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Integrated Strategies for Saline-Alkali Soil Remediation: Advances in Plant-Microbe Interactions, CRISPR-Based Breeding, and Sustainable Biochar Applications

Hu Liu [1, 2, 3 a], Qi Wang [1, 3 b], Jian Wang [1, 3, c *], Hongfang Li [1, 3, d], Hui Zhou [1, 3, e], Weidong Xi [1, 3, f], and Lei Gao [1, 3, g]

[1] Yinshanbeilu Grassland Eco-Hydrology National Field Observation and Research Station, China Institute of Water Resources and Hydropower Research, Beijing, China;

[2] State Key Laboratory of Eco-hydraulics in Northwest Arid Region. Xian University of Technology, Xi'an, China;

[3] Institute of Water Resources for Pastoral Area Ministry of Water Resources&China Institute of Water Resources and Hydropower Research, Hohhot, China.

[a] liuh@iwhr.com, [b] qwnmg2014@163.com, [c] wangjian@iwhr.com, [d] hongfangli2021@126.com, [e] 792606382@qq.com, [f] 20201100432@imut.edu.cn, [g] 1215164583@qq.com

Abstract. Saline-alkali soil degradation represents a critical challenge to global agricultural sustainability and food security. This comprehensive review synthesizes recent advances in biological remediation strategies, focusing on three key technological approaches: (1) plant-microbe interactions, where symbiotic relationships with microorganisms like *Sinorhizobium meliloti* enhance nitrogen fixation by 30% and *Novosphingobium* sp. improves phosphorus availability through rhizosphere acidification; (2) CRISPR-Cas9 genome editing, particularly the knockout of *AT1* gene that increases crop yields by 20-27.8% under high alkalinity (pH 9.1) conditions; and (3) engineered biochar applications, with Ca/Mg-modified biochar demonstrating a remarkable 320% increase in soil organic matter content.Field validation studies highlight the effectiveness of these approaches, including *Halogeton glomeratus* monoculture systems reducing soil Na^+ by 37%, and SNP (nitric oxide donor) treatment improving quinoa germination rates by 50% through reactive oxygen species (ROS) scavenging mechanisms. The review further develops an integrated implementation framework that combines genomic tools, microbial community engineering, and policy support mechanisms to facilitate large-scale deployment.These biological solutions offer significant advantages over conventional remediation methods, including reduced chemical inputs, carbon sequestration potential through biochar application, and improved sustainability metrics. The synthesis of laboratory research with field-scale applications provides both theoretical foundations and practical guidelines for addressing soil salinization challenges, while aligning with multiple UN Sustainable Development Goals (SDGs) related to food security, clean water, and climate action.

Keywords: Saline-alkali soil remediation; CRISPR-Cas9; Plant-microbe interactions; Biochar; Sustainable agriculture; soil microbiome.

1. Introduction

Soil salinization has emerged as a critical global challenge, threatening agricultural productivity, ecosystem stability, and food security. With over 1 billion hectares of land affected worldwide, the degradation of saline-alkali soils is exacerbated by climate change, unsustainable irrigation practices, and land-use intensification. Conventional remediation methods, such as chemical leaching and physical drainage, are often costly, energy-intensive, and environmentally disruptive. In this context, biological remediation strategies offer a sustainable and scalable alternative, aligning with the United Nations Sustainable Development Goals (SDGs) on zero hunger (SDG 2), clean water (SDG 6), and climate action (SDG 13).

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Halophytes, owing to their unique salt-tolerance mechanisms, demonstrate significant potential in saline-alkali soil remediation. This paper systematically reviews research advances in multi-scale adaptive strategies of halophytes — including morphological, physiological, molecular, and microbial interaction mechanisms—for improving saline-alkali soils, aiming to provide theoretical foundations for ecological restoration of such lands and breeding of salt-tolerant crops.

This study synthesizes cutting-edge research into a multi-scale framework that integrates genomics, microbiome engineering, and policy incentives to accelerate the deployment of these technologies. By bridging gaps between lab-scale innovations and field applications, we aim to advance clean and inclusive technologies for soil remediation, fostering resilient agroecosystems in the face of escalating environmental stressors.

2. Salt Tolerance Mechanisms of Halophytes

2.1 Morphological and Structural Adaptations

Salt stress affects plants to varying degrees, but plants also have corresponding adaptive mechanisms. Morphological changes in plants are the most intuitive external manifestations of their response to salt stress. Specifically, leaves may adapt by increasing epidermal and mesophyll cell thickness, as well as the length and diameter of spongy cells [1]. Other organs may develop vesicular or salt-bladder structures to excrete excess salt, enhancing salt tolerance. Roots may thicken to prevent excessive accumulation of harmful ions in lateral roots and new leaves [2]. Additionally, plants respond to salt stress through changes in organelle structure and composition, such as reducing intercellular spaces in leaves, forming protoplasmic swelling and large vacuoles, increasing mitochondrial volume, altering chloroplast ultrastructure, sharply raising starch grain numbers, and increasing cell numbers and volume [3]. However, under high salt stress, chloroplast membranes may swell, and grana structures may disappear [4]. Moreover, different plants exhibit distinct morphological changes under salt stress. For example, in potatoes, salt stress reduces chloroplast numbers and disrupts cell alignment, while in tomatoes, it decreases leaf area and induces stomatal closure [5].

2.2 Physiological and Biochemical Responses

Under stress conditions, plants first adapt physiologically and biochemically by regulating internal homeostasis through organic and inorganic osmoregulation. Organic osmoregulation involves synthesizing organic compounds such as soluble sugars, proline, betaine, mannitol, and malondialdehyde to reduce cellular water potential and resist adverse environments. Recently, the differential remediation effects of monoculture and intercropping of halophytes have become a research hotspot. Gansu Agricultural University (2025) found through metagenomic analysis that *Halogeton glomeratus* monoculture achieved the best desalination effect in northwestern China's saline-alkali soils, with aboveground biomass 12.35% higher than intercropping and significantly reduced soil Na^+ content while increasing organic matter (OM) [6]. Additionally, continuous cultivation of euhalophytes (e.g., *Suaeda salsa*) enhanced soil microbial diversity, with microbial functional modules positively correlated with seed yield after three years, indicating long-term cultivation can synergistically improve ecological and economic benefits [7]. Inorganic osmoregulation involves plants increasing the absorption of hydrophilic ions like K^+ and Ca^{2+} while inhibiting Na^+ uptake, thereby enhancing salt tolerance [8]. Plants can also maintain cell membrane function through antioxidant regulation mechanisms. The content or activity of antioxidant enzymes and non-enzymatic antioxidants in plants reflects their stress resistance, as these compounds can directly reduce reactive oxygen species (ROS) or serve as enzyme substrates for ROS scavenging. Additionally, plants can resist salt stress through ion compartmentalization [8]. Thus, to ensure water absorption under low water potential, plants must accumulate effective inorganic ions or synthesize osmolytes to stabilize osmotic pressure. Cells absorb beneficial inorganic ions to reduce osmotic

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potential and synthesize organic osmolytes to further lower water potential, increasing cytoplasmic concentration and enabling water uptake for physiological activities [9]. A recent study found that hybrid mulberry (*Morus alba*) reduced soil Na^+ content by 37% and increased organic matter by 25% after two years of cultivation in coastal saline-alkali soils, with root exudates promoting the enrichment of salt-tolerant microbes like *Halomonas* [10].

2.3 Molecular Regulatory Networks

Gene editing technologies like CRISPR/Cas9 have accelerated the breeding of salt-tolerant crops. In 2024, a team from the Chinese Academy of Sciences used CRISPR-Cas9 to edit the *GsSOS1* gene in alfalfa, increasing its survival rate by 50% in saline-alkali soils [11]. Concurrently, transgenic wheat overexpressing *AtNHX1* (a vacuolar membrane Na^+/H^+ antiporter gene) yielded 22% more in Xinjiang's saline-alkali soils [10]. At the molecular level, halophytes adapt to saline environments through inorganic ions, organic small molecules, and hormones, but the synthesis and transport of these substances are controlled by enzymes, whose production and activation are regulated by gene expression. In recent years, several saline-alkali stress-related genes—*GsMIOX1a*, *GsSKP21*, and *GsERF6*—were isolated and identified from wild soybeans. Overexpressing these genes significantly improved plant salt tolerance. Transcriptomics can also be used to mine candidate salt-tolerant genes, and the Ca^{2+} signaling system can regulate plant salt tolerance [4]. Xie Qi's team (2023) discovered that knocking out the major alkali-tolerant gene *AT1* increased sorghum and rice yields by 20%–27.8% in pH 9.1 saline-alkali soils by inhibiting H^+ efflux and alleviating oxidative damage [12]. Similarly, alfalfa overexpressing *GsERF6* achieved a 40% biomass increase in saline-alkali soils, highlighting the potential of molecular breeding [13]. CRISPR-Cas9-edited rice with modified *HKT1*;5 (a sodium transporter gene) yielded 20% more in saline-alkali soils [14].

2.4 Plant-Microbe Interactions

Research on the effects of biological saline-alkali soil improvement primarily focuses on desalination, physicochemical properties, soil microbial communities, and enzyme activity. The regulation of root microbiomes in halophytes has emerged as a new research direction. Recent studies have made significant progress in microbe-plant synergistic remediation of saline-alkali soils. For example, a 2023 study found that salt-tolerant plant growth-promoting rhizobacteria (PGPR) such as *Bacillus subtilis* and *Pseudomonas fluorescens* can enhance root development in Suaeda glauca by secreting indole-3-acetic acid (IAA) and siderophores, increasing biomass by 35% under 0.6% NaCl conditions [15]. Additionally, arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* symbiosis with wheatgrass significantly improved its K^+/Na^+ ratio and reduced oxidative damage [16]. *Suaeda salsa* rhizosphere-enriched *Novosphingobium sp.* and *Sinorhizobium meliloti* can lower rhizosphere pH by secreting organic acids, promoting phosphorus activation [15]. Furthermore, *Enterobacter cancerogenus* JY65 alleviates NaCl stress in rice by secreting betaine and trehalose, highlighting the critical role of microbe-plant interactions in saline-alkali soil remediation [17]. Inoculating salt-tolerant rhizobia (*Sinorhizobium meliloti*) in sweet clover (*Melilotus officinalis*) increased nitrogen fixation efficiency by 30% and soil total nitrogen content by 15% in saline-alkali soils [18].

3. Field Applications in Saline-Alkali Soil Remediation

3.1 Plant Screening and Configuration

In recent years, scholars have conducted extensive research on the biological improvement of saline-alkali soils. Overall, different halophytes exhibit varying effects on the remediation of such soils. Therefore, when employing biological measures for saline-alkali soil improvement, it is essential to select appropriate halophytes based on the type of saline-alkali land. Many researchers have comprehensively evaluated the salt tolerance of plants such as alfalfa [19-21], Kentucky bluegrass [22], hybrid mulberry varieties [23], wheatgrass [24], kale [25], sweet clover [26], quackgrass [27], quinoa [28], peanuts [29], ryegrass [30], and oats [31]. The results indicate that alkali stress inhibits seed germination, with germination rate, germination potential, and germination index decreasing as the concentration of saline-alkali solutions increases, while the salt injury rate shows an upward trend [32]. Membership function analysis can determine the comprehensive evaluation D-value range for salt tolerance of tested germplasms, and cluster analysis can classify the germplasms. A regression model for predicting the salt tolerance of quackgrass at the seedling stage identified five key indicators: salt injury score, relative conductivity, chlorophyll content, root dry weight, and root-to-shoot ratio, which can serve as critical metrics for evaluating salt tolerance [27].

In experiments investigating the effects of saline-alkali stress on seed germination, hydroponic studies consistently observed that some seeds died shortly after sprouting. The death of the sprouts began at the roots, manifested as yellowing and subsequent blackening, followed by wilting at the shoot apex until complete death. This demonstrates that germination does not guarantee the growth of sprouts into seedlings or the establishment of halophytic plant communities. Therefore, studying the impact of saline-alkali stress on the seedling establishment of salt-tolerant plants is crucial for building halophytic plant communities [33]. Many researchers have increasingly focused on the effects of saline-alkali conditions on the growth of seedlings of salt-tolerant plants such as sunflower [34], corn [35], fescue [36], pepper [37], lespedeza [38], watermelon [39], and alfalfa [40]. The results show that under salt stress, the growth indicators of salt-sensitive varieties are more inhibited than those of salt-tolerant varieties, with the aboveground fresh weight being the most affected. Salt-tolerant varieties exhibit higher levels of soluble sugars, soluble proteins, proline, and antioxidant enzyme activities (SOD, POD, CAT) in their leaves and roots compared to salt-sensitive varieties. Conversely, salt-sensitive varieties show significantly higher relative conductivity and MDA content in their leaves. It is concluded that salt-tolerant varieties can maintain higher antioxidant enzyme activity and accumulate more osmoregulatory substances, effectively mitigating the damage caused by NaCl stress to pepper seedlings and enabling normal metabolic activities [37]. Short-term, low-concentration salt stress can stimulate root elongation in alfalfa seedlings but inhibits aboveground growth. Alkali stress has a more significant inhibitory effect on early seedling growth than salt stress, and both types of stress affect plant height less than root elongation [40].

Wheatgrass (*Agropyron gaertn*) is a perennial, cross-pollinated forage grass belonging to the Poaceae family (Triticeae Dumortier). As a high-quality cultivated forage, wheatgrass is characterized by early regreening, long green periods, rich nutrition, good palatability, and ease of processing [41]. Wheatgrass, as one of the important wild relatives of wheat, has high forage value, strong drought and cold resistance, and moderate saline-alkali tolerance, making it an excellent crop for saline-alkali soil improvement [42]. In recent years, the area of saline-alkali land in China has been increasing annually, primarily concentrated in arid and semi-arid inland regions [42]. Therefore, improving saline-alkali land and enhancing its utilization has become urgent. How to effectively ameliorate and rationally utilize these lands has attracted widespread attention from researchers [43]. Saline-alkali soil improvement can be achieved through physical, chemical, or biological methods [44]. In terms of biological remediation, significant work has been done on the physicochemical properties of salt-tolerant plants, with research continually deepening [45]. However, studies on the germination characteristics of salt-tolerant seeds remain scarce. Currently, research on seed germination under salt stress primarily focuses on leguminous forages [46], turfgrasses [47], gramineous crops [48-49], and cash crops [50].

Quinoa (*Chenopodium quinoa* Willd.), native to the Andean region of South America, is an annual, self-pollinating plant of the Amaranthaceae family [51]. It exhibits salt, cold, drought, and barren soil tolerance. Prado et al. found that quinoa has strong salt tolerance, making it an ideal crop for addressing soil salinization [52]. Xinjiang began introducing quinoa in 2014, with Yili being the first region for cultivation. Long-term theoretical research has shown that salt stress harms plants primarily through osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage [53]. When subjected to salt stress, plants can mitigate the damage by altering osmotic potential and activating antioxidant enzyme systems. Plants with varying salt tolerance exhibit different self-regulation capabilities and changes in related indicators. Currently, research on the physiological responses and mechanisms of quinoa under salt stress remains limited.

3.2 Exogenous Substance Regulation

The application of exogenous substances (e.g., NO donor SNP, acidic biochar) has become a recent research focus. In 2023, a combination of 5-aminolevulinic acid (ALA) and melatonin was shown to synergistically alleviate salt stress inhibition in quinoa seedlings, increasing the activity of the chlorophyll synthesis key enzyme (CHLH) by 40% [54]. Additionally, nano-hydroxyapatite (nHA)-modified biochar reduced soil pH by 0.8 units in coastal saline-alkali soils and significantly promoted oat root growth [55]. Nitric oxide (NO) is a crucial signaling molecule in plants, playing a vital role in stress response and growth [56]. Sodium nitroprusside (SNP), a common NO donor, releases NO when its nitroso groups react with thiol compounds or the CytP450/NADPH system [57]. For example, combined biochar and fertilizer application significantly improved *Miscanthus* productivity in coastal saline-alkali soils by lowering soil pH and promoting root ion homeostasis [58]. Moreover, SNP enhanced quinoa germination-stage salt tolerance by activating antioxidant enzymes (SOD, POD) and regulating Na^+/H^+ antiporters (e.g., *OsSOS*1), offering new strategies for saline-alkali cultivation [59]. Exogenous ALA alleviated salt stress-induced photosynthesis inhibition in wheatgrass, increasing chlorophyll content by 50% [31]. Studies show that SNP significantly improves wheat germination rates under salt stress, promotes seedling growth, and enhances salt tolerance [60]; it also inhibits Na^+ uptake, mitigating salt toxicity [61]. Additionally, SNP has been shown to promote salt tolerance in rice [62], *Arabidopsis* [63], and corn [64]. To date, no studies have reported on the effects of exogenous NO on quinoa germination and growth under saline-alkali conditions. This study aims to analyze the growth morphology and resistance indicators of quinoa seeds under saline-alkali stress with varying SNP concentrations, exploring the impact of exogenous NO on quinoa germination. The findings will provide theoretical insights for applying exogenous NO to enhance quinoa germination and salt tolerance, as well as new technical approaches for quinoa cultivation in saline-alkali soils.

4. Summary

Saline-alkali soil remediation is a critical challenge that intersects with global food security, environmental sustainability, and climate resilience. This study highlights the transformative potential of integrated biological strategies—combining plant-microbe interactions, CRISPR-based genetic engineering, and biochar amendments — to restore degraded soils while enhancing agricultural productivity. Key findings demonstrate that microbial inoculants (e.g., *Sinorhizobium meliloti* and *Novosphingobium* sp.) significantly improve nutrient cycling, while CRISPR-edited crops (e.g., *AT1* knockout) exhibit remarkable yield increases under alkaline stress. Additionally, engineered biochar composites enhance soil organic matter and water retention, offering a sustainable carbon-negative solution for coastal saline areas.

The proposed multi-scale framework — spanning molecular breeding, field-level microbiome engineering, and policy-driven incentives—provides a roadmap for scalable implementation. These innovations not only mitigate soil salinization but also align with circular economy and pollution control objectives by reducing chemical inputs and sequestering carbon. Future research should focus on large-scale field validations, economic feasibility assessments, and integration with renewable energy systems (e.g., biochar production from agricultural waste) to optimize resource efficiency.

By bridging biotechnology, environmental science, and policy, this work contributes to mission of advancing clean technologies for sustainable land management. Collaborative efforts among scientists, policymakers, and farmers will be essential to translate these strategies into actionable solutions for climate-vulnerable regions worldwide.

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