



ADVANCES IN MOLECULAR APPROACHES FOR LENTIL (*LENS CULINARIS* MEDIK.) SALINITY TOLERANCE BREEDING UNDER A CHANGING CLIMATE



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INTRODUCTION

Lens culinaris is an important grain legume valued for its high nutritional content, including protein, dietary fibre, iron, and zinc, and for its role in sustainable agriculture through biological nitrogen fixation. It is widely cultivated in South Asia, the CWANA region, and Sub-Saharan Africa, with global production exceeding 6.5 million tons in 2025. However, soil salinity is a major abiotic stress limiting lentil productivity, causing severe yield losses even at moderate salt concentrations. Salt stress disrupts water uptake, ion balance, and metabolic processes, leading to

chlorosis, membrane damage, and premature senescence. Studies on tolerant genotypes such as PDL-1 and ILWL-137 have shown that tolerance is associated with restricted Na^+ and Cl^- uptake and maintenance of a high K^+/Na^+ ratio (Figure 1). Although these mechanisms are well understood, no commercially released salt-tolerant lentil cultivar has yet been developed through molecular breeding. Advances in marker-assisted selection, QTL mapping, transcriptomics, and genome-editing technologies offer promising opportunities for developing salt-tolerant lentil cultivars and improving productivity under saline conditions.

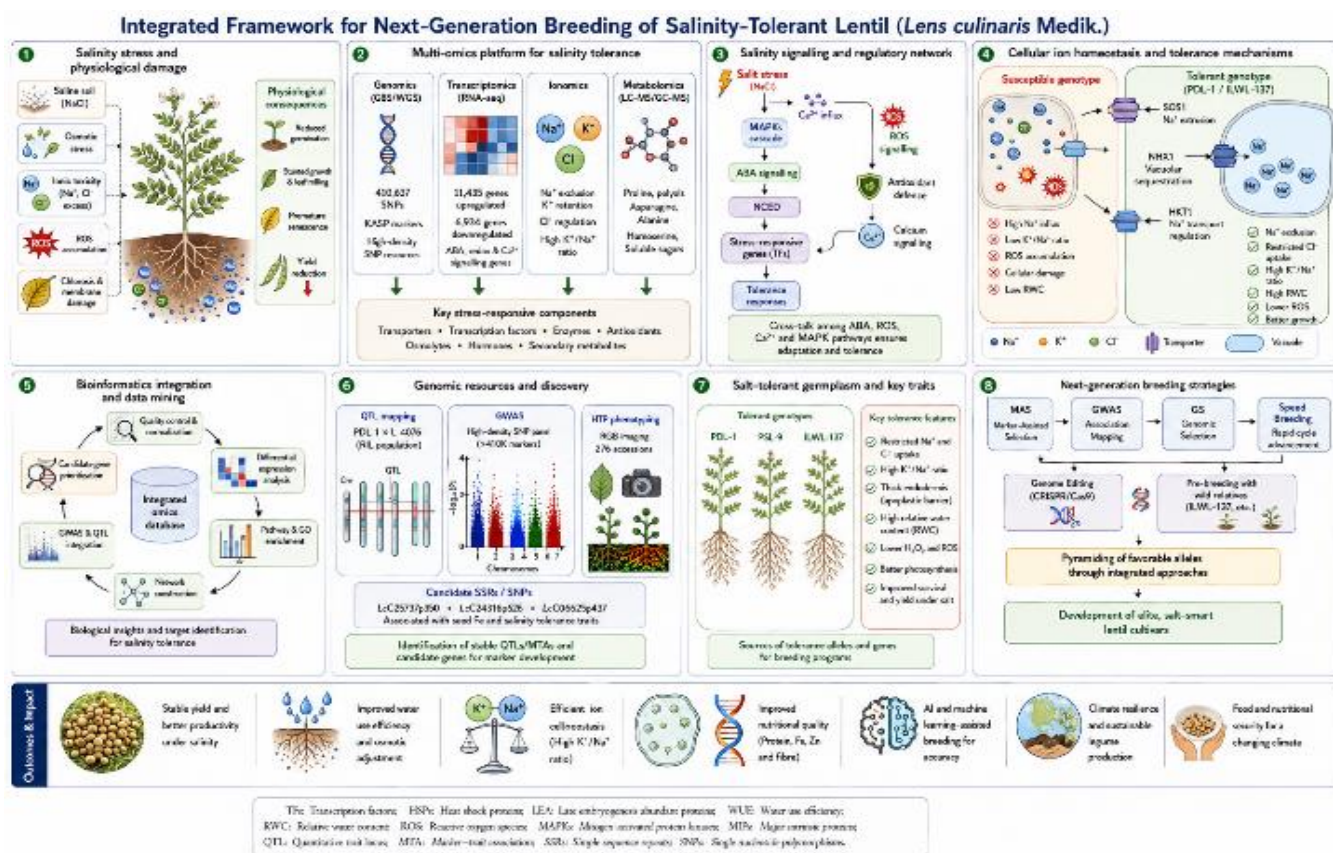


Figure 1. Framework of salt tolerant lentil breeding.

MOLECULAR MARKERS FOR SALINITY TOLERANCE

From RAPDs to High-Density SNPs

The molecular dissection of lentil began with first-generation hybridisation-based markers RFLPs, RAPDs, AFLPs, and ISSRs that revealed population structure and gene-pool boundaries across *Lens* species. These were limited in throughput and transferability. The advent of SSR markers improved reproducibility; a first genome library from genotype ILL5588 yielded 30 polymorphic SSRs later expanded to 122 genomic SSRs useful in breeding programmes.

Next-generation sequencing (NGS) transformed the landscape from 2010 onward. A landmark RNA-seq study of six lentil genotypes generated 138,106 unigene assemblies, 3,470 SNPs, and EST-SSR markers. GBS of 188 lentil genotypes subsequently yielded 410,637 SNPs, while a whole-transcriptome de novo assembly identified 26,449 EST-SSRs and 130,073 SNPs, of which 78 were converted into KASP markers deployable in marker-assisted selection (MAS).

Salt-Specific Marker-Trait Associations

A QTL associated with seedling survival under saline conditions by crossing salt-sensitive genotypes (L-4076, L-4147) with salt-tolerant lines (PDL-1, PSL-9). RGB imaging to phenotype 276 accessions, identifying tolerant genotypes based on projected shoot area, leaf colour, and compactness (Spearman $r=0.68$; $p<0.0001$).

Candidate SSR markers LcC25737p350, LcC24316p626 and LcC06625p437 showed strong associations with seed Fe content in saline environments, linking ionomics data to marker-trait networks.

QTL Mapping and GWAS for Salinity Tolerance

Bi-parental recombinant inbred line (RIL) populations have been the primary vehicle for QTL detection in lentil. The foundational QTL discovery for salinity, with markers flanking the identified locus now candidates for pyramiding into elite backgrounds. Eighteen QTLs associated with root and shoot traits under drought stress were elucidated in RIL populations from ILL6002 × ILL5588, establishing a template for multi-trait abiotic stress QTL mapping.

GWAS, enabled by high-density SNP panels, exploits historical recombination across diverse germplasm. A meta-GWAS on 292 lentil accessions identified 125 SNP markers associated with herbicide tolerance, demonstrating the scalability of the approach. For salinity, the large ionic component of stress means GWAS panels must capture both osmotic and ionic responses an integrated phenotyping challenge now being addressed by high-throughput phenotyping (HTP) platforms.

Transcriptomic Dissection of Salt Stress Response

Transcriptomics offers a window into the dynamic molecular choreography of salt stress. A

Table 1. Molecular approaches, key findings, and breeding applications for lentil salinity tolerance.

Approach	Key Finding/ Resource	Tolerance Mechanism	Breeding Application
QTL Mapping (RIL)	QTL for seedling survival under saline conditions (PDL-1 × L-4076)	Na ⁺ /Cl ⁻ exclusion, osmotic adjustment	MAS for seedling salt tolerance
GBS / SNP Panel	410,637 SNPs from 188 genotypes; population structure resolved	Genome-wide diversity capture	GWAS; genomic selection models
RNA-seq Transcriptomics	11,435 up- / 6,934 down-regulated transcripts under salt; ABA, redox, N-management pathways	Osmotic and ionic stress signalling	Gene discovery; candidate gene MAS
Image-Based HTP	276 accessions phenotyped for salt tolerance ($r=0.68$ vs. conventional)	Shoot architecture; leaf ion status	High-throughput phenotypic screening
Ionomics	Tolerant PDL-1: restricted Na ⁺ uptake; higher K ⁺ /Na ⁺ ratio; lower H ₂ O ₂	Ion homeostasis; membrane integrity	Ionomic biomarker panels
Metabolomics	Reduced organic acids; increased sugars, polyols, L-asparagine under salinity; purine catabolism active	Osmotic adjustment; C/N balance	Metabolite biomarkers for early screening
CRISPR/Cas9 (proposed)	SOS1, NHX1, HKT1, NCED identified as prime editing targets	Ion exclusion; ABA signalling; vacuolar sequestration	Precise gene editing for salt tolerance
Genomic Selection (ML)	Multi-locus SNP-based prediction of complex tolerance phenotypes	Polygenic tolerance architecture	Accelerated breeding cycles for quantitative tolerance

landmark comparative transcriptome study on salt-stressed lentils, identifying 11,435 upregulated and 6,934 downregulated transcripts and mapping 413 Gene Ontology (GO) terms across molecular, cellular, and biological processes. Under salt-specific conditions, functional annotation highlighted key roles for differentially expressed genes (DEGs) in phytohormone signalling (notably ABA), redox homeostasis, secondary metabolism, nitrogen management, and stress-response pathways.

Transcriptomic responses to combined salt and alkalinity stress, identifying ABA-responsive proteins, dehydrin 1, and 9-cis-epoxycarotenoid dioxygenase (NCED) the rate-limiting enzyme in ABA biosynthesis as central regulators. These findings open pathways for gene editing strategies targeting ABA biosynthesis and signalling components. Critically, resilient genotypes showed upregulation of genes linked to organic acid synthesis, antioxidant production, and calcium signalling cascades. The downregulation of circadian rhythm genes under long-term stress reveals an overlooked dimension: circadian disruption may impair fitness across the growing season. The SOS (Salt Overly Sensitive) pathway a Na^+ extrusion system conserved across legumes represents a prime candidate for both expression studies and CRISPR-based enhancement.

METABOLOMICS AND IONOMICS: THE BIOCHEMICAL FINGERPRINT OF TOLERANCE

Key Metabolite Signatures Under Salinity

Metabolomics captures the terminal output of gene–environment interactions and has identified several biomarkers relevant to lentil salt tolerance. Under salinity, lentil plants exhibit a characteristic metabolic shift: reduction of organic acids in leaves and roots, accumulation of sugars and polyols in leaves, and elevated L-asparagine in roots. Legumes engage the purine catabolism pathway to manage carbon and nitrogen limitation under salt stress a metabolic route that could be a target for enhancement. Identified alanine and homo serine as key biomarker metabolites for salt stress tolerance in contrasting lentil accessions, while ornithine and asparagine emerged as drought tolerance markers. Such metabolite signatures open routes to early-generation screening faster and cheaper than field evaluation.

Ionic Basis of Ion Exclusion

Ionomics — the high-throughput profiling of elemental tissue composition -has illuminated mechanisms of ion exclusion in tolerant lentil genotypes. 162 genotypes found that the tolerant line PDL-1 and wild accession ILWL-137 restricted Na^+ and Cl^- uptake by maintaining thicker endodermal and epidermal cell layers. Tolerant cultivars also showed lower H_2O_2 generation and higher soluble sugar, glycine betaine, chlorophyll, and relative water content (RWC). Confirmed that resistant genotypes use a combination of altered photosynthetic traits, decreased sodium uptake, and increased proline accumulation. Combining ionic data with genomics promises to identify gene networks governing ion transport.

EMERGING TECHNOLOGIES: CRISPR, GENOMIC SELECTION, AND AI

CRISPR/Cas9 — Precision Gene Editing

CRISPR/Cas9 enables precise, heritable edits without the regulatory burden of transgenic approaches in many jurisdictions. In lentil, the technology offers the prospect of directly editing Na^+ transporter genes (LcHKT1, LcSOS1), ABA pathway genes (LcNCED), or vacuolar antiporter genes (LcNHX) to engineer superior salt exclusion, ABA-mediated stomatal control, and Na^+ compartmentalisation. The 2021 publication of near-complete genome assemblies for CDC Redberry and wild *L. ervoides* provides essential reference scaffolds for guide RNA design.

Challenges remain in lentil transformation efficiency and regeneration protocols. However, rapid generation advancement technology which has already compressed the RIL development cycle can be coupled with editing to accelerate integration of salt-tolerance edits into elite backgrounds. Priority in planta targets include: (i) overexpression of vacuolar NHX antiporters to compartmentalise Na^+ ; (ii) loss-of-function editing of high-affinity K^+ transporter (HKT) genes to reduce Na^+ loading into shoots; and (iii) promoter engineering to enhance SOS1-mediated Na^+ extrusion at the root epidermis.

Genomic Selection and Machine Learning

Genomic selection (GS) exploits genome-wide marker data to predict breeding values for complex, quantitative traits without requiring QTL identification. For salinity tolerance governed by multiple small-effect loci distributed across the genome GS promises substantially higher

prediction accuracy than MAS based on a handful of markers. Machine learning algorithms (random forests, gradient boosting, neural networks) applied to multi-omics datasets can integrate SNP data, transcriptomic profiles, metabolite data, and environmental variables to predict salt tolerance phenotypes across diverse environments.

KEY GAPS AND A RESEARCH ROADMAP

Despite the advances reviewed above, critical gaps persist. Lentil proteomics under salt stress is virtually absent. Epigenomics and single-cell omics have not been attempted. Integration of multi-omics layers into predictive systems biology models remains nascent

genes are milestones that define the current state of the art.

Yet the field stands at an inflection point. Proteomics under salinity is essentially absent. Epigenomics has not been attempted. CRISPR editing of salt tolerance genes in lentil has not been demonstrated. Genomic selection models trained on salt phenotyping data do not exist (Figure 1). Bridging these gaps through integrated, multi-omics systems biology approaches coupled with next-generation phenotyping platforms and AI-assisted breeding is the defining challenge and opportunity for lentil science in the coming decade.

The urgency is not abstract. Soil salinity is expanding. Global demand for plant protein is

Table 2. Current gaps and recommended research priorities for lentil salinity tolerance breeding.

Omics Layer	Current Status	Key Gap	Recommended Action	Timeline
Genomics / QTL	One key QTL identified; GBS panels available	Few validated salt-specific QTLs; limited GWAS	Multi-environment GWAS with >500 accessions	Near-term
Transcriptomics	Salt-specific data growing; drought/heat better covered	No single-cell resolution; limited time-course	Single-cell RNA-seq of root cell types during salt exposure	Medium-term
Proteomics	Very limited; no salt-stress proteome published	No quantitative proteome under salinity	iTRAQ/TMT quantitative proteomics of tolerant vs. susceptible lines	Near-term
Metabolomics	Key biomarkers identified (alanine, asparagine)	No mQTL mapping for salt metabolome	mQTL mapping integrating metabolite profiles with SNP data	Medium-term
Ionomics	K ⁺ /Na ⁺ ratios characterised; key accessions known	No genome-wide ionic QTL mapping	iQTL mapping for Na ⁺ , K ⁺ , Cl ⁻ across RIL populations	Near-term
Epigenomics	Not studied in lentil under any stress	Entire layer unexplored	ATAC-seq and bisulphite sequencing under chronic salinity	Long-term
CRISPR/Editing	Proposed; not demonstrated for salt in lentil	Transformation efficiency limiting	Optimise Agrobacterium transformation; edit SOS1, NHX1	Medium-term
Genomic Selection	Models not yet trained for salinity	No multi-environment GS model exists	Train GS models using multi-location salt-phenotyped trials + SNP data	Near-term
Systems Integration	Very few multi-omics lentil studies	No regulatory network model under salinity	Build integrated gene regulatory networks from combined omics datasets	Long-term

CONCLUSION

The molecular toolkit available for lentil salinity tolerance breeding has undergone a transformation in the past decade. A near-complete reference genome, dense SNP panels, transcriptomic databases, ionic characterisation of tolerant accessions, and key metabolomics biomarkers collectively constitute a formidable foundation. The identification of a QTL for seedling survival under salinity, the characterisation of ion exclusion mechanisms in PDL-1 and ILWL-137, and the transcriptomic mapping of ABA signalling and redox homeostasis

rising. Delivering salt-smart lentil cultivars to smallholders in South Asia and the CWANA region will require the full deployment of every molecular tool reviewed here - not sequentially, but in concert.

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