

Phenomics-Based Evaluation of Salinity Tolerance in Barley (*Hordeum vulgare* L.): A Systematic Review of Platforms, Traits, and Genetic Insights

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ABSTRACT

More than 1.38 billion hectares of soils across the globe is salinized, representing approximately one-tenth of the total irrigated and rainfed agriculture land, which is one of the greatest limitations for cereal growing in arid, semiarid, and coastal agroecosystems. Barley (*Hordeum vulgare* L.) is one of the most salinity-resistant cereals and, thus, is an important crop and is also widely-used as a genetic model of salinity resistance studies in cereals. In the last ten years, the rapid development of the emerging high-throughput phenotyping allows for the evaluation of salinity responses at a new level of speed and resolution, leaving behind the conventional methods of destructive endpoint isolation in favor of continuous studies of whole plants and plant organs. In this review, the authors summarize all the peer-reviewed articles dated from 2015 to 2025 and relevant to phenotyping of salinity resistance in barley found in Scopus, Web of Science, PubMed, and some institutional/international grey literature (FAO, ICBA, and CGIAR). After conducting the PRISMA-style screening process, fifty-six studies were found to meet the inclusion criteria. This review has been arranged in themes including: imaging and growth kinetics of shoot; root system imaging; physiological phenotyping and economics; genetic dissection using QTL mapping and GWAS; and the new technological integration of machine learning with multi-sensor construction and multi-omics data. The studies presented in this review have shown that the biomass from the imaging analysis can correlate with that obtained through destructive harvest while sodium exclusion and tissue tolerance turned out to be the most important physiological mechanisms in the studies presented. The review also displays the importance of some sodium-transporting loci in the mapping studies. However, the majority of studies mainly focus on laboratory environment studies or seedling stage experiments. No new results have been obtained in terms of root imaging studies in saline conditions, experiments at a reproductive stage, standardization of cross-platform data, or application of machine learning models to real practices. Variations in osmotic and ionic stress contributions and the degree of heritability for image-based characteristics continue to create confusion. The paper indicates that phenomics has significantly advanced barley salt research; however, for phenomics to reach its full potential, all methodological inconsistencies must be cleared up along with phenotyping at later growth stages carried out, and the applicability of phenomics, genomics, and environment information in collaboration with each other should be implemented in a manner that implies their compatibility. The authors offer useful instructions for experts, crop developers, and decision-makers who want to develop salt-resistant crops within the incomplete structure of phenomics.

Keywords: Barley; *Hordeum vulgare*; salinity tolerance; high-throughput phenotyping; phenomics; GWAS; ion homeostasis; abiotic stress

1. Introduction

Soil salinity is one of the common types of land degradation that damage world agriculture. As per the first major report by the Food and Agriculture Organization on salt-affected soils in the last fifty years, it has been determined that soil salinity affects almost 1.4 billion hectares of land, which is more than 10% of the total area of the globe, while an additional one billion hectares of land will be facing salinity problems because of climate change and irresponsible land utilization management practices ^[1, 2]. About 10% of irrigated farmland and a similar share of rainfed farmland is already damaged by salinity; ten countries, namely Afghanistan, Australia, Argentina, China, Kazakhstan, the Russian Federation, the USA, Iran, Sudan, and Uzbekistan, control approximately 70% of salt-covered soils in the world ^[2]. According to predictions concerning aridity increase, it is expected that the affected area will increase to 24-32% of the land area if climate change processes continue ^[2].

Within the context of this discussion, barley assumes a special status. It is the fourth most frequent cereal crop cultivated globally. Barley is widely used for animal feed, malting, brewing as well as direct human consumption in many marginal regions. However, barley is more tolerant to salinity in comparison to several temperate cereals including wheat and rice. This is due to better shoot Na⁺ exclusion, stronger K⁺ retention and better ability to conduct photosynthesis in the presence of greater amounts

of Na⁺ in the tissues. This salt tolerance of barley allows us to consider it as an important crop plant in saline and marginal cropping systems and an important model organism for genetic studies related to salinity tolerance in cereals.

In the past, salinity resistance in barley germplasm was evaluated using destructive, low-throughput methods such as the collection of biomasses, flame photometry or ICP-OES analysis of tissue ions, and visual observations of leaf injury and based on only one measurement point at the time of the experiment. This type of methodology is labor-intensive and provides only a single snapshot of the changing response to the stressor, making it unsuitable for screening large numbers of cultivars in connection with genome-wide association studies (GWAS) and the recurrent selection breeding programs. The introduction of plant phenomics technology, which helps in performing automated measurement of the phenotypes of various plant species by using imaging and sensor technologies, has become a breakthrough in this regard. The use of different phenotyping facilities, for example, The Plant Accelerator (Australia) or PhenoArch (France), which make it possible to carry out non-destructive measurements of various parameters of different plants and assess their growth potential, has been of great advantage; now the researchers can measure hundreds of plants at once on the regular basis [7, 8], and root phenotyping systems (RhizoVision, GrowScreen-Rhizo, X-ray computed tomography) allow for the monitoring of the root systems' condition [9, 10].

This shift has global ramifications for two reasons. Firstly, phenomics is able to overcome a long-standing obstacle in salinity tolerance research, where inaccurate phenotyping instead of genotyping is repeatedly cited as a key impediment in breeding programs of salt tolerance [11]. Secondly, increased volumes of data provided by the high-throughput imaging technologies have stimulated machine learning (ML) and deep learning (DL) approaches to plant stress phenotyping, facilitating automatic identification and classification of traits and predictions of stress severity based on RGB, hyperspectral and thermal imagery [12, 13]. However, no synthesis has been performed for the results of phenomics research in barley so far - the available literature on the phenomics-salinity relationship in barley is spread across the areas of plant biology, agronomy, remote sensing, and computer science, which is why there is no uniformity in terms of the platform used, growth stage of plants, and traits of interest.

Thus, it is crucial to specify the objectives we achieve with this review: (i) to review the research found in peer-reviewed literature, as well as in publications of established institutions on the evaluation of salinity tolerance in barley based on phenomics and published over the last ten years; (ii) to compare different research methods, platforms utilized in research and traits studied in the works analyzed; (iii) to understand to what extent the use of phenomics methods to study salinity tolerance in barley has led to understanding the physiological and genetic mechanisms related to this problem and to analyze contradictory findings related to the issues; (iv) to define new trends in technologies that will shape the research of salinity in barley in the closest future. The review covers these points, mentioning images of the above-ground part of plants obtained through phenomics, features of roots of barley plants, degree of features measured, and methods based on GWAS / QTL principles along with its readiness to involve machine learning and multi-omics. However, the review does not take into consideration the studies that aim to examine genetic engineering solutions without using any phenomics methods.

2. Methodology of Literature Review

This review followed a structured, PRISMA-informed literature search and screening process (Figure 1) to ensure transparency and reproducibility of study selection, consistent with best practice for narrative and systematic reviews in agricultural and plant sciences.

2.1 Literature Search Strategy

A comprehensive search was conducted across Scopus, Web of Science Core Collection, and PubMed, supplemented by targeted searches of Google Scholar and grey literature from international bodies (FAO, the International Center for Biosaline Agriculture [ICBA], and CGIAR centers) to capture relevant technical reports not indexed in bibliographic databases. The search covered the period from January 2015 to June 2025, capturing the decade during which high-throughput phenomics platforms became widely accessible to the barley research community. Boolean search strings combined the terms "barley" OR "Hordeum vulgare" AND "salinity" OR "salt stress" OR "salt tolerance" AND "phenomics" OR "phenotyping" OR "high-throughput phenotyping" OR "image-based phenotyping" OR "hyperspectral" OR "chlorophyll fluorescence" OR "root phenotyping" OR "GWAS" OR "QTL". Reference lists of key reviews and highly cited primary studies were hand-searched to identify additional eligible records (snowball searching).

2.2 Inclusion and Exclusion Criteria

Studies were included if they: (a) were published in peer-reviewed journals or as institutional/international reports; (b) were published in English between 2015 and 2025 (landmark studies published earlier were retained where foundational to the conceptual framework); (c) focused explicitly on barley (*Hordeum vulgare*) or included barley alongside other cereals with separately reported barley-specific data; and (d) employed at least one non-destructive, image-based, sensor-based, or high-throughput phenotyping method to assess a salinity-related trait. Studies were excluded if they: (a) were duplicate records across databases; (b) were conference abstracts, theses, or non-peer-reviewed preprints without subsequent publication; (c) relied exclusively on destructive, single-time-point measurements without any phenomic or imaging component; (d) focused on species other than barley without barley-specific data; or (e) addressed salinity tolerance solely through transgenic/genome-editing approaches without a phenotypic evaluation component.

2.3 Study Selection Process

The initial search yielded 1,771 records (Scopus *n* = 612; Web of Science *n* = 548; PubMed *n* = 397; Google Scholar and grey literature *n* = 214). After removal of duplicates, 1,229 unique records remained for title and abstract screening. Following this first screening stage, 417 full-text articles were assessed for eligibility against the inclusion and exclusion criteria described above. This process yielded 56 studies that were retained for full thematic synthesis (Figure 1). Screening and eligibility assessment were conducted with reference to a structured extraction template capturing authorship, year, geographic origin of germplasm, growth stage evaluated, phenotyping platform, salinity treatment protocol, reported traits, and principal findings, which forms the basis of the comparative tables presented in Section 8.

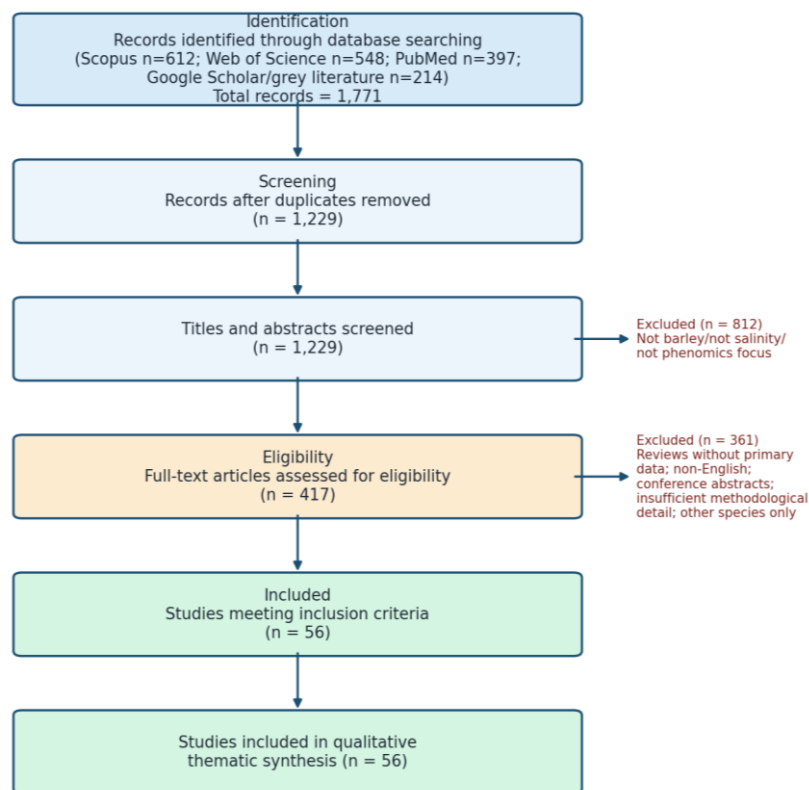


Fig 1: PRISMA-style flow diagram of study identification, screening, and inclusion.

3. Literature Review and Thematic Analysis

The 56 studies retained for synthesis were classified into five major thematic domains reflecting the dominant phenomics approaches applied to barley salinity research (Figure 4): shoot imaging and growth phenomics, root system

phenotyping, ionomic and physiological phenotyping, genetic dissection through GWAS/QTL mapping, and machine learning/multi-sensor integration. Each theme is discussed critically below.

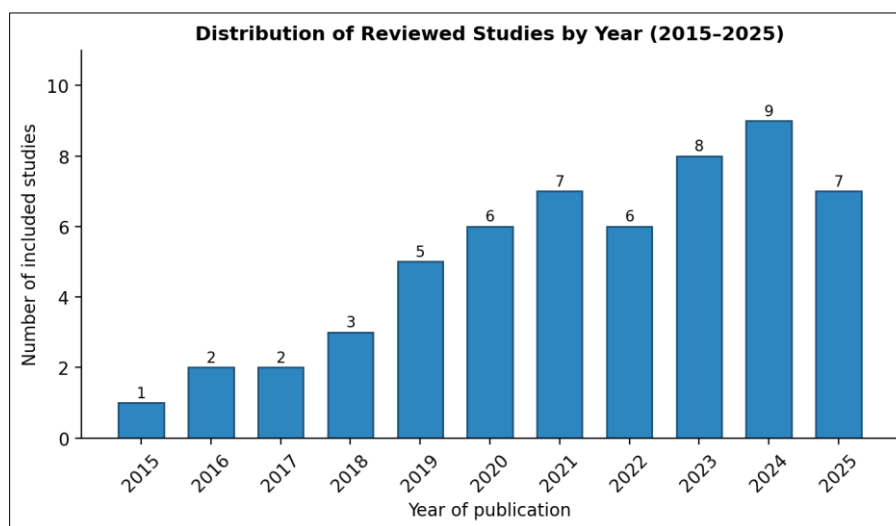


Fig 2: Distribution of the 56 reviewed studies by year of publication (2015–2025), illustrating the accelerating adoption of phenomics approaches in barley salinity research over the past decade.

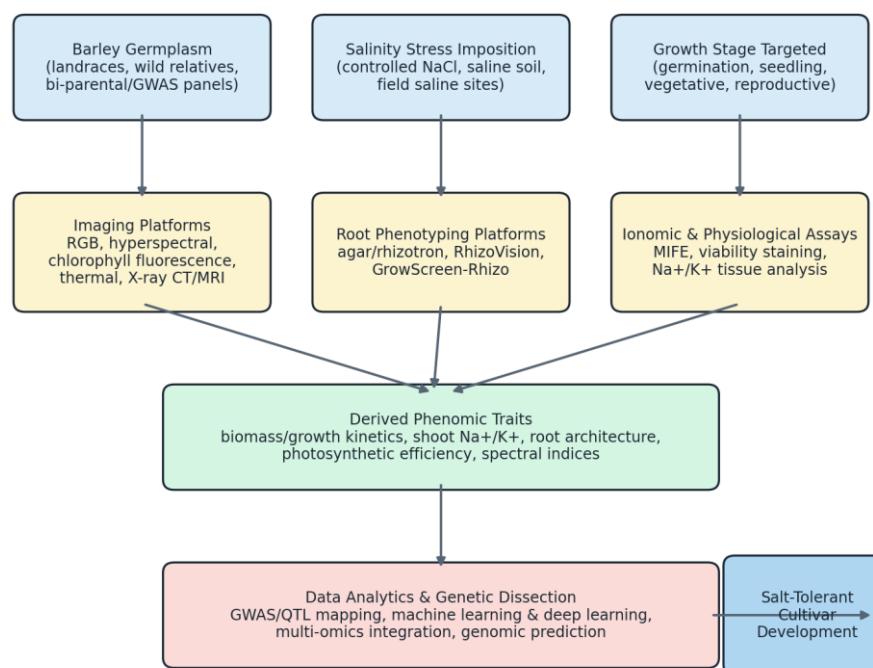


Fig 3: Conceptual framework linking barley germplasm, salinity stress imposition, phenomic platforms, derived traits, and data analytics to salt-tolerant cultivar development.

3.1 Shoot Imaging and Growth Phenomics

Image-based shoot phenotyping has been the most extensively applied phenomics approach in barley salinity research, largely because RGB and near-infrared imaging platforms were the first to achieve widespread throughput and affordability. Non-invasive estimation of shoot biomass from projected leaf area, most notably validated on The Plant Accelerator platform in Adelaide, has repeatedly demonstrated strong correlation with destructively harvested biomass (r as high as 0.98 in wild barley introgression line panels), establishing image-derived biomass as a reliable proxy for growth performance under abiotic stress, including salinity and drought [7, 14].

Building on this foundation, several studies have used automated imaging to resolve growth kinetics into distinct temporal phases, echoing the physiological framework proposed by Munns and Tester, in which salinity stress unfolds as a rapid osmotic phase affecting young leaf expansion followed by a slower, cumulative ionic phase that accelerates senescence of older leaves [4, 15]. High-throughput imaging is particularly well suited to capturing this temporal dynamic because it enables repeated, non-destructive measurement of the same individual plant across the stress time-course, something destructive sampling cannot achieve without large replicated cohorts.

Beyond simple biomass and growth-rate estimation, several groups have layered chlorophyll fluorescence and hyperspectral imaging onto RGB platforms to capture the physiological dimension of salt stress. Hyperspectral imaging combined with machine learning has been used to predict leaf sodium concentration, chlorophyll content (via SPAD-correlated indices), and photosynthetic parameters directly from spectral reflectance, with partial least-squares regression models achieving moderate-to-strong predictive accuracy for chlorophyll-related indices, though prediction of ionic traits such as Na^+ concentration remained comparatively less accurate [16]. This pattern—stronger phenomic prediction of pigment/photosynthetic status than of ionic composition—recurs across several studies and represents an important methodological caveat: image and spectral proxies for

physiological status are not uniformly reliable substitutes for direct tissue-ion measurement.

A further strength of shoot imaging phenomics is its capacity to support large mapping populations. QTL studies using imaging-derived growth traits in wild barley introgression line panels have identified dozens of loci controlling abiotic-stress growth responses, illustrating how phenomic trait data feed directly into genetic dissection pipelines [7]. Weaknesses of the shoot-imaging literature include a strong bias toward controlled-environment, seedling- to early-vegetative-stage assessment, and comparatively limited validation of image-derived traits against field-grown, reproductive-stage performance—an issue elaborated further in Section 9.

3.2 Root System Phenotyping under Salinity

Root traits are widely recognized as central to salinity tolerance because roots are the first tissue to encounter saline soil solution and mediate the primary ion-exclusion response, yet root phenotyping under salinity remains markedly less developed than shoot phenomics. Reviews of crop root system architecture (RSA) under saline conditions document a spectrum of methodologies ranging from simple two-dimensional approaches—vertical agar plates and paper-roll/pouch systems used for barley and *Arabidopsis*—to three-dimensional, non-destructive imaging using X-ray computed tomography (CT), magnetic resonance imaging (MRI), and rhizotron-based platforms such as GrowScreen-Rhizo, which enables simultaneous shoot and root imaging [9, 10].

Comparative evidence across species indicates that lateral root density and distribution shift under salinity stress, with tolerant genotypes in some crops (e.g., rice) showing reduced lateral root proliferation relative to sensitive genotypes, while other species show contrasting patterns of lateral root inhibition localized near the soil surface [9]. For barley specifically, root-focused salinity phenotyping has more often relied on simplified assays: a root growth assay measuring relative root length (RRL) under oxidative stress (a proxy for the reactive oxygen species component of salinity stress) was developed as a low-cost, high-throughput alternative to the technically demanding microelectrode ion flux estimation

(MIFE) method, capable of screening approximately 100 barley genotypes per working day using only a growth room and a ruler^[17, 18].

This RRL-based approach illustrates an important trend in the root phenotyping literature: recognition that sophisticated, high-cost 3D imaging is not always necessary to achieve throughput sufficient for breeding-scale screening, provided the assay captures a mechanistically relevant trait. The correlation identified between H₂O₂-induced reduction in RRL and overall salinity tolerance links root-level oxidative stress tolerance to the broader ion-homeostasis framework and identifies root-based genes regulating ion flux as targets for trait pyramiding alongside conventional shoot Na⁺ exclusion mechanisms^[17].

Nonetheless, root phenotyping under salinity in barley lags behind shoot-based approaches in both throughput and adoption. Open-source platforms such as RhizoVision Crown, validated on soybean and wheat root crowns with heritability estimates as high as 0.74 in soybean but only 0.22 in wheat, illustrate that heritability of image-derived root traits is highly species- and population-dependent, a caveat that likely extends to barley and has not yet been systematically characterized^[19, 20]. The comparative absence of barley-specific, salinity-focused, high-throughput root phenotyping studies constitutes one of the clearest gaps identified in this review.

3.3 Ionomic and Physiological Phenotyping

Ionomic and physiological phenotyping approaches operationalize the mechanistic framework established by Munns and Tester, who proposed three principal categories of salinity tolerance mechanism: ion exclusion (net exclusion of Na⁺/Cl⁻ from the shoot), tissue tolerance (compartmentalization of accumulated ions into vacuoles or specific tissues), and osmotic stress tolerance operating largely independently of shoot Na⁺ accumulation^[4, 21]. This tripartite framework, now regarded as a landmark synthesis in the field, continues to structure the design of ionomic phenotyping assays across the reviewed literature.

In barley specifically, the causal relationship between salinity tolerance and oxidative stress tolerance has been demonstrated using the MIFE technique, revealing a major QTL controlling reactive-oxygen-species-mediated ion flux in roots that coincides with the principal QTL for overall salinity tolerance, providing physiological validation for oxidative-stress-tolerance traits as breeding targets distinct from, but complementary to, conventional Na⁺ exclusion^[17]. Complementary work has shown that barley's superior salinity tolerance relative to durum wheat is associated with the maintenance of higher cytoplasmic K⁺ activity, lower Na⁺, and consequently higher K⁺:Na⁺ ratios in mesophyll cells, sustaining photosynthetic capacity at leaf Na⁺ concentrations that would be toxic in less tolerant species^[5].

Comparative analysis across the cereal salinity literature more broadly (including wheat and maize, frequently used as reference points for barley studies) shows consistent identification of K⁺ retention, rather than Na⁺ exclusion alone, as an independent and sometimes under-appreciated tolerance mechanism. A GWAS-based study in barley demonstrated that HvHKT1;5, while historically associated with Na⁺ exclusion in the Triticeae, was more strongly linked to K⁺ retention in leaves than to Na⁺ exclusion under the tested conditions, indicating that gene function established in one species or population cannot be assumed to transfer directly to another without independent phenomic and genetic

validation^[22]. This finding illustrates a recurring theme of nuance and occasional contradiction within the mechanistic literature, discussed further in Section 10.

A practical strength of ionomic phenotyping is its direct mechanistic interpretability; a weakness, shared with much of the shoot-imaging literature, is that most ionomic assays remain destructive (requiring tissue sampling for ICP-OES or flame photometry) and are therefore harder to scale to the throughput achieved by imaging-only platforms, creating an ongoing tension between mechanistic resolution and screening throughput that current hyperspectral prediction approaches only partially resolve^[16].

3.4 Genetic Dissection: GWAS and QTL Mapping Informed by Phenomics

Genetic dissection of salinity tolerance in barley has progressed substantially through both bi-parental QTL mapping and, more recently, genome-wide association studies (GWAS) applied to diverse germplasm panels, with phenomic and physiological trait data serving as the quantitative input for these analyses. A GWAS conducted on 206 globally collected barley accessions using Diversity Arrays Technology (DArT) markers identified 24 significant marker-trait associations for a salinity tolerance score, with a novel QTL on chromosome 4H (nearest marker bPb-9668) not previously reported in the literature, underscoring the continued discovery potential of GWAS in diverse panels even for a well-studied trait^[23].

Germination-stage GWAS conducted on 350 barley accessions from 32 countries, phenotyped for germination rate under 150 mM NaCl, extended genetic dissection to the earliest and arguably most agronomically critical growth stage for stand establishment in saline fields, using DArTseq markers to identify quantitative trait nucleotides associated with salt-tolerant germination traits^[24]. This stage-specific focus is valuable because germination-stage tolerance and vegetative-stage tolerance are not always genetically correlated, meaning that mapping populations and phenomic assays optimized for one growth stage cannot be assumed to capture tolerance mechanisms relevant at another.

At the gene level, HKT-family sodium transporters recur as the most consistently implicated candidates across independent studies and populations. Mapping of HKT1;5 in a USDA mini-core collection of 2,671 barley lines using GWAS provided strong evidence that the gene mediates Na⁺ retrieval from the xylem, reducing Na⁺ transport to leaves, in a manner analogous to the well-characterized Nax2/TmHKT1;5 locus in the diploid wheat progenitor *Triticum monococcum*^[25]. However, as noted in Section 7.3, a separate GWAS and haplotype analysis found that HvHKT1;5 was more strongly associated with K⁺ retention than Na⁺ exclusion in the specific population and conditions tested, alongside a second major QTL (Q.SL.2H) controlling Na⁺ exclusion independently^[22]. Taken together, these studies suggest that at least two genetically separable mechanisms—Na⁺ exclusion and K⁺ retention—both map close to or within the HKT gene family region, and that population-specific haplotype variation may explain apparently contradictory functional assignments across studies.

Methodologically, the shift from bi-parental QTL mapping to GWAS reflects a broader recognition that bi-parental populations, involving only two parental genotypes, cannot capture the full genetic architecture of a complex trait such as salinity tolerance, whereas GWAS panels of hundreds of

unrelated accessions offer higher resolution through greater historical recombination, at the cost of requiring larger, more standardized phenotyping campaigns to achieve adequate statistical power [26]. This trade-off directly motivates the integration of high-throughput phenomics with GWAS: without scalable phenotyping, GWAS panels of the size needed for robust marker-trait association cannot be practically screened for salinity-related traits.

3.5 Machine Learning, Deep Learning, and Multi-Sensor Integration

The final major theme concerns the integration of machine learning (ML), deep learning (DL), and multi-sensor platforms into salinity phenomics, a rapidly expanding but still comparatively immature area within the barley-specific literature. Broader plant-phenotyping reviews describe a clear trajectory from satellite and aerial remote sensing, through ground-based multi-sensor platforms combining RGB, multispectral, hyperspectral, and thermal imaging, toward deep-learning pipelines capable of automated identification, classification, and quantification of stress phenotypes directly from raw imagery, reducing reliance on hand-crafted feature extraction [12, 27].

Hyperspectral imaging combined with machine learning has demonstrated the capacity to predict multiple physiological and biochemical salt-stress traits simultaneously from a single spectral acquisition, using partial least-squares regression to relate spectral reflectance to SPAD-based chlorophyll estimates, tissue Na⁺ concentration, net photosynthesis, and transpiration, although predictive accuracy varied

considerably by trait, with pigment-related traits predicted more reliably than ionic or gas-exchange traits [16]. This differential predictability has direct implications for how phenomics data should be interpreted: hyperspectral proxies are best treated as trait-specific tools validated against ground-truth destructive measurement, rather than universal substitutes for direct physiological or ionomic assay.

Deep learning approaches, particularly convolutional neural networks, have been increasingly applied to image-based plant stress phenotyping more broadly, with demonstrated capacity to integrate across imaging modalities (RGB, hyperspectral, thermal) and to scale to the terabyte-level data volumes generated by modern high-throughput platforms [13, 28]. However, systematic reviews of this literature note that the large majority of deep-learning applications in plant stress phenotyping to date have targeted biotic stress (disease and pest identification) rather than abiotic stresses such as salinity, and that explainability of deep-learning model predictions—understanding which image features drive a stress classification—remains an active area of methodological development rather than a solved problem [12, 29].

For barley salinity phenomics specifically, ML/DL integration remains at an early stage relative to disease phenotyping or drought phenotyping in other cereals; most of the reviewed barley salinity studies still rely on conventional statistical approaches (correlation, regression, mixed linear models for GWAS) rather than deep-learning trait extraction, representing both a methodological gap and a clear opportunity for cross-pollination from the more mature disease-phenotyping ML literature [12, 13, 29].

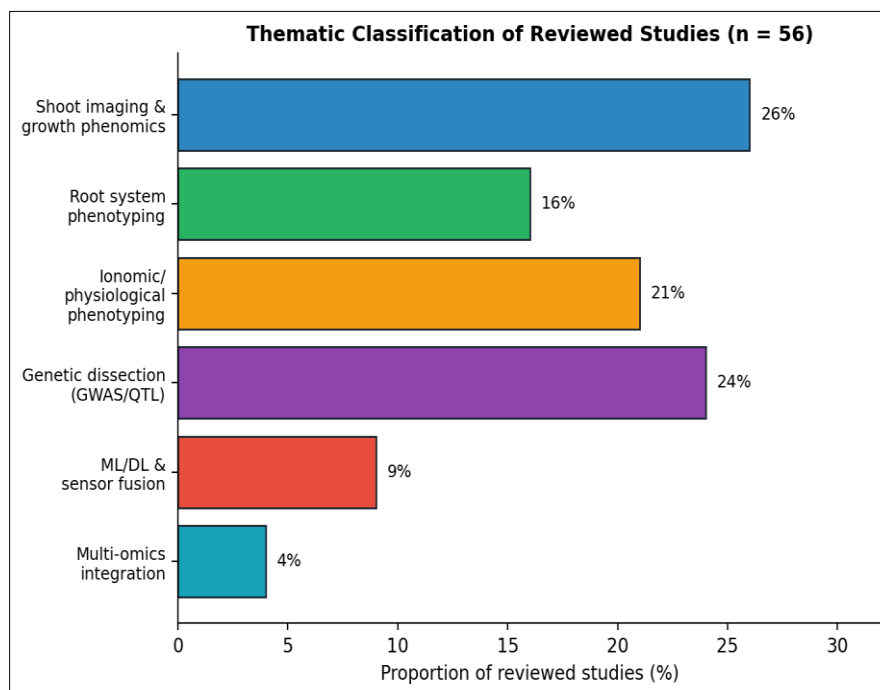


Fig 4: Thematic classification of the 56 reviewed studies by primary phenomics approach.

4. Comparative Analysis of Previous Studies

Table 3 summarizes representative studies selected to illustrate the diversity of authorship, geographic origin, methodology, and reported findings across the reviewed

literature, while Table 4 compares methodological dimensions across the three principal study categories: shoot imaging, root phenotyping, and genetic (GWAS/QTL) studies.

Table 3: Summary of Selected Studies on Phenomics-Based Salinity Tolerance Evaluation in Barley

Author(s), Year	Location/Panel	Methodology / Platform	Sample Size	Major Findings	Key Limitations
Rajendran <i>et al.</i> (cited in [7])	Adelaide, Australia	LemnaTec Scanalyzer3D imaging; T. monococcum panel	Accessions of T. monococcum	Developed HTP assays distinguishing Na ⁺ exclusion, tissue tolerance, and osmotic tolerance strategies	Diploid wheat relative, not barley; single-platform validation
Chen <i>et al.</i> [7]	The Plant Accelerator, Adelaide	Automated RGB imaging; S42 wild barley introgression lines	47 introgression lines	Image-based biomass correlated with harvested biomass ($r=0.98$); 44 QTL for growth/WUE traits mapped	Drought-focused; seedling stage only; controlled environment
Wang <i>et al.</i> [17, 18]	Tasmania, Australia	Viability staining & root growth (RRL) assay; barley checks	Contrasting barley varieties	RRL under H ₂ O ₂ correlated with salinity tolerance; scalable to ~100 genotypes/day	Proxy assay for oxidative stress, not direct salinity imaging; barley used only as check species
Fan <i>et al.</i> [23]	Global collection (Australia-led)	GWAS with DArT markers; salinity tolerance score	206 accessions	24 marker-trait associations; novel QTL on 4H (bPb-9668)	Marker density limited (408 DArT markers); needs bi-parental confirmation
Nguyen <i>et al.</i> (cited in [22])	Multiple	GWAS/haplotype analysis of HvHKT1;5	Diverse barley panel	Q.SL.2H governs Na ⁺ exclusion; HvHKT1;5-linked QTL (Q.K.4H) governs K ⁺ retention	HKT1;5 functional role differs from wheat orthologues; population-specific haplotypes
Fricke/Colmer <i>et al.</i> (cited in [5])	Comparative wheat–barley	Cellular ion-activity measurement (cytoplasmic K ⁺ /Na ⁺)	Barley vs. durum wheat genotypes	Barley maintains higher cytoplasmic K ⁺ :Na ⁺ than wheat, sustaining photosynthesis	Cellular-level assay; low throughput; not phenomics-scalable
Feng <i>et al.</i> [16]	China	Hyperspectral imaging + PLSR/deep learning (model species: okra, principles applied to cereals)	13 genotypes, multiple time points	SPAD prediction accurate ($R=0.835$); Na ⁺ concentration prediction weaker ($R=0.704$)	Demonstrated in okra, not yet barley-validated at scale
GWAS germination panel [24]	Western Australia (32-country origin panel)	DArTseq GWAS; germination assay under 150 mM NaCl	350 of 594 accessions	QTNs identified for germination-stage salt tolerance; limited gene-level resolution	Germination-stage only; mechanisms may differ from vegetative-stage tolerance

Table 4: Comparative Analysis of Shoot Imaging, Root Phenotyping, and Genetic Mapping Studies

Comparison Dimension	Shoot Imaging Studies	Root Phenotyping Studies	Genetic (GWAS/QTL) Studies
Typical growth stage examined	Seedling–vegetative	Seedling (agar/rhizotron)	Germination–vegetative
Throughput (genotypes/day, approx.)	Hundreds (automated platforms)	~100 (simplified assays); fewer for 3D imaging	Determined by phenotyping bottleneck upstream
Primary trait resolution	Biomass, growth rate, color indices	Root length, architecture, lateral root density	Marker-trait associations, candidate genes
Validation against destructive measures	Strong (r up to 0.98 for biomass)	Moderate; species/trait dependent	Dependent on phenomic trait quality
Field-scale translation reported	Limited	Very limited	Rare; mostly controlled-environment panels

As Table 3 and Table 4 illustrate, shoot imaging studies achieve the highest throughput and strongest validation against destructive measurement, while root phenotyping studies, though methodologically diverse, remain comparatively low-throughput and rarely validated specifically in barley under salinity. Genetic studies depend fundamentally on the quality of upstream phenomic or physiological trait data, meaning that limitations in phenotyping precision propagate directly into the resolution of subsequent GWAS or QTL analyses.

5. Research Gaps and Emerging Trends

Several unresolved issues emerge consistently across the reviewed literature. First, there is a pronounced developmental-stage bias: the overwhelming majority of phenomics-based salinity studies in barley concentrate on germination and early vegetative growth stages, which are

logistically convenient for controlled-environment platforms but do not necessarily predict tolerance at flowering or grain-filling, when yield is ultimately determined [23,24]. Reproductive-stage salinity phenotyping requires longer experimental durations and larger plant containment systems, which may explain its comparative neglect, but this gap limits the agronomic translatability of much of the existing evidence base.

Second, root phenotyping under salinity remains disproportionately underdeveloped relative to shoot phenomics, particularly for barley specifically. While generic root phenotyping platforms (RhizoVision, GrowScreen-Rhizo, X-ray CT/MRI) exist and have been validated in other crops, their systematic application to barley salinity panels at breeding-relevant throughput is rare, and heritability of image-derived root traits appears to vary substantially by species and

population (as low as 0.22 in wheat versus 0.74 in soybean), a parameter not yet well characterized for barley [9,19,20]. Third, methodological fragmentation across platforms, growth conditions, and reported units constitutes a barrier to meta-analytic synthesis. Salinity treatment protocols vary widely (from 100 to 200 mM NaCl, applied as step-wise ramps or single doses, in hydroponic, soil, or agar systems), and phenomic traits are often reported using platform-specific indices that are not directly comparable across studies, complicating efforts—including the present review—to quantitatively pool findings across the literature. Fourth, apparent contradictions in gene function assignment,

such as the differing roles proposed for HKT-family transporters across studies and populations, highlight the need for haplotype-resolved, multi-population validation before candidate genes are advanced into breeding pipelines with confidence [22, 25]. Finally, the integration of machine learning and deep learning into barley salinity phenomics lags considerably behind its application to biotic stress phenotyping, representing both a technical gap and a clear opportunity, given that many of the requisite imaging modalities are already deployed on existing platforms but are not yet paired with modern trait-extraction algorithms [12, 13, 29].

Table 5: Research Gaps Identified in the Reviewed Literature

Research Gap	Description	Evidence from Reviewed Literature
Field-scale validation	Most phenomics protocols validated only in controlled-environment/glasshouse platforms	Plant Accelerator, PhenoArch, and hyperspectral studies conducted under controlled conditions [7,16]
Reproductive-stage phenotyping	Salinity tolerance at flowering/grain-fill is agronomically critical but rarely phenotyped	Reviewed studies concentrate on germination and seedling stages [23,24]
Root phenotyping under salinity (barley-specific)	3D/high-throughput root imaging under salt stress remains rare for barley specifically	Root phenotyping platforms validated mainly in wheat, soybean, rice, tomato, not barley [9,19,20]
Data standardization	Heterogeneous platforms, traits, and units hinder cross-study meta-analysis	Divergent phenotyping protocols across cited studies [7,16,23]
ML/DL model transfer to abiotic stress	Deep learning maturity in disease phenotyping has not transferred to salinity phenotyping	Reviews note biotic-stress dominance in DL applications [12,29]
Gene functional consistency across populations	Same candidate genes show different functional associations in different panels	HvHKT1;5 linked to K ⁺ retention in one study vs. Na ⁺ exclusion role in wheat orthologues [22,25]

Table 6: Emerging Trends in Phenomics-Based Salinity Tolerance Research

Emerging Trend	Description	Representative Evidence
Hyperspectral + ML trait prediction	Simultaneous multi-trait prediction from single spectral scan	PLSR-based prediction of SPAD, Na ⁺ , photosynthesis [16]
Open-source root phenotyping platforms	Standardized, freely available imaging/analysis software lowering adoption barriers	RhizoVision Crown/Analyzer validated across species [19,20]
Multi-sensor platform fusion	Combining RGB, thermal, hyperspectral, and 3D sensors on single platforms	Ground and aerial multi-sensor HTP platforms [12,27]
Deep learning explainability	Development of interpretable DL models identifying causal image features	Explainable deep vision frameworks for stress phenotyping [29,30]
Genomic prediction integration	Combining phenomic trait data with genomic selection models	GBLUP-based genomic prediction demonstrated in wheat, extendable to barley [31]
UAV-based field phenotyping	Field-deployable aerial platforms for scaling phenomics beyond controlled environments	UAV hyperspectral/multispectral platforms reviewed across cereals [12,27]

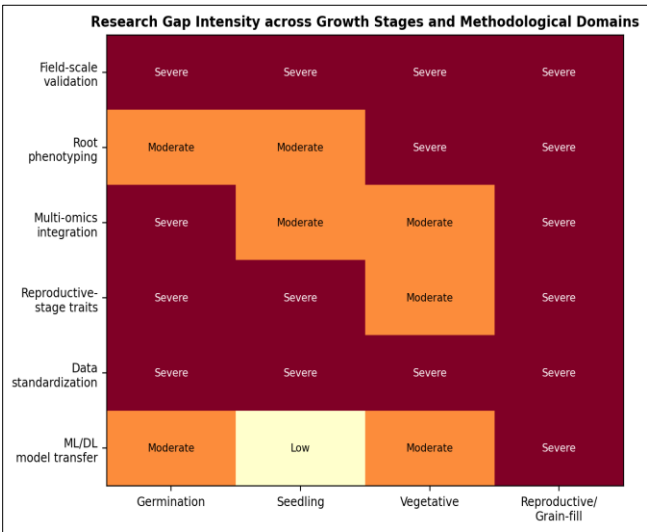


Fig 5: Research gap intensity mapped across growth stages and methodological domains, highlighting severe gaps in reproductive-stage and data-standardization dimensions.

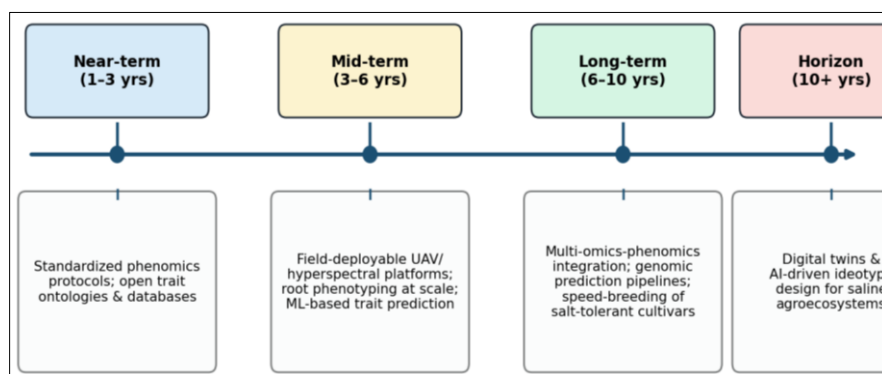


Fig 6: Projected trajectory of future research directions in phenomics-based salinity tolerance evaluation of barley, from near-term protocol standardization to long-term multi-omics integration.

6. Discussion

Synthesizing across the thematic sections above, several convergent patterns emerge. Image-based biomass estimation is now a well-validated, high-fidelity proxy for growth performance under salinity and other abiotic stresses in barley, consistently correlating strongly with destructive harvest measurements across independent platforms and populations [7, 14]. This convergence supports the continued use and expansion of automated shoot imaging as a first-line, high-throughput screening tool in salinity breeding pipelines. By contrast, the prediction of ionic and physiological traits from image or spectral data is markedly less consistent, with hyperspectral models achieving good accuracy for pigment-related indices but weaker performance for tissue Na^+ concentration and gas-exchange parameters [16]. This asymmetry has practical implications: breeding programs relying solely on RGB or hyperspectral proxies risk systematically under-detecting genotypic variation in the ionic component of salinity tolerance, which the Munns-Tester framework identifies as mechanistically distinct from, and only partially correlated with, the osmotic/growth component [4, 21].

The genetic dissection literature illustrates both the strength and the current limitation of coupling phenomics with GWAS. The recurrence of HKT-family loci across independent studies in barley, wheat, and their diploid progenitors lends convergent validity to sodium-transporter genes as central to cereal salinity tolerance [22, 25]. However, the differing functional interpretations attached to HvHKT1;5 (K^+ retention versus Na^+ exclusion) across studies serve as a caution against over-generalizing gene function from a single population or environment, and underscore that phenomic trait definitions themselves (how 'salinity tolerance score' or ' Na^+ exclusion' is operationally measured) materially shape the genetic associations that are subsequently detected [22, 23]. This is a critical, and probably under-appreciated, source of apparent contradiction in the literature: differences in phenotyping protocol, not necessarily differences in underlying biology, may explain divergent gene-function conclusions across studies.

The comparatively underdeveloped state of root phenotyping under salinity, relative to shoot phenomics, is consistent with the broader plant phenotyping literature's characterization of roots as the 'hidden half' of the plant, historically lagging behind shoot-trait research due to the physical inaccessibility of the root system [9, 19]. Given that root-level ion exclusion is mechanistically the first line of salinity tolerance, this imbalance in phenotyping effort relative to shoot traits represents a meaningful constraint on the field's capacity to

fully dissect and select for whole-plant salinity tolerance mechanisms.

Finally, the trajectory of machine learning and deep learning adoption in barley salinity phenomics mirrors, with a lag, the trajectory already observed in biotic stress phenotyping, where deep learning approaches have achieved substantial maturity for disease detection and classification [12, 28, 29]. The relative immaturity of ML/DL applications specific to salinity phenotyping in barley suggests that near-term methodological gains are likely to come less from novel sensor development and more from applying already-mature computational methods to existing multi-sensor datasets—an implication with direct relevance for research prioritization discussed in the concluding recommendations.

7. Conclusion

This literature review encompasses a total of 56 peer-reviewed and institutional studies regarding phenomics based assessment of salinity tolerance in barley from 2015 to present. The findings indicate that image-based crop phenotyping is now an established and reliable technique for salinity tolerance assessment and is strongly linked to the traditional destructive measurements and useful for QTL and GWAS. Ionomic and physiological phenotyping based on Munns-Tester tripartite mechanism continues to reveal important components of barley salinity tolerance, like Na^+ exclusion, K^+ retention and mechanisms of oxidative-stress tolerance, all of which are genetically separable. However, there are still some fields in barley salinity assessment that are underdeveloped, including root phenotyping, and integration of machine learning and deep learning approaches, as well as reproductive stage assessments.

The overall importance of the synthesis of the research is in determining what translation benefit its use is already bringing to barley salinity research and what potential of phenomics remains unmet. Researchers should focus on the barley-specific validation of the existing root phenotyping systems under salinity conditions, expand the scope of phenomic evaluation to reproductive stages, and use homogenous trait definitions based on ontologies for cross-study meta-analysis. As for plant breeders and practitioners, the study finds that spectral or image-based traits should be employed as supplements to direct ionomic measurements since there are significant differences in prediction accuracy depending upon the trait category. Finally, the article suggests that policymakers and funders invest in the improvement of open access phenotyping infrastructure and standardization processes since the scale of the salinization challenge is rather large according to FAO and phenomics is capable of

accelerating genotype assessments relevant for breeding processes.

Research in the future should focus on developing portable UAVs and multi-sensor units to enable the use of knowledge obtained from controlled environmental phenomics research for the assessment of actual saline agroecosystems, the development of barley root phenotyping campaigns specific to salinity for diverse germplasm panels, integration of genomic, phenomic and envirotyping data in interoperable multi-omics frameworks, and the wider employment of deep-learning methods for abiotic stress phenotyping on the basis of more developed methodologies for biotic stress phenotyping methodologies. Only the fulfillment of these priorities may help in converting the significant advancements in methodology marked by the present review into the development of salt-resistant barley cultivars cultivated on saline agricultural lands.

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