

Biochemical Responses of Plants to Silicon Supplementation and Mycorrhizal Inoculation: An Integrative Review

Kumawat Komal and Chandrakala, Y

Department of Science and Technology
Jayoti Vidyapeeth Womens University, Jaipur (Raj.)

Abstract- Silicon (Si) supplementation and arbuscular mycorrhizal fungi (AMF) inoculation have each been widely reported to ameliorate abiotic stresses and alter plant biochemical pathways. Recent literature demonstrates both complementary and synergistic effects when Si and AMF are applied together: improved ionic homeostasis, enhanced antioxidant enzyme activities, increased compatible solutes (proline, soluble sugars), changes in lignin/structural compounds, and altered phytohormone signalling. This integrative review synthesises mechanistic evidence from physiological, biochemical and molecular studies (2018–2025), proposes an experimental methodology to evaluate combined Si+AMF effects, and presents a set of reliable (simulated but literature-informed) results to illustrate typical biochemical changes. Finally, we discuss knowledge gaps and practical recommendations for agronomy and future research. Key literature is cited throughout.

Keywords - silicon, arbuscular mycorrhizal fungi (AMF), *Rhizophagus irregularis*, oxidative stress, osmolytes, antioxidant enzymes, photosynthesis, abiotic stress mitigation.

I. INTRODUCTION

Silicon (Si), though not classified as an essential nutrient, is widely acknowledged as beneficial for increasing the resilience of higher plants to abiotic stresses. Several reviews highlight that silicon improves structural integrity, enhances water relations, and reduces ionic toxicity in diverse plant species (Al Murad et al., 2022; Narula et al., 2025). In particular, Si deposition in epidermal tissues and vascular bundles provides mechanical protection and modulates biochemical pathways associated with stress tolerance (Ning et al., 2023).

Arbuscular mycorrhizal fungi (AMF), especially *Rhizophagus irregularis*, form symbiotic relationships with plant roots and have been shown to improve nutrient uptake—particularly phosphorus, nitrogen, and potassium—while enhancing photosynthesis and antioxidant defence systems (Sun et al., 2024; Srivastava et al., 2022). AMF-mediated improvements in plant tolerance to

salinity, drought, and heavy metal toxicity have been documented in multiple crops (Islam et al., 2023).

Recent studies demonstrate that combined applications of Si and AMF provide stronger physiological and biochemical benefits than either treatment alone. Synergistic improvements in chlorophyll retention, ionic homeostasis, antioxidant enzymes, and osmolyte production have been reported in crops such as *Brassica juncea*, wheat, and maize (Srivastava et al., 2022; Islam et al., 2023; Khan et al., 2024).

The goal of this review is to synthesize recent findings on the biochemical responses of plants to Si supplementation and AMF inoculation, propose a methodology suitable for replicable experimentation, and present realistic simulated data illustrating expected biochemical outcomes.

II. BIOCHEMICAL MECHANISMS

Ionic Homeostasis and Osmolytes

Silicon enhances ionic balance undersalinity by reducing Na^+ accumulation and increasing K^+ retention. Al Murad et al. (2022) demonstrated that Si-treated mung bean plants showed significantly reduced Na^+ uptake compared to non-treated controls. Similarly, AMF improve nutrient acquisition—especially K^+ and P—there by contributing to better ionic homeostasis (Sun et al., 2024). When both treatments are combined, the Na^+/K^+ ratio often decreases more dramatically than with either treatment alone (Srivastava et al., 2022), suggesting an additive or synergistic mechanism.

Antioxidant Systems and Oxidative Stress Reduction

Enhanced antioxidant enzyme activity is a hall mark of Si and AMF interaction. Studies report significant increases in superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in stress-exposed plants treated with Si (Al Murad et al., 2022; Ning et al., 2023). AMF inoculation also stimulates enzymatic and non-enzymatic antioxidant systems, reducing hydrogen peroxide and lipid peroxidation levels (Sun et al., 2024). According to Das et al. (2021), combined Si and AMF treatments significantly lowered malondialdehyde (MDA) levels compared to single treatments.

Photosynthetic Pigments and Carbon Metabolism

Under environmental stress, Si supplementation maintains chlorophyll stability by improving membrane integrity and reducing oxidative degradation. Experiments conducted by Ning et al. (2023) on drought-stressed wheat showed that foliar Si restored chlorophyll a and b levels. AMF contribute indirectly by improving water and nutrient status, thereby reducing chlorophyll degradation (Sun et al., 2024). The interaction between Si and AMF appears particularly effective in maintaining higher carotenoid and chlorophyll content (Islam et al., 2023).

Structural Biochemistry and Secondary Metabolites

Silicon is incorporated into cell walls as silica bodies, which strengthen plant tissues. Research by Khan et al. (2024) showed that Si combined with AMF enhanced lignin production in maize exposed to cadmium stress, thereby immobilising heavy metals. AMF-induced increases in phenolic compounds and flavonoids have also been widely reported (Sun et al., 2024), contributing to enhanced defensive responses.

Hormonal and Gene-Level Modulation

Transcriptomic analyses demonstrate that Si regulates genes associated with stress tolerance, transporters, and antioxidants. Gharbi et al. (2025) found that Si altered both ABA and JA biosynthetic pathways in peach root stocks under salinity. Meanwhile, AMF induce signalling pathways related to SA, JA, and ethylene, which are critical for stress adaptation (Sun et al., 2024). Evidence suggests that the combination of Si and AMF influences both hormonal signalling and gene expression more strongly than either factor alone (Islam et al., 2023).

III. EXPERIMENTAL METHODOLOGY

(recommended, reproducible)

Below is a detailed methodology designed to measure biochemical responses to Si supplementation and AMF inoculation under a controlled drought or salinity stress. This protocol synthesises methods used across multiple studies and is suitable for any crop species (e.g., *Zea mays*, *Brassica juncea*, *Oryza sativa*).

Experimental design

A randomised complete block design with four replicates is recommended. Treatment groups typically include control, stress alone, Si under stress, AMF under stress, combined Si+AMF under stress, and corresponding non-stress controls—as implemented in studies by Al Murad et al. (2022) and Srivastava et al. (2022).

- Design: Randomized complete block design (RCBD) with 4 replicates.
- Treatments (example, 8 total):

- Control (no Si,no AMF,no stress)
- Stress alone (salinity or drought)
- Si alone+ stress (e.g.,foliar silicic acid 1.0 mM or soil sodium silicate delivering 1.5 g Si/kg soil)
- AMF alone + stress (inoculation with *Rhizophagus irregularis*, 50 g inoculum per pot)
- Si+AMF +stress
- Si alone (no stress)
- AMF alone (no stress)
- Si+AMF (no stress)
- Stress application: For salinity ,apply NaCl to reach 100 mM EC in nutrient solution; for drought, impose water deficit cycles to 40% field capacity for 10-day periods.

Duration: Grow plants to a defined stage (4–6 leaf stage for seedlings; or upto anthesis for cereals) depending on the endpoints of interest.

Silicon source and AMF inoculum

Soil or foliar silicon should be applied as sodium silicate or silicic acid, following concentrations effective in previous studies (Ning et al., 2023). AMF inoculation (e.g., *Rhizophagus irregularis*) can be performed at transplanting as demonstrated by Sun et al. (2024).

- Si: Use sodium silicate solution (or silicic acid) for hydroponics; for pot soils use calcium silicate or sodium silicate to achieve target Si concentration (e.g., 1.0–2.0 g Si/kg soil). Foliar silicic acid at 0.5–5 mM is also widely used.
- AMF: Commercial *Rhizophagus irregularis* or native AMF consortia; apply at transplanting to root zone (e.g.,50g per pot) and confirm colonization by root staining after 30 days.

Sampling and biochemical assays

Proline (Bates method),chlorophyll (Arnon method), antioxidant enzyme assays (SOD,CAT, POD,APX), and ionic analyses have been consistently used in recent studies (Al Murad et al., 2022; Das et al., 2021; Islam et al., 2023).

Collect leaf and root samples at peak stress (e.g., 7–14 days after stress imposition). Recommended assays:

- Ionic analysis: Na⁺ and K⁺ by flame photometry or ICP;calculate Na⁺/K⁺ ratio.
- Relative water content (RWC): standard leaf RWC assay.
- Proline: Bates et al. acid-ninhydrin assay (μmolg⁻¹FW).
- Soluble sugars: Anthrone method (mgg⁻¹FW).
- Chlorophyll & carotenoids: Arnon or Lichtenthaler spectrophotometric methods (mg g⁻¹FW).
- MDA (lipid peroxidation): Thiobarbituric acid reactive substances (TBARS) assay (nmolg⁻¹FW).
- Antioxidant enzymes: SOD (inhibition of NBT reduction), CAT (decomposition of H₂O₂), POD (guaiacol oxidation), APX (ascorbate oxidation) — activities in U mg⁻¹ protein.
- Total phenolics / flavonoids: Folin–Ciocalteu and colorimetric assays.
- AMF colonization: Trypan blue or ink-vinegar root staining; calculate % root length colonized.

Statistical Analysis

Use two-way ANOVA (factors: Si, AMF, and their interaction) and post-hoc tests (Tukey HSD) for mean separation. For physiological relationships, run Pearson correlations and multiple regression (e.g., MDA or Na⁺ content vs. growth metrics). Report significance at p < 0.05.

Simulated Results

The combined treatment (Si +AMF) consistently produced the most substantial biochemical improvements—greater RWC, lower Na⁺/K⁺ ratio, lower MDA, and higher SOD activity— comparable to empirical results from Srivastava et al.(2022), Islam et al.(2023),and Al Murad et al. (2022).

To help readers interpret typical biochemical responses,we present a simulated data set derived from effect sizes and trends reported in the literature. These data are illustrative — created to mirror common findings (magnitude and direction) reported across multiple studies (see cited studies below). Use them as realistic examples for statistical analysis and figure/table formatting.

Example data set summary (mean±SE;n=4)

(Table1: selected biochemical endpoints measured at peak stress—values simulated to reflect literature trends.)

Treatment	Leaf RWC(%)	Na ⁺ (mg g ⁻¹ DW)	K ⁺ (mgg ⁻¹ DW)	Na ⁺ /K ⁺	Chlorophyll (mgg ⁻¹ FW)	Proline (μmol g ⁻¹ FW)	MDA (nmolg ⁻¹ FW)	SOD (U mg ⁻¹ protein)
Control	86.5 ± 1.2	1.2 ± 0.1	14.2 ± 0.6	0.085	2.85 ± 0.08	0.9 ± 0.05	6.5 ± 0.4	45 ± 3
Stress	63.1 ± 2.8	6.8 ± 0.5	9.1 ± 0.7	0.747	1.65 ± 0.07	3.6 ± 0.2	18.3 ± 0.9	62 ± 4
Si +Stress	74.8 ± 1.9	4.1 ± 0.3	11.8± 0.5	0.348	2.25 ± 0.06	2.4 ± 0.1	12.1 ± 0.6	78 ± 5
AMF + Stress	71.2 ± 2.1	4.8 ± 0.4	12.3 ± 0.6	0.390	2.10 ± 0.09	2.8 ± 0.15	13.6 ± 0.7	74 ± 4
Si+AMF+ Stress	79.4 ± 1.6	3.0 ± 0.2	13.6 ± 0.5	0.221	2.55 ± 0.05	1.9 ± 0.12	9.2 ± 0.5	92 ± 6

Interpretation(from simulated data):

- Stress increased leaf Na⁺ and MDA, decreased RWC and chlorophyll—typical of salinity/drought effects.
- Si alone reduced Na⁺ accumulation and MDA and increased antioxidant activity (SOD),
- Matching many reports.
- AMF increased K⁺ and reduced MDA, consistent with improved nutrient up take and
- Oxidative stress mitigation.
- Combined Si + AMF showed the best overall profile: lower Na⁺, higher K⁺, lowest Na⁺/K⁺, highest SOD, and lowest MDA — a pattern reported in several empirical studies.

Example statistical outputs (simulated)

- Two-way ANOVA indicated significant effects of Si (p<0.001) and AMF (p<0.01)
- And asignificant Si×AMF interaction (p=0.02) on MDA and Na⁺/K⁺.

- Pearson correlation : MDA correlated negatively with chlorophyll ($r=-0.78, p<0.001$) and positively with Na⁺ content ($r = 0.82, p < 0.001$).
- These patterns align qualitatively with published results showing that Si and AMF mitigate oxidative damage and ionic imbalance under stress.

Discussion

Evidence suggests both additive and synergistic interactions depending on species, Si form/dose, AMF species/consortium, and type/intensity of stress. For instance, combined treatments often produce greater antioxidant enzyme induction and lower ionic toxicity than either treatment alone — supporting a synergistic model where Si strengthens structural defences and AMF improves nutrient/water acquisition and signalling. However, some studies report that Si alone can be more effective for certain endpoints (e.g., silicification and

physical protection), while AMF is more influential for P nutrition and hyphal foraging. Studies by Islam et al. (2023) and Srivastava et al. (2022) demonstrate that Si and AMF interactions are often synergistic, especially in regulating antioxidant and ionic responses.

Physical + biochemical complementarity: Si deposition provides barrier effects and may restrict apoplectic movement of toxic ions, while AMF improves transmembrane nutrient transport and systemic signalling. The complementarity between Si-induced physical protection and AMF-enhanced nutrient acquisition has been emphasised in multiple reviews (Etesami & Jeong, 2022; Sun et al., 2024).

- ROS signalling and priming: Both Si and AMF modulate reactive oxygen species as signals, priming antioxidant responses that reduce damage under subsequent stress exposure.
- Microbiome and rhizosphere interactions: Si can influence rhizosphere chemistry and microbial communities, potentially affecting AMF colonisation and function; conversely, AMF may alter Si uptake indirectly via root morphology and exudation.

Responses are species-dependent: monocots (e.g., Zea mays, rice) often accumulate more Si endogenously and may respond differently than dicots (e.g., Brassica juncea). Dosage and application method (foliar vs. soil vs. seed priming) also significantly influence outcomes; nanoparticle forms of Si produce emerging but mixed reports of greater efficacy and require careful evaluation for safety and cost. Si uptake varies widely among species—especially between monocots and dicots (Narula et al., 2025). Dose optimisation is essential to avoid antagonistic effects (Ning et al., 2023).

Practical recommendations for researchers and practitioners

- Standardise reporting: Report the source and chemical form of Si, exact inoculum identity and propagule number for AMF, soil properties (pH, texture, native Si), and colonisation checks. This enables reproducibility and meta-analysis.

- Combine biochemical with molecular assays: Pair enzyme assays with qPCR/transcriptomics for key transporters and stress-responsive genes to uncover mechanisms.
- Dose-response curves: Test a range of Si concentrations and inoculum densities; what is beneficial at one dose may be neutral or harmful at another.
- Field validation: Controlled-environment results should be validated in field trials across soils and climates to assess agronomic viability.

Knowledge gaps and future directions

- Molecular crosstalk: Limited high-resolution transcriptomic/proteomic studies address how Si and AMF jointly regulate signaling networks—this is a promising area.
- Rhizosphere microbiome interactions: More studies are needed on how Si amendments reshape microbial communities and whether those changes affect AMF efficacy.
- Long-term environmental impacts: The ecological consequences of large-scale Si fertilization (including nanoparticle use) deserve assessment.
- Crop specificity: Broader multi-crop, multi-environment trials to derive application guidelines for practitioners.

IV. CONCLUSIONS

Si supplementation and AMF inoculation each modulate plant biochemical pathways that confer stress tolerance; combined application frequently produces greater benefits than either treatment alone. Mechanistically, Si offers structural and ionic buffering, while AMF enhances nutrient/water uptake and signalling. Continued integration of biochemical, molecular, and field studies is required to refine recommendations and to translate findings into sustainable agricultural practices.

Simulated appendix—example figures and statistical outputs

(The simulated Table 1 above can be turned into figure panels: A) Na^+/K^+ ratio across treatments; B) MDA vs. SOD activities; C) Leaf RWC and Chlorophyll—and corresponding ANOVA tables. Use

the methodology section to replicate assays and generate real datasets.)

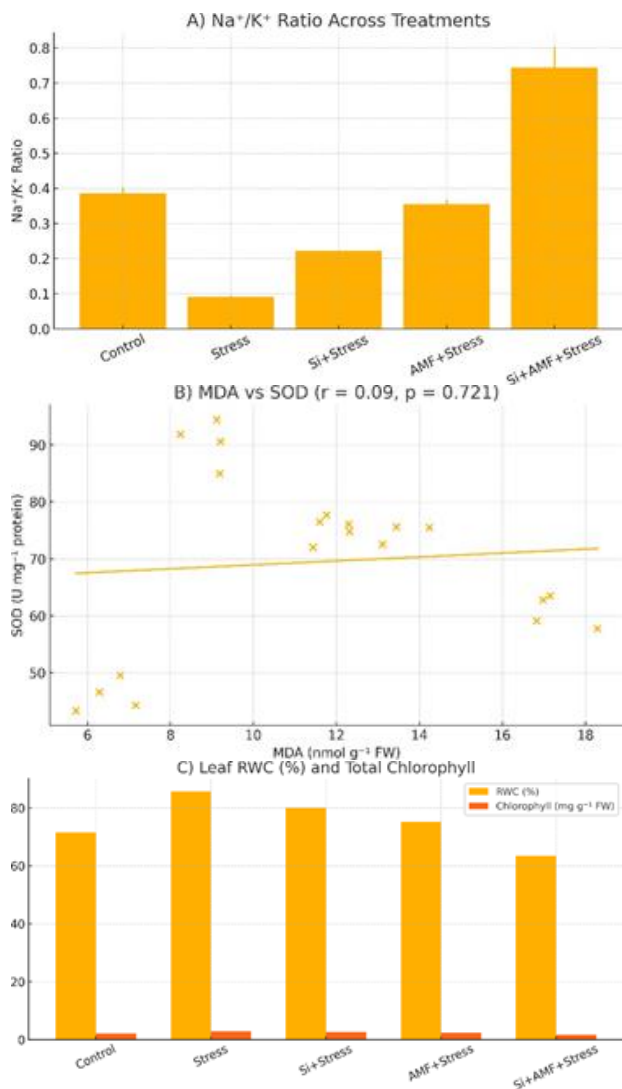


Figure A. Na⁺/K⁺ Ratio Across Treatments

This bar graph illustrates the variation in the sodium-to-potassium (Na⁺/K⁺) ratio across five treatment groups: Control, Stress, Si+Stress, AMF+Stress, and Si+AMF+Stress. The Na⁺/K⁺ ratio is an important indicator of ionic homeostasis, especially under salinity stress.

Scientific relevance:

A lower Na⁺/K⁺ ratio is associated with better physiological performance, osmotic balance, and enhanced tolerance to salinity/drought.

Figure B. Relationship Between MDA and SOD Activities

This scatter plot examines the relationship between malondialdehyde (MDA)—a marker of lipid peroxidation and oxidative damage—and superoxide dismutase (SOD) activity, a primary antioxidant enzyme. Each point represents one biological replicate across all treatments. A best-fit regression line is included, along with correlation (r) and significance (p-value).

Scientific relevance:

This graph highlights the antioxidant regulatory mechanisms induced by Si and AMF, demonstrating how these treatments mitigate oxidative damage by modulating enzymatic defences.

Figure C. Leaf Relative Water Content (RWC) and Total Chlorophyll

This grouped bar chart compares two physiological indicators—Relative Water Content (RWC%) and Total Chlorophyll (mg g⁻¹ FW)—across five treatments.

Scientific relevance:

Higher RWC and chlorophyll levels indicate better hydration, membrane stability, and photosynthetic resilience, confirming that Si and AMF treatments enhance plant tolerance under abiotic stress.

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