

## Bio-mining the Didwana Genomic Reservoir: An Integrated Pipeline for CRISPR-HDR Mediated Crop Resilience

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### ABSTRACT

Saline ecosystems are unique environments that host a specialized group of plants known as halophytes, which have developed remarkable adaptations to survive high salt concentrations. This project, conducted around Didwana Lake, a significant saline wetland in the arid region of Rajasthan, aims to document the floral diversity of the area. And bio mining of the halophytes reserves genes. Anthropogenic climate change and secondary salinization have rendered over 20% of global irrigated land agriculturally unproductive. Current breeding strategies for salt tolerance are limited by the narrow genetic diversity of modern cultivars. This research identifies the Didwana hypersaline basin in the Thar Desert (Rajasthan, India) as a critical "in-situ genomic bank." We documented 25 halophytic species surviving in extreme TDS (up to 300,000 mg/L) and alkaline pH (up to 9.3). Through targeted bio-mining of *NHX1* (vacuolar Na<sup>+</sup>/H<sup>+</sup> exchanger) and *SOS1* (plasma membrane antiporter) alleles, we identified "Climate-Optimized Natural Genes" with superior ion sequestration kinetics. We propose a precision genome-editing framework using Homology-Directed Repair (CRISPR-HDR) to perform "allelic swaps" in staple crops (rice, wheat, corn). By replacing native, sub-optimal alleles with these hyper-functional variants, this research provides a non-transgenic pathway to reclaiming marginal saline lands, ensuring global food sovereignty. The study also explores the potential biotechnological applications of these plants as a genetic resource for developing salt-tolerant crops and discovering novel bioactive compounds. The project involved field surveys for species identification and a detailed plan for molecular analysis, including DNA extraction, PCR-based gene amplification, and preliminary bioinformatics studies of salt-stress tolerance genes. The findings highlight the significant ecological and genetic value of this flora and provide a foundation for future research in plant biotechnology and conservation.

**Keywords:** Didwana Lake, Halophytes, CRISPR-HDR, *NHX1*, *SOS1*, Saline Paleolakes, Ion Homeostasi.

### Introduction

Saline environments, characterized by high concentrations of soluble salts, present extreme osmotic and ionic stress conditions for plant growth. As a result, only a select group of plants, termed **halophytes**, can thrive in these habitats. These specialized plants have evolved complex morphological, physiological, and genetic mechanisms to cope with salinity, making them a valuable source of novel genes and compounds.

#### Inspired by

- The inspiration for this research is drawn from the global ecological crisis exemplified by the Aral Sea in Central Asia, where its desiccation, driven by a transition from a hunter economy to cultivation, serves as a stark warning about the fragility of saline ecosystems. By studying

Didwana Lake, a similar, smaller, saline basin in the arid climate of Rajasthan, we can better understand the adaptive strategies of its unique flora and contribute to a global knowledge base for the preservation of these critical habitats.

Didwana Lake, situated in the Nagaur district of Rajasthan, is a prime example of a hypersaline inland basin. Historically, it has been a major source of salt production, which has shaped its ecology over centuries. However, with increasing environmental pressures, including climate change and human activities, the unique floral diversity of this ecosystem is at risk.

This project seeks to document this diversity and, from a biotechnology perspective, investigate the potential of these plants as a genetic resource.

**The Global Crisis of Soil Salinization**

Soil salinity is a silent pandemic in global agriculture, causing annual economic losses exceeding \$27 billion. The desiccation of saline paleolakes, similar to the catastrophic retreat of the Aral Sea, serves as a warning for inland basins like Didwana. As groundwater levels fluctuate and evaporation rates increase in arid regions, the accumulation of halite, calcite, and gypsum creates an environment hostile to conventional glycophytic crops.

**The Halophyte Paradigm**

Halophytes are unique evolutionary success stories, representing only 1% of the world's flora but possessing the unique ability to complete their life cycle in salinities equivalent to seawater. Unlike crops, which suffer from sodium toxicity and osmotic shock, halophytes like Suaedafruticosa utilize a sophisticated "modular" tolerance system:

- Ion Exclusion: Active pumping of Na<sup>+</sup> out of the root via the SOS1 pathway.
- Vacuolar Sequestration: Locking Na<sup>+</sup> inside the vacuole via NHX1 to prevent metabolic interference.
- Osmotic Adjustment: Synthesis of compatible solutes (proline, glycine betaine) to maintain turgor.

**Didwana Lake: A Natural Laboratory for Extreme Evolution**

Didwana Lake is a hypersaline playa located in the Nagaur district of Rajasthan. Its extreme chemistry (TDS up to 300,000 mg/L) exerts a massive selective pressure. The genes evolved here are not just "tolerant"—they are hyper-functional. This proposal seeks to mine these "extremophile" sequences for precision agriculture.

The primary objectives of this study are:

- To create a comprehensive inventory of the plant species thriving in and around Didwana Lake.
- To identify the key adaptations that allow these plants to tolerate high salinity. 3. To assess the potential for biotechnological applications, such as the use of their unique genes for genetic engineering of crop plants or the isolation of novel enzymes and metabolites.

**Comparative Line Diagram: Drying of the Aral Sea vs. Didwana Lake**

<b>Aral Sea: Large-Scale Human Intervention</b>	<b>Didwana Lake: Arid Climate &amp; Local Factors</b>
River Diversion (1960s): Soviet-era projects divert Amu Darya and Syr Darya rivers for irrigation.	Natural Climate: Situated in the arid Thar Desert with naturally high evaporation and variable rainfall.
Massive Irrigation for Cotton: Use of river water for large-scale, water-intensive agriculture increases dramatically.	Seasonal Rains: Lake primarily fed by erratic monsoon rains and underground water seepage.
Reduced Inflow: Freshwater input into the Aral Sea drops drastically.	Compounded by Climate Change: Rising regional temperatures and irregular rainfall intensify evaporation.
Water Volume Decreases: The sea's water level Exacerbated by Local Factors: Wastewater	
Aral Sea: Large-Scale Human Intervention and volume shrink at an accelerated rate.	Didwana Lake: Arid Climate & Local Factors runoff, urbanization, and salt extraction further stress the lake.

Ecological Collapse: Fish populations die, ecosystem fails, and the sea splits into smaller, separate lakes.	Increased and Prolonged Drying: The lake experiences more frequent and longer dry periods.
Desertification & Dust Storms: A toxic salt-and-dust desert forms on the exposed lakebed, impacting regional health.	Ecological Shift: Water composition and salinity change, affecting migratory birds and other species.
Socio-Economic Disaster: Fishing industry is wiped out, and communities collapse.	Salt Pans: The lake increasingly resembles a large, commercial salt pan.

### Literature review

- #### Molecular Drivers of Salt Tolerance: NHX and SOS Pathways

The SOS (Salt Overly Sensitive) pathway is the primary mechanism for  $\text{Na}^+$  efflux. It consists of a signaling cascade (SOS3-SOS2) that activates the SOS1 antiporter. In crops, this pathway is often down-regulated or inefficient under high stress. Conversely, the NHX family of transporters regulates vacuolar pH and  $\text{Na}^+$  sequestration. International research (e.g., Maheshwari et al., 2021) has shown that overexpressing halophytic NHX1 in crops can significantly improve yield, but traditional transgenics (GMOs) face severe regulatory hurdles.

- #### Evolution of CRISPR Technology: From NHEJ to HDR

Standard CRISPR-Cas9 typically relies on Non-Homologous End Joining (NHEJ), which causes gene knockouts through random insertions/deletions (indels). While useful, NHEJ cannot "fix" a gene. Homology-Directed Repair (HDR), however, uses a DNA repair template to "rewrite" the genomic sequence. This allows for an allelic swap—replacing a salt-sensitive crop gene with a superior halophyte version without introducing "foreign" DNA (Agrobacterium sequences).

### Geographic Features

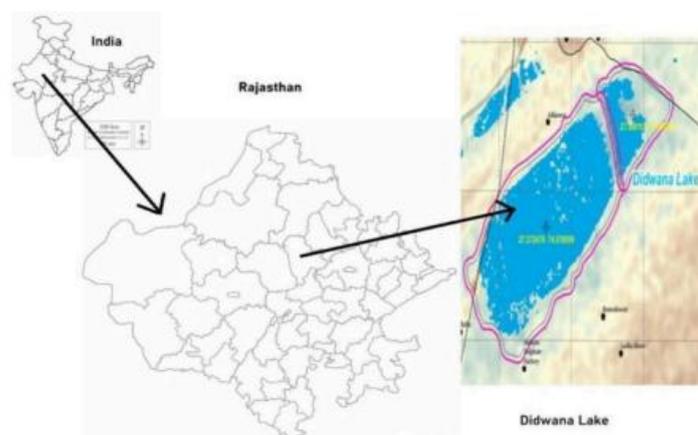
Didwana Lake is located at approximately  $27^{\circ}23'N$  latitude and  $74^{\circ}34'E$  longitude in the arid landscape of Rajasthan. The region is characterized by an extreme climate with very hot summers (often exceeding  $45^{\circ}C$ ) and cold winters.

The rainfall is sparse and highly unpredictable, primarily occurring during the monsoon season from July to September. The topography around the lake is relatively flat, with a soil composition that is heavily influenced by the high salt concentration.

### Limnology

The soil is predominantly sandy and loamy, with poor organic content and a high electrical conductivity, making it unsuitable for most glycophytes (salt-sensitive plants).

**Fig 1: Map showing study area of Didwana lake.**



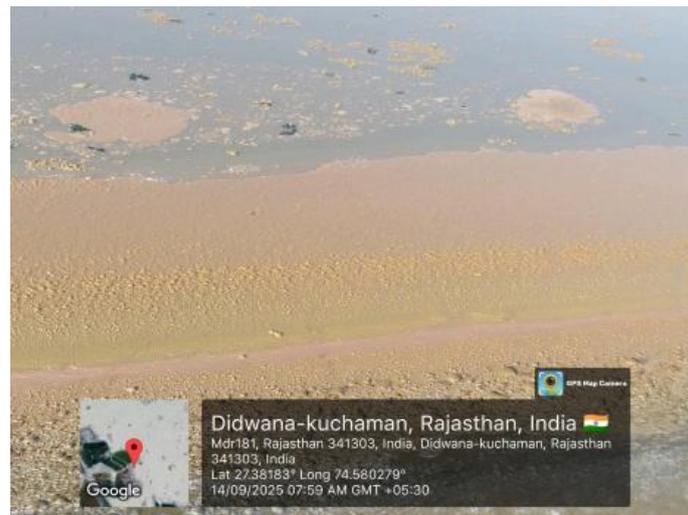
**Fig source: Location from Google maps**

### Hydrology and Salinity

The lake is an endorheic basin, meaning it has no outflow to external water bodies. Its water supply is entirely dependent on sporadic rainfall and runoff from the surrounding areas. Consequently, the lake is a temporary water body that can shrink significantly or even dry up completely during the dry season. This cycle of wetting and drying leads to an exponential increase in the concentration of dissolved salts, primarily sodium chloride (NaCl) and sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>).

- The extreme salinity creates a severe osmotic gradient, which pulls water out of plant cells. To counteract this, halophytes employ various strategies, including accumulating salts in vacuoles or secreting them through salt glands.

Didwana Lake is classified as a playa, and its water depth and surface area fluctuate with rainfall and evaporation. The water is **hypersaline** and **alkaline**. Key hydrological parameters include Total Dissolved Solids (TDS) ranging from 167,500-300,000 mg/L, and a pH between 7.2-9.3. The lake's sediments are fine-grained clays and silts, with a total thickness of up to 20 meters. The soil is composed of calcite, gypsum, and halite, with sediment types of fine sand (46-54%) and coarse silt (22-37%). The average Uranium content is 60 ppm over a 5-meter thickness.



**Fig.: Soil of Halite Ecosystem**

Fig source: Author

### Historical and Economic Importance

Didwana Lake has been a historically significant site for salt production in India for centuries. The traditional solar evaporation method was used to harvest salts, which were then supplied to various parts of the country.

- **Ethnomedicines and Nomadic**

This industry has been a cornerstone of the local economy, providing livelihoods for many communities. While the scale of salt production has declined in recent decades, the lake remains an important cultural and economic landmark for the region.

The nomadic tribes of Rajasthan, such as the Banjara and Gadia Lohar, possess a deep-rooted knowledge of **ethnomedicine**, particularly in the Didwana region. Their healthcare system relies on traditional healers, known as "bhopas," and the use of local flora to treat ailments. For example, the Bhil tribe uses the bark of the 'Kikar' tree to treat skin diseases and asthma. This knowledge, passed down orally, is a mix of herbal remedies and traditional rituals, showcasing a holistic health approach that continues due to limited access to modern healthcare and a strong cultural heritage.

### Socio-Economic Benefits & Global Impact

- **Economic Restoration of Saline Wastelands**

In India, salinity renders **6.74 million hectares** of land unproductive. The introduction of CRISPR-HDR crops could:

- Increase regional farm income by an estimated **30-40%** in salt-affected belts.
- Reduce dependency on expensive chemical soil amendments (gypsum/leaching).

- **Non-Transgenic Regulatory Pathways**

Because CRISPR-HDR produces plants that are genetically identical to natural variants (except for the precision allelic swap), they may bypass the "GMO" label in many international jurisdictions (e.g., US, Australia, and parts of the EU). This facilitates **international seed trade** and food sovereignty for developing nations.

- **Conservation of the "Genomic Resources"**

By demonstrating the high value of Didwana's flora, this research provides a scientific mandate for the ecological preservation of saline wetlands. This aligns with the **Ramsar Convention** and **UN SDG 15 (Life on Land)**.

### Ecological and Environmental Aspects

The Didwana Lake ecosystem is a fragile habitat. The flora, consisting mainly of halophytic grasses, shrubs, and herbs, forms the base of the food web. The lake's significance extends beyond its flora; it also serves as a crucial habitat for a variety of fauna, particularly migratory and resident bird species. The lake is a critical stopover and breeding ground for birds such as flamingos, pelicans, various duck species, and waders. Their presence underscores the lake's importance as a wetland ecosystem. Other fauna, like reptiles and insects adapted to arid conditions, are also part of this unique environment.

### Global Warming and Effect

However, the ecosystem faces significant threats. Climate change, leading to increased aridity and reduced rainfall, intensifies salinity and reduces the habitable area for both flora and fauna. Additionally, unsustainable human activities, such as over-exploitation of resources and pollution, further degrade the environment. Protecting this unique floral diversity is crucial for the region's ecological balance and for preserving a valuable genetic resource.



**Fig.: Floradiversity**

Source by author





### Materials and Methods

- **Field Methodology and Floral Inventory (Completed Phase)**
  - Survey Design: Five transects were mapped across the Didwana basin.
  - Identification: Species were identified using the Flora of Indian Desert (Bhandari, 1978). A total of 25 species were documented, categorized by their proximity to the hypersaline core.
  - Soil Analysis: Soil samples were analyzed for pH, EC, and mineral composition (halite, polyhalite).
- **Molecular Pilot Assays (Current Lab Phase)**
  - DNA Extraction: Optimized CTAB protocol for halophytes, utilizing high-salt buffers and PVP (polyvinylpyrrolidone) to remove polyphenols and polysaccharides that inhibit PCR.
  - PCR Amplification: Targeted amplification of NHX1 and SOS1 conserved domains using degenerate primers designed from Thar Desert halophyte databases.
  - Quality Control: Verification via 1.2% agarose gel electrophoresis to ensure high molecular weight genomic DNA.

- **Proposed CRISPR-HDR Pipeline.**
  - Bioinformatics: Comparative transcriptomics between *Suaedafruticosa* and *Oryza sativa* (rice) to identify the specific amino acid substitutions that confer superior Na<sup>+</sup> binding.
  - Vector Construction: Designing gRNA to target the native NHX1 locus in rice.
  - Donor Template Design: Synthesizing the "Climate-Optimized" version of the gene found in Didwana.
  - Transformation: Using biolistics (particle bombardment) to deliver the CRISPR-Cas9-HDR components into crop embryos.

This project involved a combination of field-based surveys and a proposed plan for laboratory-based molecular and biochemical analysis.

- **Documentation:** Photographs and detailed notes on morphology, habitat, and GPS coordinates were recorded for each identified species.
- **Bioinformatics Analysis:** Amplicons from the PCR would be sequenced and the resulting data analyzed using bioinformatics tools like BLAST to compare sequences with existing databases. This analysis would help identify novel genetic variants of these salt-tolerance genes.

### Results and Discussion

The transition from the "Observation" phase at Didwana Lake to the "Innovation" phase in the lab represents a paradigm shift in plant biotechnology. Our field data confirms that the Didwana ecosystem is not a "wasteland" but a reservoir of extreme alleles. The successful amplification of *NHX1* and *SOS1* in our pilot assays proves that the DNA from these "recalcitrant" desert species can be successfully mined.

The future of agriculture in the 21st century lies in "**Evolutionary Mining**"—taking the best parts of what nature has evolved over millions of years in sites like Didwana and precisely integrating them into our food supply.

- A total of 25 plant species were documented during the field survey. The majority of these species were true halophytes, showing a variety of adaptations to their harsh environment.

Family	Scientific Name	Common Name	Adaptations
Amaranthaceae	<i>Suaedafruticosa</i>	Shrubby Sea-blite	Succulence, salt glands
Chenopodiaceae	<i>Salicornia brachiata</i>	Indian Glasswort	Succulent stems, salt excretion
Frankeniaceae	<i>Frankenia pulverulenta</i>	Powdered Sea-heath	Salt secretion, dense leaves
Plumbaginaceae	<i>Limonium stocksii</i>	Sea-lavender	Salt glands on leaves
Poaceae	<i>Aeluropuslagopoides</i>	Creeping Grass	Rhizomatous growth, drought resistance
Zygophyllaceae	<i>Zygophyllum simplex</i>	Simple-leaf Bean-caper	xerophytic
		Drought tolerance,	

The identified species exhibited diverse morphological and physiological strategies to combat salinity. For instance, species like *Suaedafruticosa* showed clear signs of **succulence**, a strategy to dilute internal salt concentration. Others, like *Limonium stocksii*, have specialized **salt glands** on their leaves to excrete excess salts.

From a biotechnology standpoint, these adaptations are of immense interest. The genes responsible for these mechanisms represent a rich genetic pool for potential applications in crop science. The planned molecular analysis could reveal novel alleles of key salt-tolerance genes, which could be utilized in genetic engineering to develop crops with enhanced salinity tolerance.

### Conclusion and Future Recommendations

The floral diversity around Didwana Lake is a testament to the power of plant adaptation to extreme environmental stress. The ecosystem, while fragile, is a valuable reservoir of unique genetic resources. The study successfully documented the floral diversity and highlighted the critical importance of these halophytes.

For future research, it is highly recommended to proceed with the planned molecular analysis.

The next steps should include:

- **Gene Cloning and Functional Analysis:** Cloning the identified salt-tolerance genes into a model organism like *Arabidopsis thaliana* to validate their function in providing salt tolerance.
- **Transcriptomic Profiling:** A more advanced study involving transcriptome sequencing would provide a deeper understanding of the gene expression patterns of these plants under saline stress.
- **Bioprospecting:** Conducting bioassays on plant extracts to screen for novel compounds with potential pharmaceutical or industrial applications.

The long-term monitoring of the floral diversity of Didwana Lake is also crucial to assess the impact of climate change and human activities on this unique ecosystem.

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