

Mustard and salinity tolerance:

A resilient oilseed for the future

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*Salinity is one of the major abiotic stresses limiting crop production and productivity, particularly in the arid and semi-arid regions of India. Indian mustard (*Brassica juncea*), a leading oilseed crop valued for its culinary and medicinal properties, is highly susceptible to salt stress, which causes significant yield losses. The adverse effects include reduced seed germination, stunted plant growth, decreased seed yield, and a decline in oil content. Exploring the genetic variation within mustard germplasm offers opportunities for developing salt-tolerant cultivars. A comprehensive understanding of morphological, physiological, and molecular mechanisms underlying salt tolerance is crucial for this purpose. Strategies such as exploiting existing germplasm, adopting improved breeding techniques, implementing effective agronomic practices, and applying advanced technologies like genome editing hold great potential for enhancing salt tolerance in mustard. Integration of conventional breeding with modern genomic tools and genome-editing approaches will facilitate the identification and functional validation of key candidate genes and regulatory networks, thereby accelerating the development of resilient mustard cultivars for saline environments.*

Keywords: Crop improvement, Genome editing, Indian mustard, Salinity stress, Salt tolerance mechanism

INDIAN mustard (*Brassica juncea*) is one of the major oilseed crops of India, valued for its high demand in edible oil production. It offers significant nutritional and economic benefits and remains a preferred cooking oil in eastern India, including the states of Bihar, West Bengal, Odisha, Jharkhand, as well as Rajasthan, Punjab, and Madhya Pradesh. Edible oils derived from Indian mustard contribute approximately 27.8% to the country's total oilseed economy. Despite its versatile applications and substantial contribution to India's agricultural economy, the nation continues to rely on the import of edible oils. This dependence is primarily attributed to the low productivity of mustard crops, which suffer significant yield losses due to abiotic stresses driven by changing climatic conditions and deteriorating soil health. Among these challenges, soil salinity poses a serious constraint, particularly in regions with low annual rainfall, poor-quality irrigation, and suboptimal land management practices. Salinity adversely affects mustard by impairing seed germination, vegetative growth, photosynthetic efficiency, lipid accumulation,

ultimately reducing seed yield and oil quality. To address this issue, salt-tolerant varieties such as CS60 and CS58 have been developed by ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal. These genotypes have demonstrated superior seed and oil yields compared to other widely cultivated varieties like CS54, Kranti, and Giriraj under saline conditions.

Given the escalating problem of soil salinization, exacerbated by climate change, unsustainable irrigation practices, and excessive fertilizer application, it is imperative to investigate the physiological, biochemical, and molecular mechanisms underlying salinity tolerance in mustard. Such insights will be pivotal for developing improved, climate-resilient cultivars capable of sustaining productivity in salt-affected agro-ecosystems.

Impact of salinity on mustard cultivation

Soil salinity is a major constraint affecting the productivity of Indian mustard crops, particularly in poorly irrigated, arid, and semi-arid regions. It results

from the excessive accumulation of soluble salts, primarily sodium chloride (NaCl), in the soil. Saline soils are characterized by elevated concentrations of various salts, including sodium, calcium, magnesium, potassium, carbonates, bicarbonates, chlorides, sulphates, borates, and lithium compounds.

The excessive build-up of these salts disrupts plant water uptake, leading to reduced growth, wilting, and, ultimately, plant mortality. High salt concentrations impair seed germination and hinder seedling establishment, resulting in poor field emergence and reduced plant populations. Additionally, salinity disturbs the nutrient balance within plants. The accumulation of sodium (Na⁺) and chloride (Cl⁻) ions interferes with the uptake of essential nutrients such as potassium (K⁺) and calcium (Ca²⁺), causing nutrient imbalances and subsequent physiological disorders. Salinity also raises osmotic potential of the soil, inducing osmotic stress that limits water availability to plants. This stress promotes the overproduction of reactive oxygen species (ROS), leading to oxidative damage to cellular structures and metabolic dysfunctions. Moreover, salinity negatively affects photosynthetic efficiency by reducing chlorophyll content and impairing enzyme activities associated with photosynthesis, thereby severely limiting plant growth and yield.

Oil quality in mustard is also compromised under saline conditions, as salt stress alters the fatty acid composition of the oil, diminishing both its commercial value and nutritional properties. Interestingly, *B. juncea* has demonstrated potential for phytoremediation of metal-contaminated saline soils, garnering attention as a candidate for sustainable agriculture and soil restoration strategies. Given these challenges, it is crucial to develop salt-tolerant mustard genotypes and systematically explore existing germplasm resources to identify and harness naturally occurring salinity tolerance traits for breeding programmes and crop improvement initiatives.

Mustard's natural salinity tolerance mechanisms

Mustard exhibits low to moderate salt tolerance, attributed to several inherent adaptive mechanisms. Natural genetic variation for salinity tolerance exists within the extensive germplasm collections of mustard, offering valuable resources for crop improvement. It is essential to identify and select elite germplasm with superior adaptability to varying climatic and saline conditions through conventional breeding approaches. In this context, ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal has reported several mustard genotypes exhibiting moderate to high salinity tolerance with minimal yield compromise under salt-affected environments. Several physiological, biochemical, and molecular traits contribute to the ability of mustard plants to withstand soil salinity:

- **Deep root system:** Certain mustard germplasm possesses deeper root architectures, enabling plants to access water from subsoil layers while avoiding the detrimental effects of surface salt accumulation.
- **Osmotic adjustment:** To mitigate osmotic stress,

mustard plants accumulate osmoprotectants such as proline, glycine betaine, and polyamines. These compatible solutes help maintain cellular osmotic balance and protect cellular structures under saline conditions.

- **Antioxidative defense system:** Salinity-induced oxidative stress is alleviated through the enhanced activity of antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD). Additionally, the introduction and expression of the *codA* gene encoding choline oxidase from *Arthrobacter globiformis* in *B. rapa* spp. have been shown to improve photosynthetic efficiency under high salinity stress by enhancing oxidative stress tolerance.
- **Ion homeostasis and transport regulation:** Mustard plants maintain ionic balance by regulating ion transport and minimizing toxic sodium (Na⁺) accumulation. This is achieved through the action of ion transporters and antiporters such as SOS1, SOS2, SOS3, ENH, and NHX proteins, which play pivotal roles in maintaining intracellular ion homeostasis and nutrient balance under saline conditions.

Harnessing these adaptive traits through systematic germplasm evaluation and targeted breeding strategies holds promise for developing high-yielding, salt-tolerant mustard cultivars suitable for saline and marginal agro-ecosystems.



Variation in the root length in mustard germplasm

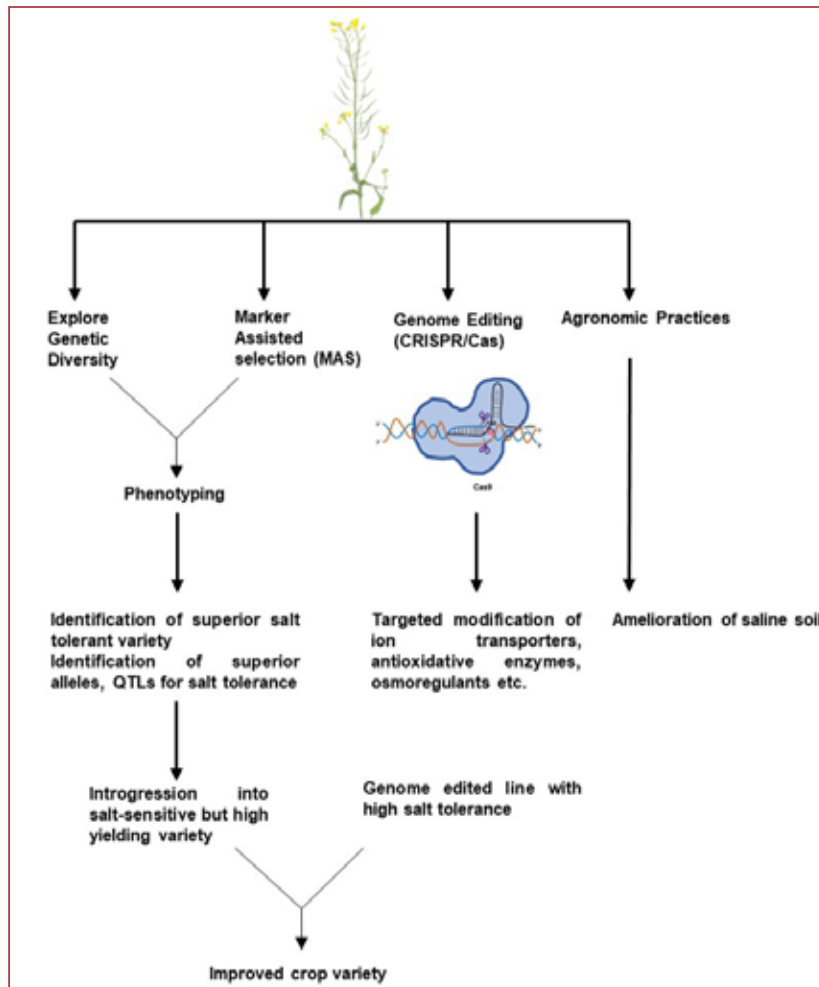
Strategies for enhancing salinity tolerance in mustard

To reduce the nation's dependence on edible oil imports and safeguard the national economy, it is essential to achieve self-sufficiency in oilseed production, particularly mustard. In light of increasing soil salinization and unpredictable climatic conditions, the development of salt-tolerant, high-yielding mustard varieties has become a priority. Several strategies can be employed to enhance salinity tolerance in mustard crops:

- **Conventional breeding:** Significant genetic variability for salinity tolerance exists within local landraces, wild relatives, and traditional mustard cultivars. Systematic identification and selection of elite germplasm under saline and adverse climatic conditions can lead to the discovery of naturally tolerant lines. These tolerant genotypes can be characterized and evaluated in salt-affected soils to assess their adaptability and agronomic performance. Subsequently, selected tolerant lines can be hybridized with high-yielding but salt-sensitive cultivars to develop superior varieties with enhanced salinity tolerance through conventional breeding approaches.
- **Marker-assisted selection (MAS):** The existing genetic variability in mustard can be efficiently exploited using molecular markers such as RFLP,

AFLP, SSR, and SNP, which reveal polymorphism and are closely associated with genes or quantitative trait loci (QTLs) governing salinity tolerance. Marker-assisted selection allows for the precise identification of plants carrying desirable alleles, bypassing the need for time-consuming and labour-intensive phenotypic screening under saline conditions. Furthermore, marker-assisted backcross breeding (MABC) can be applied to introgress identified salt tolerance QTLs into high-yielding, salt-sensitive genetic backgrounds, thereby combining salinity resilience with desirable agronomic traits.

- **Genome editing using CRISPR/Cas technology:** Genome editing offers a precise and efficient approach to enhance stress tolerance traits in mustard. The CRISPR/Cas9 system enables targeted modification of genes by utilizing a 20 bp single-guide RNA (sgRNA) specific to the gene of interest. The Cas9 nuclease introduces a double-strand break near the Protospacer Adjacent Motif (PAM) sequence, and during the DNA repair process, insertions, deletions, or targeted modifications are introduced. In mustard, CRISPR/Cas9 technology can be utilized to target key genes involved in ion transport (e.g. SOS1, NHX), osmoregulation (e.g. proline and glycine betaine biosynthesis pathways), and antioxidative defense mechanisms, thereby improving plant's resilience to salinity stress.



Strategies to develop salt tolerance mustard variety

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- **Agronomic practices and nanotechnology:** In addition to genetic improvement, integrated agronomic strategies can play a critical role in managing salinity stress. Intercropping mustard with salt-tolerant cover crops can reduce surface soil salinity and improve soil structure. Application of soil amendments such as gypsum, organic matter, and biochar helps neutralize excessive salts, enhancing soil fertility and water-holding capacity. Adoption of efficient irrigation methods, such as drip or alternate furrow irrigation, minimizes salt accumulation near the root zone. Utilization of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) enhances nutrient uptake, root growth, and stress tolerance under saline conditions. Recent advancements in nanotechnology have introduced the application of nano-encapsulated nutrients and engineered nanoparticles, which improve nutrient absorption, physiological efficiency, and plant growth under saline environments.

SUMMARY

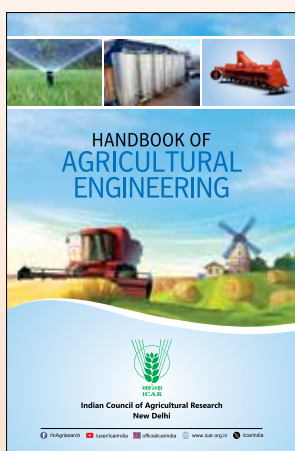
Indian mustard is a moderately resilient crop that requires relatively less irrigation and can tolerate moderate levels of soil salinity, making it a promising crop for cultivation in salt-affected areas. However, to fully harness its potential, extensive reservoir of mustard germplasm must be systematically explored to identify genotypes with superior resilience and adaptability to saline soils, without compromising seed yield or oil quality. There are relatively few reports available on the identification of novel genes and quantitative trait loci (QTLs) associated with salinity tolerance in mustard. Limited genetic and molecular information constrains the development of salt-tolerant cultivars through molecular breeding and biotechnological approaches. Therefore, there is a pressing need for comprehensive studies aimed at identifying and characterizing salt-responsive genes, QTLs, and regulatory networks in mustard. This will not only advance our understanding

of the genetic basis of salinity tolerance but also facilitate the development of molecular markers and gene targets for breeding programmes focused on improving salinity resilience in mustard.

To achieve this, it is crucial to integrate conventional breeding approaches with modern biotechnological tools and sustainable agronomic practices. Advanced technologies such as genome editing, in combination with omics-based approaches including genomics, transcriptomics, proteomics, and metabolomics hold immense potential to unravel the complex mechanisms underlying salinity tolerance. The integration of these cutting-edge tools can accelerate the development of high-yielding, salt-tolerant mustard cultivars, thereby revolutionizing mustard cultivation in salt-affected agro-ecosystems and contributing to the nation's edible oil security.

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