

INNOVATIVE TECHNIQUES TO EVALUATE EARLY SEEDLING VIGOUR

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ABSTRACT

Seedling vigour, the capacity of a seed lot to produce rapid, uniform, and robust seedlings under a wide range of field conditions, is a critical determinant of crop establishment, yield potential, and ultimately food security. Traditional vigour assays, including the standard germination test, accelerated ageing test, electrical conductivity test, cold test, and tetrazolium test, remain the backbone of seed testing laboratories worldwide, yet they are frequently destructive, labour-intensive, subjective, and slow, limiting their utility for high-throughput breeding and quality-control pipelines. Over the past two decades, an expanding suite of innovative, largely non-destructive technologies has emerged to complement and, in some cases, replace conventional bioassays. This review synthesises recent advances in image-based phenotyping and computer vision, machine learning and deep learning classification, hyperspectral and multispectral imaging, near-infrared and Raman spectroscopy, chlorophyll fluorescence imaging, X-ray radiography and micro-computed tomography, root system architecture phenotyping, thermal imaging and unmanned aerial vehicle (UAV)-based field emergence monitoring, electrophysiological assays, and omics-based biomarker discovery (proteomics, metabolomics, transcriptomics, and epigenomics). The principles, analytical workflows, reported accuracies, crop applications, and comparative strengths and limitations of each approach are discussed, alongside emerging trends in multi-sensor data fusion and artificial intelligence-driven decision support. The review concludes by identifying key bottlenecks constraining commercial adoption, including throughput, cost, standardisation, and cross-laboratory reproducibility, and outlines priority research directions for translating laboratory-scale innovations into routine, non-destructive seedling vigour evaluation for the global seed industry.

KEYWORDS: seed vigour, seedling vigour, image analysis, hyperspectral imaging, machine learning, X-ray computed tomography, chlorophyll fluorescence, phenomics.

INTRODUCTION

Seed quality is a composite trait encompassing genetic purity, physical purity, physiological potential, and health status, of which physiological potential commonly expressed through the twin concepts of germination and vigour is the most dynamic and agronomically consequential (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). While the standard germination test estimates the maximum percentage of seeds capable of producing normal seedlings under optimal laboratory conditions, it provides little information about how a seed lot will perform under the sub-optimal, heterogeneous conditions typical of production fields. Seed vigour was formally defined by the International Seed Testing Association (ISTA) as "the sum of those properties that determine the potential level of activity and performance of the seed during germination and seedling emergence" (ISTA, 2021), encompassing rate and uniformity of germination and seedling growth, emergence capacity under unfavourable environments, and post-storage performance.

Early seedling vigour specifically refers to the speed, uniformity, and robustness with which a germinated seed develops into an autotrophic seedling capable of establishing itself in the field. High seedling vigour translates into rapid canopy closure, greater competitiveness against weeds, more efficient resource capture, and improved resilience to abiotic stresses such as chilling, drought, and soil crusting (Finch-Savage & Bassel, 2016). Consequently, the accurate, rapid, and objective evaluation of seedling vigour is of paramount importance to plant breeders selecting superior genotypes, seed companies conducting quality control, and certification agencies enforcing seed-lot standards.

Conventional vigour tests, several of which are codified in the ISTA Handbook of Vigour Test Methods and the Association of Official Seed Analysts (AOSA)/Society of Commercial Seed Technologists (SCST) Seed Vigor Testing Handbook, have historically relied on destructive sampling, manual scoring, and prolonged incubation periods (AOSA/SCST, 2009). These constraints are increasingly incompatible with the scale of modern breeding programmes, which may require phenotyping of tens of thousands of genotypes per season, and with the throughput demands of commercial seed processing lines. This has catalysed a wave of methodological innovation over the past fifteen years, drawing on advances in digital imaging, spectroscopy, robotics, and

artificial intelligence, to develop non-destructive, rapid, and increasingly automated alternatives for seedling vigour assessment (Rajjou et al., 2012; ElMasry, Mandal, Al-Rejaie, & Belin, 2019).

2. Conventional Approaches to Seedling Vigour Assessment: A Baseline

Before considering emerging technologies, it is useful to summarise the conventional vigour tests against which innovative methods are typically benchmarked. The standard germination test, conducted under controlled temperature and moisture on paper substrates, remains the reference method but is a poor discriminator among seed lots of comparably high germinability (Marcos-Filho, 2015). The accelerated ageing (AA) test, developed by Delouche and Baskin (1973) and later adapted as a formal vigour test by McDonald and Phaneendranath (1978), exposes seeds to high temperature (40–45 °C) and high relative humidity (90–100%) for a fixed duration before germination, with the rationale that low-vigour seeds deteriorate more rapidly under these accelerated stress conditions. ISTA has validated the AA test specifically for soybean (*Glycine max*) (Basu & Groot, 2023).

The electrical conductivity (EC) test, one of only two vigour tests formally incorporated into the ISTA Rules for Seed Testing, quantifies the leakage of electrolytes from imbibing seeds into deionised water as an inverse proxy for membrane integrity and, by extension, vigour (Matthews & Powell, 2006). Seed lots exhibiting high conductivity readings, indicating extensive electrolyte leakage through compromised plasma membranes, are classified as low vigour, and the test correlates strongly with field emergence in species such as pea, soybean, and maize (Matthews & Powell, 2006; ISTA, 2021). The controlled deterioration test, cold test, and radicle emergence test provide additional species-specific stress-based assessments, while the tetrazolium (TZ) test exploits the reduction of colourless 2,3,5-triphenyl tetrazolium chloride to red formazan by dehydrogenase enzymes in metabolically active tissue to infer both viability and, through the pattern and intensity of staining, vigour (Kittock & Law, 1968; Basu & Groot, 2023).

Growth-based vigour indices, such as the Abdul-Baki and Anderson vigour index (product of germination percentage and seedling length, or seedling dry weight), and seedling growth rate assays, offer a more direct read-out of early seedling performance but require several days of incubation and manual or semi-automated seedling measurement (Marcos-Filho, 2015). Collectively, these conventional assays, though robust and widely standardised, share common limitations: destructiveness, subjectivity in visual scoring, sensitivity to operator training, long turnaround times (days to weeks), and, in several cases, poor reproducibility between laboratories (ISTA, 2021). These shortcomings constitute the principal motivation for the innovative approaches reviewed in the following sections.

3. Innovative and Emerging Methods for Seedling Vigour Evaluation

3.1 Digital Image Analysis and Computer Vision

Digital image analysis has become one of the most widely adopted non-destructive approaches for quantifying seedling morphological traits associated with vigour, including radicle and hypocotyl length, growth rate, seedling area, greenness, and uniformity. Commercial platforms such as the Seed Vigour Imaging (SVI) system and Video Test-Morphology software automate the capture and measurement of germinating seeds and seedlings on standardised paper substrates, substantially reducing the labour and subjectivity associated with manual ruler-based measurement, as reported in recent syntheses of automated seed-quality inspection methods. In tomato, an image-based node detection and internode length estimation pipeline was developed to assess seedling vigour rapidly by tracking the pattern of internode elongation over time using machine-learning-assisted node identification and affinity propagation for node ordering, achieving a relative error below 15.4% in internode length estimation despite variable lighting and complex backgrounds (Yamamoto, Guo, & Ninomiya, 2016).

Interactive and traditional machine learning pipelines have also been applied directly to RGB images of soybean seeds and seedlings, correlating seed-coat appearance with subsequent seedling classification and achieving overall classification accuracy as high as 0.94, using low-cost, free-access image analysis software accessible to breeding programmes without specialised imaging hardware (Nagel et al., 2020). More broadly, computer-vision-based seed and seedling phenotyping pipelines integrate segmentation, feature extraction (colour, texture, shape descriptors), and statistical or machine-learning classifiers to translate raw images into quantitative vigour indices at a throughput of hundreds to thousands of seeds per hour, vastly exceeding manual capacity (Silva & Cicero, 2018).

A recurring theme in this literature is the trade-off between imaging simplicity (RGB cameras under uniform lighting) and information content: while RGB-based systems are inexpensive and readily deployable, they are primarily sensitive to seedling geometry and surface colour and may fail to detect internal physiological deterioration that precedes visible morphological symptoms. This limitation has motivated the parallel development of spectral and hyperspectral imaging approaches.

3.2 Machine Learning and Deep Learning Classification Frameworks

The integration of machine learning (ML) and deep learning (DL) with imaging and spectral data has transformed seed and seedling vigour phenotyping from simple threshold-based image processing into sophisticated pattern-recognition problems capable of capturing subtle, multivariate signatures of vigour. Classical ML algorithms, including linear discriminant analysis (LDA), support vector machines (SVM),

random forest (RF), and partial least squares discriminant analysis (PLS-DA), are commonly applied to extracted spectral or morphological features, whereas convolutional neural networks (CNNs) and vision transformers increasingly operate directly on raw image data, learning discriminative features without manual feature engineering (Zhang & Zhao, 2023).

A notable example is MsiFormer, an end-to-end deep learning pipeline for rice seed vigour testing that integrates a YOLO-based object detector for seed localisation with a vision-transformer-based vigour prediction model applied to 19-waveband multispectral images; this approach achieved a classification accuracy of 94.17%, outperforming three alternative deep learning benchmarks by 2.5–18.3 percentage points (Zhao et al., 2023). In hyperspectral applications, deep and shallow learning models coupled with hyperspectral imaging of artificially aged soybean seeds have enabled rapid, non-destructive vigour classification based on spectral signatures linked to germination percentage, germination energy, and germination index (Liu et al., 2025).

For smooth brome grass (*Bromus inermis*), a comparison of five multivariate methods principal component analysis (PCA), LDA, SVM, random forest, and normalised canonical discriminant analysis (nCDA) applied to multispectral seed images found that LDA consistently outperformed the other algorithms, achieving vigour prediction accuracies of 94.2–99.6% across different seed maturities and harvest years, although classification of viability (normal versus dead seeds) proved more challenging, particularly across different seed maturities (Zhang et al., 2023). These findings illustrate a broader pattern in the literature: while deep learning models often achieve the highest raw accuracy, particularly with large, well-labelled datasets, simpler discriminant-based classifiers remain competitive and more interpretable for smaller sample sizes typical of many seed-testing laboratories.

Deep learning is also increasingly applied to time-series imaging for dynamic vigour assessment. Bidirectional long short-term memory (BiLSTM) networks, optimised using metaheuristic algorithms such as dung beetle optimisation, have been used to classify chilling-injury severity in tomato seedlings from chlorophyll fluorescence image sequences, illustrating how temporal deep learning architectures can capture stress-response dynamics relevant to seedling robustness (Chen et al., 2024).

3.3 Spectroscopic and Multispectral/Hyperspectral Imaging Techniques

Spectroscopic methods exploit the interaction of electromagnetic radiation, typically in the visible, near-infrared (NIR), and short-wave infrared regions, with seed and seedling tissue to infer biochemical composition and physiological state without physical damage. Near-infrared spectroscopy (NIRS) measures diffuse reflectance or transmittance across a spectral range (commonly 950–1650 nm or broader), generating spectra that encode information on moisture, protein, oil, and carbohydrate content, which are in turn correlated with germination and vigour through chemometric models such as partial least squares (PLS) regression (Al-Amery, Geneve, Sanches, Grover, & Nakhoul, 2018). The general principle, instrumentation, and workflow of NIR-based seed vigour assessment are illustrated in Figure 1.

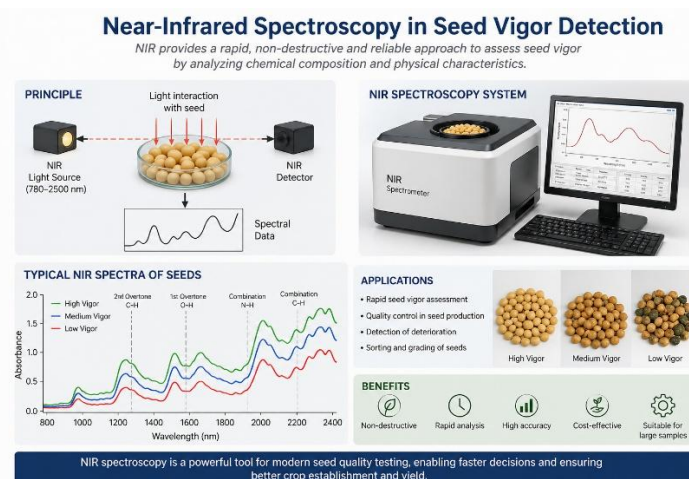


Figure 1 Near-Infrared Spectroscopy for Rapid Seed Vigor Assessment

In a benchmark study using over 80 commercial soybean seed lots, NIRS-based qualitative prediction models correctly classified low- and high-germination lots with 85.7–89.7% accuracy; for vigour, a two-category classification scheme (low vigour versus combined medium-and-high vigour) achieved 80–100% correct classification of low-vigour lots and 96.3–96.6% correct classification of medium/high-vigour lots, although three-category models struggled to separate medium from high vigour (Al-Amery et al., 2018). Similar approaches have been extended to rapeseed, where Savitzky–Golay smoothing combined with synergy interval partial least squares and random frog wavelength selection, feeding a support vector regression model, achieved coefficients of determination of 0.86 (calibration) and 0.84 (prediction) for germination rate estimation (Liu, Zhang, & Wang, 2023).

Multispectral and hyperspectral imaging extend spectroscopic principles into the spatial domain, capturing spectral information at each pixel of a seed or seedling image and thereby combining morphological and

biochemical information. Instruments such as the Videometer Lab, which captures 19 discrete wavebands, have been used to generate vigour gradients in rice seeds subjected to 0–20 days of accelerated ageing, from which deep learning models such as MsiFormer were trained (Zhao et al., 2023). In soybean, hyperspectral imaging combined with machine learning classifiers applied to seeds subjected to artificial ageing enabled rapid, non-destructive vigour-class prediction, offering an alternative to destructive germination assays for high-throughput seed sorting (Liu et al., 2025).

Multispectral imaging has similarly been applied to distinguish chicory seed maturity classes and predict germination rate and vigour based on principal component and quadratic discriminant analysis of chemometric image features (Boelt et al., 2018). Raman spectroscopy, though less widely applied to vigour per se, has demonstrated utility for predicting crude protein and oil content in intact soybean seeds, biochemical traits closely linked to seed quality and storage potential (Lee et al., 2013; Schulmerich et al., 2012). Collectively, spectroscopic and hyperspectral technologies offer the advantage of probing biochemical determinants of vigour that are invisible to conventional RGB imaging, at the cost of more expensive instrumentation and more complex chemometric analysis pipelines.

3.4 Chlorophyll Fluorescence Imaging

Chlorophyll fluorescence imaging (CFI) exploits the principle that chlorophyll retained in immature or stressed seed tissues fluoresces under specific excitation wavelengths, providing a rapid, non-invasive proxy for seed maturity and, indirectly, vigour. Jalink, van der Schoor, Frandas, van Pijlen, and Bino (1998) demonstrated in *Brassica oleracea* that seeds exhibiting the lowest chlorophyll fluorescence produced the highest percentages of germination and normal seedlings, establishing the inverse relationship between residual seed chlorophyll and physiological quality that underlies commercial chlorophyll-fluorescence-based seed sorters (Basu & Groot, 2023).

In chicory, chlorophyll fluorescence imaging of the pericarp and pappus was used to track seed maturation, with fluorescence intensity following a logistic decline correlated negatively with germination rate; the technique proved most discriminating for vigour differences between 21 and 36 days after flowering (Muller, Bertin, & Chantre, 2011). Similarly, optical imaging integrating chlorophyll fluorescence with chemometric multispectral analysis has been applied to tomato and carrot seeds, where excitation-emission combinations around 645/700 nm enabled cultivar discrimination linked to seedling emergence and vigour indices (Boelt, Shrestha, & Carstensen, 2020).

Beyond seed-level assessment, chlorophyll fluorescence imaging of intact seedlings, quantifying parameters such as minimal fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v), and the derived F_v/F_m ratio (an index of photosystem II photochemical efficiency), provides a rapid, high-throughput screen for physiological perturbation. In a foundational demonstration, *Arabidopsis* seedlings grown in 96-well microtitre plates were screened using chlorophyll fluorescence imaging to detect herbicide-induced metabolic perturbations before any visible growth effects emerged, illustrating the sensitivity of this approach for early stress detection relevant to vigour assessment under sub-optimal conditions (Barbagallo et al., 2003). More recent applications employ automated CFI platforms, such as the SeedReporter system, to quantify F_v/F_m dynamics in soybean seeds subjected to controlled ageing, revealing physiological deterioration signatures, including altered chlorophyll a and b content and lignin accumulation, that precede detectable changes in conventional germination and emergence tests (Diniz et al., 2021).

3.5 X-ray Radiography and Micro-Computed Tomography

X-ray-based imaging techniques provide non-destructive visualisation of internal seed structure, enabling detection of embryo malformation, cavities, mechanical damage, and insect infestation that are not discernible externally and that strongly influence subsequent seedling vigour. Conventional two-dimensional X-ray radiography has long been used to characterise internal morphology in species such as tomato, muskmelon, and maize, correlating radiographic density patterns with subsequent germination outcomes (Van der Burg, Aartse, van Zwol, Jalink, & Bino, 1994; Dell'Aquila, 2007). A representative radiographic seed-quality analysis workflow is shown in Figure 2.

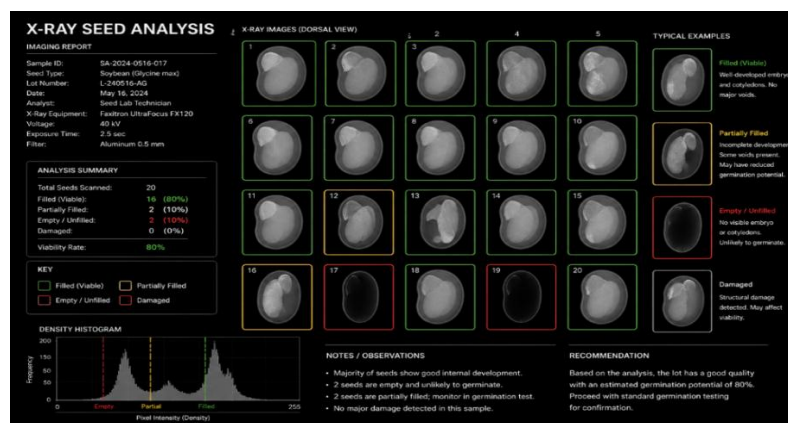


Figure 2 X-Ray Radiographic Analysis of Seed Viability and Internal Structure

More recently, X-ray micro-computed tomography (micro-CT) has enabled three-dimensional, high-resolution reconstruction of internal seed architecture. In tomato, micro-CT coupled with discriminant analysis of morphometric traits, including three-dimensional free-space percentage and Sauter diameter, correctly classified 96.3% of normal seedlings, 83.3% of ungerminated seeds, and 63.6% of abnormal seedlings, yielding an overall predictive accuracy of 91.9% for germination outcome from internal seed morphology alone (Pace, De Bellis, & Rascio, 2019). A specific caveat identified in this work is that increasing X-ray exposure levels were associated with a rise in the proportion of abnormal seedlings, underscoring the need to calibrate radiation dose carefully when micro-CT is used for phenotyping rather than purely diagnostic purposes (Pace et al., 2019).

High-throughput X-ray CT phenotyping platforms, such as the 3DPheno-Seed&Fruit system, integrate specially designed multi-seed sample containers with automated three-dimensional image segmentation software to quantify up to eight morphological phenotypes including internal air-space volume, embryo size, and seed-coat thickness across large seed batches without manual measurement (Wang et al., 2020). Complementary work has applied X-ray microscopy with phase-contrast computed tomography to resolve cellular-level structural changes in embryos of small, live seeds during early germination stages, offering unique insight into the structural dynamics underpinning early vigour without disrupting the germinating tissue (Kim et al., 2020). Collectively, X-ray and micro-CT approaches provide a level of internal structural detail unmatched by surface imaging or spectroscopy, though at a substantially higher instrumentation cost and lower throughput compared with RGB or NIR-based systems, restricting current use primarily to research settings rather than routine seed-testing laboratories (Gomes, dos Santos Bento, Martins, & Machado, 2019).

3.6 Root System Architecture Phenotyping

Because early root growth determines a seedling's capacity for water and nutrient acquisition and anchorage, root system architecture (RSA) is an increasingly important, though technically demanding, target for vigour phenotyping. High-throughput imaging platforms designed for *Arabidopsis* and crop seedlings grown on transparent agar media, such as the High Resolution Root Scanner (HIRROS), enable automated, non-destructive, time-resolved visualisation of root growth in controlled growth chambers, capturing dynamic traits including primary root elongation rate, lateral root emergence timing, and branching density (Aravind et al., 2022).

Automated analysis pipelines combining image registration with topological tracking algorithms have been developed to resolve complex, overlapping root systems in two-dimensional time-lapse image sequences, addressing a long-standing challenge in root phenotyping caused by root crossovers and occlusions (Aravind et al., 2022). In field-relevant contexts, semi-hydroponic and rhizotron-based root phenotyping platforms have linked seedling root traits, such as total root length and root:shoot ratio, to variation in seed yield and nutrient capture in field-grown oilseed rape, demonstrating that seedling-stage root architecture measurements can be predictive of mature-plant performance (Thomas et al., 2016).

A persistent methodological challenge is the inference gap between seedling-stage root phenotypes, typically measured in Petri dishes or agar columns over days to weeks, and mature root system performance in soil. Root architecture simulation models, such as RootBox, parameterised using seedling-stage traits from pea genotypes phenotyped in both Petri-dish and sand-column systems, have been used to bridge this gap, improving the prediction of mature root traits, including tap root length and lateral branching, compared with direct extrapolation from seedling measurements alone (Postma, Kuppe, Owen, Mellor, Griffiths, Bennett, Lynch, & Watt, 2017). In soybean, computer-vision and machine-learning pipelines integrating time-series image capture, barcode-based seedling identification, and convolutional autoencoder-based segmentation have enabled root phenotyping of hundreds to thousands of seedlings, substantially increasing throughput relative to manual root tracing methods (York, 2018).

3.7 Thermal Imaging and UAV-Based Field Emergence Phenotyping

While laboratory-based imaging methods excel at controlled-condition phenotyping, field-based seedling vigour, expressed as emergence rate, uniformity, and stand establishment, requires scalable remote sensing approaches. Unmanned aerial vehicles (UAVs) equipped with RGB, multispectral, and thermal sensors have rapidly become a preferred platform for high-throughput field emergence phenotyping, replacing labour-intensive manual stand counts (Feng et al., 2023). Figure 3 summarises the integrated aerial phenotyping platforms, sensor types, and downstream data products used in this approach.

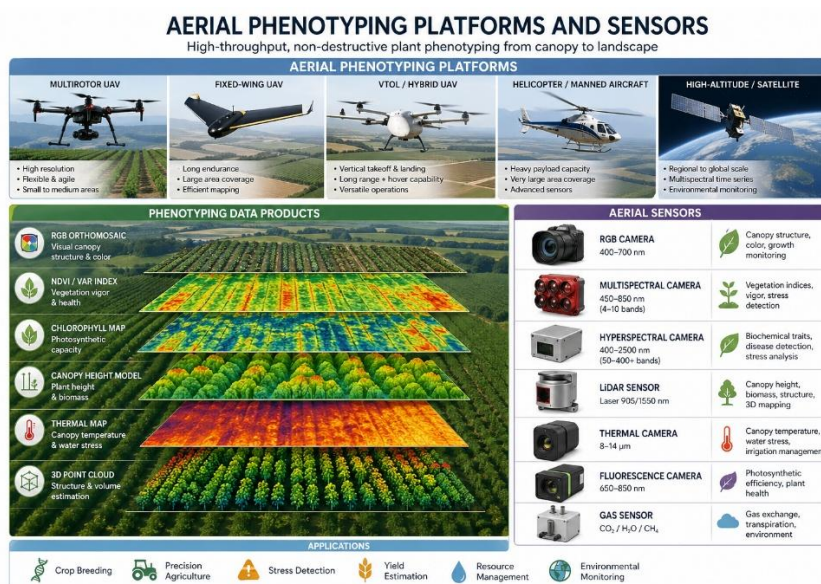


Figure 3 Integrated Overview of Aerial Phenotyping Platforms, Sensors, and Data Products for Precision Agriculture

In maize, a deep-learning-based system for quantifying seedling emergence from UAV RGB imagery was developed to recognise seedling location, spacing, and size, additionally deriving indices of emergence uniformity beyond simple plant counts; this framework was validated across three experimental stations over two growing seasons, demonstrating robustness to variable field and lighting conditions (Feng et al., 2023). An analogous ensemble learning model for soybean integrated seedling detection with an improved AlexNet-based growth-stage discrimination module to determine emergence proportion across VE (emergence), VC (cotyledon), and V1 (first-node) stages, achieving 99.92% seedling counting accuracy and 99.07% growth-stage classification accuracy (Zhang & Zhao, 2023).

Corn emergence uniformity has similarly been estimated and spatially mapped using UAV imagery and deep learning, building on earlier ground-based approaches that quantified uniformity via metrics such as growing degree units to 50% emergence and the Gibb's Index of categorical variability (Wang, Wu, Chen, & Shi, 2022). More recent work has explored lightweight, field-deployable object-detection architectures, such as YOLOv8n, for real-time seedling detection and counting from UAV RGB imagery, aiming to reduce computational demands sufficiently for on-board, real-time deployment (Li et al., 2025).

Thermal infrared imaging complements RGB and multispectral UAV sensing by detecting canopy or leaf temperature differentials associated with transpirational stress and stomatal function, which can reveal physiological vigour differences not visible in structural imagery, and has been integrated with meteorological data in related crop-monitoring applications (Zhang et al., 2025). While UAV-based phenotyping dramatically increases spatial and temporal throughput relative to manual field scoring, reported accuracies remain sensitive to flight altitude, sensor resolution, canopy overlap at later growth stages, and illumination variability, and most published models remain crop- and site-specific, limiting immediate cross-environment transferability (Feng et al., 2025; Li et al., 2025).

3.8 Electrophysiological and Biophysical Assays

Beyond the classical electrical conductivity bulk-leachate test described in Section 2, refined electrophysiological methods have been developed to increase sensitivity and reduce sample destructiveness. The single-seed conductivity test measures electrolyte leakage from individual seeds rather than bulk seed-lot samples, enabling detection of within-lot heterogeneity that bulk conductivity measurements obscure, and is recognised within the AOSA/SCST Seed Vigor Testing Handbook alongside the potassium leakage test, which specifically quantifies potassium ion efflux as a sensitive indicator of membrane damage (AOSA/SCST, 2009). Comparative studies across species have consistently demonstrated strong negative correlations between electrolyte leakage and seed germination performance; for example, in wall-rocket (*Diplotaxis* spp.) seeds subjected to artificial ageing, conductivity was negatively correlated with germination ($R^2 = 0.85$) and positively correlated with the time to 50% germination, T50 ($R^2 = 0.77$), with a conductivity threshold of 1.4–2.2 mS g⁻¹ dry weight identified as indicative of an 80% reduction in germination potential (Orsenigo et al., 2015). In *Lotus corniculatus*, optimisation of sample size and soak duration for the leakage conductivity test identified 100 seeds soaked for 24 hours as the configuration yielding the strongest correlation ($r = 0.93$ – 0.98) with subsequent seedling emergence in soil (Gonzalez-Zertuche & Vazquez-Yanes, 2009).

Emerging biophysical approaches include bioimpedance and electrical impedance spectroscopy, which probe tissue-level electrical properties across a range of frequencies to characterise cell membrane capacitance and extracellular resistance in imbibing seeds and developing seedlings, offering potentially greater physiological

resolution than single-frequency conductivity measurements, though these remain at an earlier stage of validation for routine seed-vigour testing compared with the conventional EC test (Matthews & Powell, 2006).

3.9 Omics-Based Biomarker Discovery: Proteomics, Metabolomics, Transcriptomics, and Epigenomics

A fundamentally different, molecular-level approach to vigour assessment seeks to identify biochemical and genetic biomarkers that predict or explain variation in seedling vigour, moving beyond phenotypic correlation toward mechanistic understanding. The landmark review by Rajjou, Duval, Gallardo, Catusse, Bally, Job, and Job (2012) synthesised evidence that germination vigour depends on the coordinated interplay of stored messenger RNA quality, proteostasis, and DNA integrity established during seed maturation, with sulfur amino acid metabolism identified as a key biochemical determinant of the seed's commitment to germination.

Comparative proteomic studies have identified specific protein biomarkers associated with vigour loss and its reversal through priming. In sugarbeet, eighteen proteins were found to be up-regulated during hydropriming (an invigoration treatment) and down-regulated during controlled-deterioration ageing, with the down-regulation reversible upon subsequent priming of aged seeds, while isocitrate lyase, a glyoxylate-cycle enzyme, emerged as a particularly robust candidate diagnostic marker of seed vigour, showing consistent depletion in deteriorated seeds across species (Catusse et al., 2011). In poplar, proteomic analysis linked declining seed vigour to oxidative modification of stored proteins, reduced translational capacity, and repression of energy metabolism enzymes, echoing patterns previously described in *Arabidopsis* subjected to controlled versus natural ageing protocols (Ma, Zhao, & Li, 2015; Rajjou et al., 2008).

Metabolomic profiling, typically using gas chromatography-mass spectrometry (GC-MS), has been applied to identify small-molecule biomarkers of seed ageing and vigour loss. In hybrid rice, metabolomic comparison of seed lots differing in storability identified a defined panel of metabolites, drawn from 89 detected compound peaks, that tracked with degree of natural and artificial ageing, offering candidate rapid biochemical assays for storability and vigour screening (Xin, Tao, & Zhou, 2021). Complementary work profiling primary carbon metabolism has highlighted specific enzymes and metabolites, including those linked to protein synthesis initiation and elongation, as promising quality biomarkers reflecting the metabolic reactivation that occurs during early imbibition (Domergue et al., 2019).

Multi-omics integration is increasingly used to resolve the hormonal and metabolic regulatory networks governing emergence vigour in direct-seeded rice, in which classical gibberellin-abscisic acid antagonism is modulated by additional cytokinin signalling affecting cell division and dormancy release, with integrated transcriptome-metabolome analyses revealing dynamic regulatory hubs coordinating amino acid, starch, and sucrose metabolism during the transition from seed to seedling (Chen, Wang, & Liu, 2026). Beyond proteomics and metabolomics, -omics-based biomarker classifications encompassing molecular, biochemical, physiological, metabolic, mitochondrial, and morphological signals of seed ageing have been systematically catalogued across seventeen species, providing a framework for prioritising candidate biomarkers for future high-throughput vigour diagnostics (Fu et al., 2019).

Although omics-based biomarker discovery currently requires specialised analytical infrastructure (mass spectrometry, sequencing platforms) that precludes routine deployment in seed-testing laboratories, these approaches provide the mechanistic foundation upon which more accessible, targeted rapid assays, such as antibody-based biomarker strips or PCR-based expression assays for key vigour-associated transcripts, may eventually be developed (Rajjou et al., 2012; Wang et al., 2015).

3.10 Other Emerging Technologies

Several additional technologies, while less extensively validated for seedling vigour specifically, show emerging promise. Electronic nose (e-nose) systems, which detect volatile organic compound emissions from imbibing or deteriorating seeds using arrays of gas sensors, have been explored as rapid, non-destructive proxies for seed physiological status, capitalising on the association between lipid peroxidation, membrane deterioration, and volatile aldehyde and alcohol release during seed ageing (Domergue et al., 2019). Terahertz spectroscopy and imaging, which probe rotational and vibrational molecular transitions at frequencies between microwave and infrared, are being investigated for moisture and internal-defect detection in seeds, offering potential complementary information to NIR spectroscopy, though validation specifically against vigour outcomes remains limited (Al-Amery et al., 2018).

Single-seed near-infrared sorting technology, increasingly integrated into commercial seed-conditioning lines, enables real-time culling of low-vigour seeds from a processing stream based on NIR spectral signatures, effectively converting a laboratory diagnostic method into an operational quality-improvement tool (Boelt et al., 2018). Similarly, chlorophyll-fluorescence-based commercial seed sorters, grounded in the maturity-fluorescence relationship established by Jalink et al. (1998), are now used industrially to remove immature, low-vigour seeds from vegetable seed lots prior to sale (Basu & Groot, 2023).

4. Multi-Sensor Data Fusion and Artificial Intelligence-Driven Integration

A clear trajectory across the innovative methods reviewed above is the trend toward combining multiple data streams, morphological, spectral, thermal, and increasingly molecular, within unified machine learning frameworks to capture complementary dimensions of seedling vigour that no single sensor modality can resolve alone. The rice vigour testing pipeline MsiFormer exemplifies this trend, fusing object detection

(spatial/morphological information) with vision-transformer-based analysis of 19-waveband multispectral data (biochemical information) within a single end-to-end deep learning architecture (Zhao et al., 2023). Similarly, integrated optical imaging studies in tomato and carrot combined chlorophyll fluorescence measurements with chemometric multispectral image analysis, demonstrating that fusing fluorescence-based maturity information with reflectance-based compositional information improved cultivar and vigour-class discrimination beyond either modality alone (Boelt et al., 2020). In UAV-based field phenotyping, the integration of RGB, multispectral, and thermal imagery, alongside meteorological covariates, is increasingly used to model emergence and early vigour as a function of both plant-level structural traits and environmental stress indicators, moving field phenotyping from simple stand counting toward predictive, environment-aware vigour modelling (Feng et al., 2025).

From a computational standpoint, these data fusion strategies rely heavily on advances in deep learning architectures capable of handling heterogeneous, high-dimensional inputs, including multi-branch CNNs, attention-based transformer models, and ensemble learning frameworks that combine the outputs of multiple specialised sub-models (Zhang & Zhao, 2023). A practical constraint on wider adoption of these fused, AI-driven pipelines is the substantial labelled training data required, typically necessitating destructive reference measurements, such as standard germination or accelerated ageing outcomes, to calibrate and validate the models, meaning that even the most sophisticated non-destructive AI systems remain fundamentally dependent on conventional vigour assays as ground truth (Liu et al., 2025; Al-Amery et al., 2018).

5. Comparative Evaluation of Methods

The principal innovative methods reviewed above according to their underlying principle, non-destructiveness, relative throughput, and primary limitations, providing a comparative reference for method selection according to laboratory resources and application context, are summarised in Table 1.

Table 1 Comparative Evaluation of Innovative Seedling Vigour Assessment Methods

Method	Principle	Destructive	Relative Throughput	Key Limitation
Digital image analysis / computer vision	Seedling morphology (length, area, colour) from RGB images	No	High	Limited to external morphology; cannot detect internal deterioration
Machine learning / deep learning classification	Pattern recognition on image or spectral features	Depends on input data	High (after training)	Requires large labelled training datasets; limited interpretability
NIR / hyperspectral / multispectral imaging	Spectral reflectance linked to biochemical composition	No	Medium–High	High instrument cost; complex chemometric modelling
Chlorophyll fluorescence imaging	Residual chlorophyll / photosystem II efficiency	No	High	Species- and maturity-stage-dependent sensitivity
X-ray radiography / micro-CT	Internal structural density and morphology	No	Low–Medium	Expensive equipment; possible radiation exposure effects
Root system architecture phenotyping	Time-resolved imaging of root growth in agar/rhizotron	No (in vitro)	Medium	Petri-dish traits do not always predict field/mature root traits
UAV / thermal field phenotyping	Aerial RGB, multispectral, thermal imagery of field stands	No	Very High (field scale)	Sensitive to flight altitude, canopy overlap, weather
Electrophysiological assays (EC, impedance)	Electrolyte leakage / membrane integrity	Partially (imbibed seeds)	Medium	Bulk EC masks within-lot heterogeneity unless single-seed
Omics biomarkers (proteomics, metabolomics)	Molecular signatures of deterioration/vigour	Yes	Low	Requires specialised infrastructure; not yet routine

6. Challenges and Limitations

Despite substantial technological progress, several challenges continue to constrain the translation of innovative vigour-assessment methods from research settings into routine seed-testing and breeding practice. First, standardisation remains limited: unlike the ISTA and AOSA-codified conventional tests, most image-based, spectroscopic, and machine-learning methods lack universally agreed protocols for sample preparation, imaging conditions, and model validation, leading to reported accuracies that are often specific to the crop, cultivar, instrument, and dataset used in a given study (ISTA, 2021; Al-Amery et al., 2018).

Second, most machine learning and deep learning models require substantial labelled training datasets generated using destructive reference tests, meaning that the promise of fully non-destructive vigour assessment is, in practice, contingent on an initial, often extensive, destructive calibration phase; furthermore, models trained on

one crop, cultivar set, or environment frequently generalise poorly to new genetic material or growing conditions without retraining (Liu et al., 2025; Feng et al., 2025).

Third, instrumentation cost and technical expertise present significant adoption barriers, particularly for hyperspectral imaging systems, X-ray micro-CT scanners, and omics platforms, which remain financially and technically inaccessible to many small- and medium-sized seed companies and public breeding programmes, especially in resource-limited settings (Gomes et al., 2019; Rajjou et al., 2012). Fourth, the biological complexity of vigour itself, a multifactorial trait shaped by genotype, maternal environment during seed development, harvest and storage conditions, and mechanical handling, means that no single sensor modality is likely to fully capture vigour variation, reinforcing the need for, but also the complexity of, multi-sensor data fusion approaches (Finch-Savage & Bassel, 2016; Basu & Groot, 2023).

Finally, the inference gap between controlled laboratory or in vitro seedling phenotypes and actual field emergence and establishment performance remains a persistent concern across virtually all innovative methods, from root system architecture phenotyping in agar plates to chlorophyll fluorescence imaging under artificial lighting, underscoring the continued necessity of field validation studies to confirm that laboratory-derived vigour indices translate into meaningful predictions of agronomic performance (Postma et al., 2017; Thomas et al., 2016).

7. Future Directions

Several priority research directions are likely to shape the next generation of seedling vigour assessment technologies. Continued development of low-cost, portable spectral and imaging sensors, potentially integrated into smartphone-based platforms, could substantially broaden access to non-destructive vigour phenotyping beyond well-resourced laboratories, extending these tools to smallholder-oriented seed systems in developing regions (Al-Amery et al., 2018; Nagel et al., 2020).

Expanding shared, open-access image and spectral datasets, annotated with corresponding destructive reference measurements across diverse crops, genotypes, and environments, would help address the generalisability limitations of current machine learning models and facilitate the development of transfer-learning approaches capable of adapting pre-trained models to new species or conditions with minimal additional calibration data (Zhang & Zhao, 2023; Liu et al., 2025).

Greater emphasis on multi-modal data fusion, combining morphological, spectral, thermal, and where feasible targeted molecular biomarker data within unified predictive frameworks, is likely to yield vigour indices more robust than any single modality, particularly if guided by mechanistic insight from omics-based biomarker discovery regarding which physiological processes are most diagnostic of vigour loss (Rajjou et al., 2012; Boelt et al., 2020). Closer integration between root system architecture phenotyping and mechanistic growth-simulation models, following the approach demonstrated for pea root systems, offers a promising route to reconcile rapid seedling-stage phenotyping with predictions of mature-plant field performance (Postma et al., 2017).

Finally, continued engagement between seed technology researchers and international standard-setting bodies such as ISTA and AOSA will be essential to establish validated protocols and inter-laboratory reproducibility benchmarks for the most mature innovative methods, a necessary step for their formal incorporation into international seed testing rules alongside the long-established conventional vigour tests (ISTA, 2021; AOSA/SCST, 2009).

8. CONCLUSION

Early seedling vigour is a multifactorial, physiologically complex trait that exerts a disproportionate influence on crop establishment and productivity, yet its accurate and rapid assessment has historically been constrained by the destructive, subjective, and time-consuming nature of conventional vigour tests. Over the past two decades, an increasingly diverse toolkit of innovative technologies, spanning digital image analysis, machine learning and deep learning classification, hyperspectral and multispectral imaging, chlorophyll fluorescence imaging, X-ray radiography and micro-computed tomography, root system architecture phenotyping, UAV-based field emergence monitoring, refined electrophysiological assays, and omics-based biomarker discovery, has substantially expanded the capacity to evaluate seedling vigour non-destructively, rapidly, and at scale.

No single method currently offers a complete replacement for conventional vigour testing; rather, each technology captures a partial, complementary dimension of the multifactorial vigour phenotype, from external morphology and biochemical composition to internal structural integrity and molecular deterioration signatures. The most promising path forward lies in the thoughtful integration of these modalities within multi-sensor, AI-driven frameworks, calibrated against robust destructive reference standards and validated across diverse genetic and environmental contexts. Realising the full potential of these innovations for global seed quality assurance and crop improvement will require sustained investment in standardisation, open data sharing, and accessible instrumentation, ensuring that the benefits of these technological advances extend beyond well-resourced research institutions to the broader seed industry and, ultimately, to farmers worldwide.

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