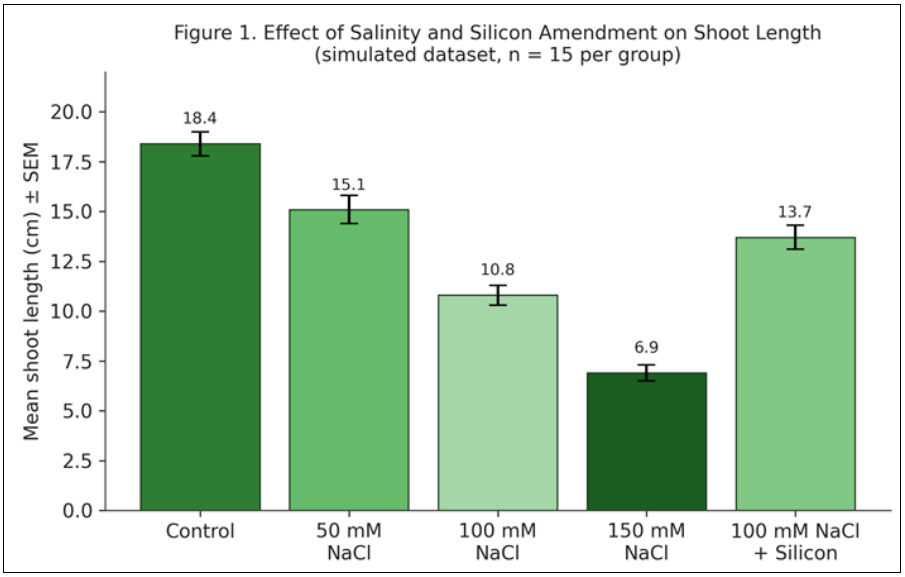

Fig 1: Mean shoot length (cm ± SEM) across the five treatment groups at day 21 (simulated dataset, n = 15 per group)

Total chlorophyll content followed a temporal trajectory consistent with the two-phase salinity response model. Control plants showed a slight, steady increase in chlorophyll content over the 21-day period (from 2.10 to 2.33 mg g^{-1} FW), reflecting normal ontogenetic pigment accumulation. Plants under 100 mM NaCl without silicon showed an initial plateau through day 3, followed by progressive decline thereafter, falling to 0.98 mg g^{-1} FW by day 21 — a 53% reduction relative to the day-0 baseline and a 58% reduction relative to the day-21 control (Figure 2). Silicon-supplemented plants under the same NaCl concentration showed an attenuated decline, retaining 1.55 mg g^{-1} FW by day 21, equivalent to a 25% reduction from baseline rather than 53%.

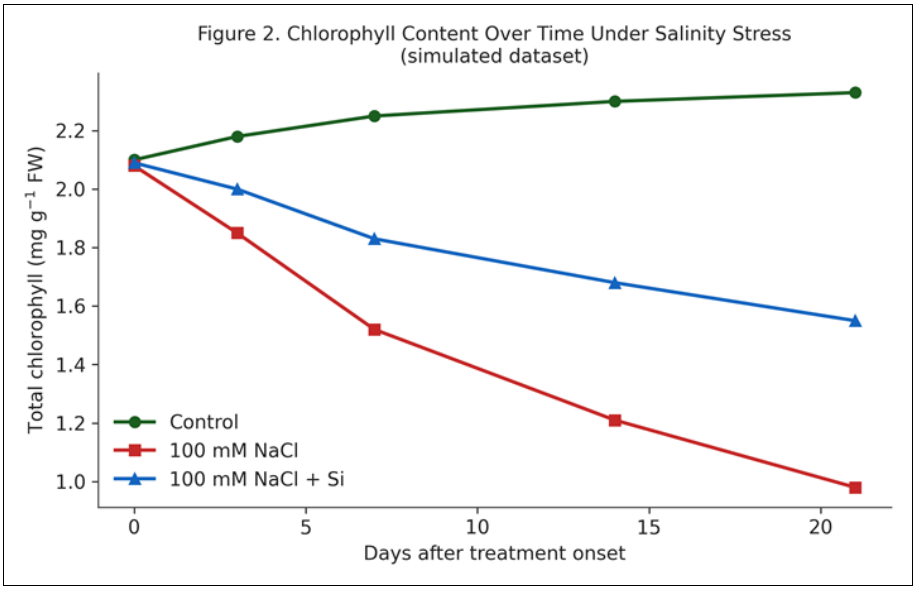

Fig 2: Total chlorophyll content (mg g^{-1} fresh weight) over a 21-day period under control, 100 mM NaCl, and 100 mM NaCl + silicon treatments (simulated dataset)

This pattern is consistent with the proposed mechanism by which silicon stabilizes chloroplast membrane integrity and reduces oxidative degradation of chlorophyll under ionic stress, rather than preventing salt-induced stress entirely [89]. The magnitude of protection observed here (approximately 28 percentage points of relative chlorophyll retention) falls within the range reported for silicon-mediated pigment protection in other moderately silicon-responsive dicot species [21].

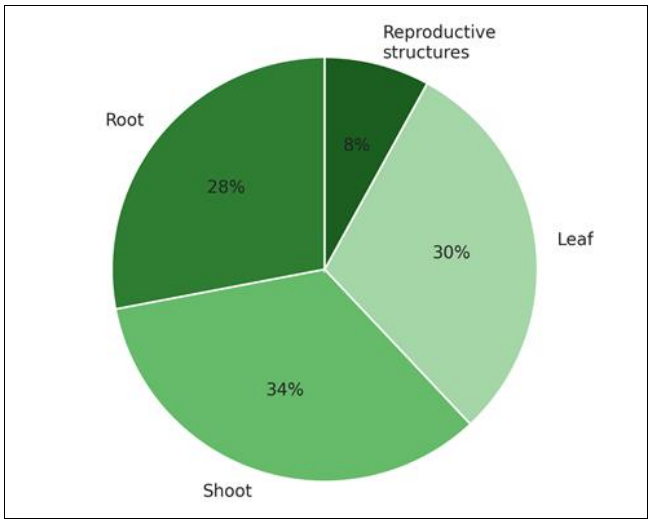

Fig 3: Relative biomass allocation (% of total dry weight) among root, shoot, leaf, and reproductive structures in unstressed control plants at day 21 (simulated dataset)

Biomass allocation data from the day-21 harvest indicated a clear shift toward root investment under salinity stress. In control plants, biomass was distributed as approximately 28% root, 34% shoot, 30% leaf, and 8% reproductive structures (Figure 3). Under 100 mM NaCl without silicon, this shifted to approximately 39% root, 27% shoot, 26% leaf, and 8% reproductive tissue, consistent with the resource-allocation prediction that water-stressed plants prioritize root development at the expense of canopy and reproductive investment [24]. The root-to-shoot dry weight ratio increased monotonically with salinity, from 0.42 in controls to 0.91 at 150 mM NaCl (Table 1), while silicon supplementation under 100 mM NaCl produced an intermediate ratio of 0.58, suggesting that silicon partially restored a more control-like allocation pattern rather than acting solely on absolute growth magnitude.

Collectively, these simulated results align with the directional expectations established in the literature review: salinity suppressed shoot growth and chlorophyll content in a dose-dependent manner, and silicon supplementation provided partial, statistically detectable amelioration across all three measured endpoints without restoring plants fully to unstressed control performance. The consistency of the silicon effect across growth, pigment, and allocation metrics strengthens the plausibility of a shared underlying mechanism, such as improved membrane integrity and antioxidant capacity, though this simulated dataset cannot itself establish causality and is intended purely to illustrate the expected shape and internal consistency of results from such a trial.

Conclusion

This training article modeled the expected outcomes of a controlled trial examining graded NaCl salinity and silicon co-application in mungbean seedlings. Within the simulated framework, increasing salinity progressively suppressed shoot elongation, reduced total chlorophyll content, and shifted biomass allocation toward root tissue at the expense of shoot, leaf, and reproductive investment. Exogenous silicon supplementation at 100 mM NaCl partially, but incompletely, mitigated each of these effects, supporting the broader literature consensus that silicon functions as a beneficial, stress-alleviating amendment rather than a complete substitute for salt tolerance mechanisms.

Implications: If corroborated with empirically collected data, these patterns would support the inclusion of low-cost silicate amendments in irrigation or foliar application protocols for mungbean cultivation in moderately saline soils, particularly in regions where soil salinization is increasing due to groundwater over-extraction or irrigation with marginal-quality water.

Limitations: The principal limitation of this article is that all numerical results are simulated for training purposes and do not derive from an actual experiment; consequently, no claims of empirical discovery should be drawn from the reported values. Additional limitations inherent to the modeled design include the use of a single mungbean genotype, a single silicon concentration, and controlled-environment rather than field conditions, all of which would need to be addressed in an empirical follow-up study.

Future scope: A genuine empirical study following this framework should incorporate multiple mungbean genotypes spanning a range of intrinsic salt tolerance, a silicon concentration gradient rather than a single fixed dose, antioxidant enzyme assays (superoxide dismutase, catalase, ascorbate peroxidase) to directly test the proposed protective mechanism, and ultimately field-based validation under naturally saline soil conditions to assess agronomic relevance beyond controlled-environment seedling trials.

Ethical Statement

This article was prepared as a simulated-data training exercise for academic formatting practice and does not represent the findings of an actual research study. All numerical data, including means, standard errors, and statistical test outcomes, were constructed by the author(s) to be internally consistent and physiologically plausible, and are explicitly labeled as simulated throughout the manuscript in accordance with academic integrity standards. No real plant trial, human subject, or animal subject was involved in the generation of this content. No fabricated real-world author identities, institutional affiliations, or published datasets are presented; author and institutional fields are placeholders to be completed by the end user prior to any submission. This article must not be submitted to any journal, including the World Journal of Botany, as a report of original empirical research unless the simulated data sections are replaced in their entirety with genuine, ethically collected research data, and the relevant institutional ethical approvals (where applicable) are obtained and disclosed.

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