www.sasinsights.com E-ISSN: 3107-135X
SCIENCE AND SOCIETY INSIGHTS
Sci Soc Insights (2026), 5: 26-43
Review Article

Genetic Improvement for Resource-Use Efficiency and Sustainable Crop Management

Ali Murtaza[1*], Ayaz Haider Khan[1], Malaika Imtiaz[1], Laiba Ansari[2] and Muhammad Haris Shahid[2]

[1]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan
[2]Department of Agronomy, University of Agriculture Faisalabad, Pakistan
**Corresponding author*: mralimurtazarai@gmail.com

Article History: 26-004 Received: 28 Nov 2025 Revised: 01 Jan 2026 Accepted: 10 Jan 2026 Published Online: 2026

Citation: Murtaza A, Khan AH, Imtiaz M, Ansari L and Shahid MH, 2026. Genetic improvement for resource-use efficiency and sustainable crop management. Sci Soc Insights, 5: 26-43. https://doi.org/10.65822/j.sasi/2026.003

ABSTRACT

Improving the resource-use efficiency (RUE) is a corner stone to develop sustainable and climate resilient crops. This review is a synthesis of the physiological and genetic basis of nitrogen (NUE), phosphorus (PUE) and water use efficiency (WUE) as well as strategies for optimizing photosynthesis. We describe the important roles played by nutrient remobilization, root system architecture, stomata regulation and osmotic adjustment. The paper goes on to discuss higher-order genetic manipulation, such as the use of transgenic approaches and precision genome editing techniques such as multiplexing, base and prime editing, in the engineering of key transporters, transcription factors (e.g. Dof1, PHR1, DREB), and hormonal pathways, in order to deconstruct these complex traits. We highlight the need to combine these improved genotypes with sustainable management practices such as precision agriculture and agro ecology and show their synergistic potential to minimize environmental footprints through Life Cycle Assessment. Finally, we discuss the ongoing challenges in genotype-by-environment interactions and regulatory issues and call for future research focusing on predictive breeding using integrated pan-genomics, phonemics' and machine learning for accelerating the breeding of the next-generation, resource-efficient crops.

Keywords: Resource-Use Efficiency, Nitrogen Use Efficiency (NUE), Water Use Efficiency (WUE), Genome Editing, Root System Architecture, Predictive Breeding and Sustainable Agriculture.

INTRODUCTION

The growing demands of a global population and the escalating volatility of climatic changes that we are witnessing are posing an unprecedented challenge to agricultural sustainability and food security. Conventional agricultural practices, which rely heavily on the use of high inputs of synthetic fertilizers and irrigation, have managed to increase yields, but at a considerable environmental cost, including greenhouse gas emissions, water scarcity and eutrophication (Kumar et al., 2024; Ma et al., 2022). This has added to the urgency of developing crop varieties capable of producing more food with fewer resources - one of the key positions of sustainable intensification. At the center of this quest is the complex trait of resource-use efficiency (RUE), which includes the ability of the plant to successfully acquire and use basic resources, such as nitrogen, phosphorus, and water, and to convert them to biomass by means of the process of photosynthesis.

The physiological and genetic basis of RUE are complex, and are determined by a complex network of processes such as nutrient uptake, assimilation and remobilization and sophisticated sensing and response mechanisms to abiotic stresses. For example, nitrogen use efficiency (NUE), which is critically dependent not only upon uptake and assimilation but also remobilization (NREM) of nitrogen from senescing leaves to developing grains, this process depends on specific proteases and autophagy which can account for more than 50-90% of grain nitrogen (Du et al., 2024; W. Wang et al., 2023). Similarly, phosphorus use efficiency (PUE) is attained by changes in root architecture and phosphate transporters regulation & water use efficiency (WUE) by genetic control of stomatal patterning, deep root systems and osmotic adjustment (Z. Li et al., 2021; Uga, 2021).

Recent advances in genetic and genomic technologies have greatly increased the speed with which these complex characteristics can be dissected and manipulated. The advent of high-throughput sequencing, genome-wide association studies (GWAS), and especially advanced genome-editing technologies such as the ones offered by especially advanced genome-editing technologies such as CRISPR-Cas9, Cas13, and Cas14, as well as base editing and prime editing, has changed the face of precision breeding (Huang et al., 2021). 6 These tools allow the direct deconstruction of polygenic traits, the fine-tuning of plant architecture as well as the production of novel and enhanced alleles, opening the door to the implementation of a "Green Revolution 2.0" (Huang et al., 2021). Furthermore, there is a synergy among genetically improved crops and sustainable management practices, including precision agriculture and agro ecological systems, providing a transformative path towards reducing the environmental footprint of food production (Legzdiņa et al., 2022; Singh et al., 2025).

This review is an attempt to provide a synthesis of the existing knowledge about the genetic and physiological mechanisms of resource-use efficiency in major crops. We will give an overview of the progress made in genetic manipulation (i.e. transgenic and genome-editing) to improve macro-nutrient efficiency, drought tolerance, and photosynthetic capacity. A major goal is to investigate the integration of such improved genotypes with sustainable crop management processes and to evaluate their impact in combination with the sustainability approach (Life Cycle Assessment, LCA). Finally, we will discuss and critically analyze the remaining technical, regulatory and socio-economic challenges that need to be overcome and future research and policy directions required in order to realize the full potential of next generation, resource efficient crops.

The Physiological and Genetic Foundations of Resource-Use Efficiency

At the center of the effort for sustainable crop improvement is the search for resource-use efficiency, which is a complex trait with complex physiological and genetic bases. This pursuit is focused on the most fundamental processes of nitrogen, phosphorus, and water use as well as the fundamental engine of plant growth: photosynthesis. For nitrogen, efficiency is not only up to the uptake and assimilation, but importantly to the remobilization (NREM) of nutrients from senescing leaves to grains, which is a process catalyzed by specific proteases and autophagy. Similarly, phosphorous efficiency is pursued using a two-fold approach of altering root architecture with a view to increasing soil exploration and controlling phosphate transporters for efficient acquisition. Concurrently, water use efficiency is maximized by genetic regulation of stomatal patterning, development of deep root systems for water mining and elaborate osmotic adjustment mechanisms. Finally, improving the photosynthetic efficiency is itself a huge possibility, with attempts being made to engineer solutions for overcoming the yield limiting process-photorespiration, as well as optimizing light capture- Rubisco engineering and optimized canopy architecture. Together, these interconnected pathways make up a whole blueprint for producing next-generation crops that can thrive with fewer inputs.

Nitrogen Use Efficiency (NUE): Uptake, Assimilation, and Remobilization

Uptake

The genetic dissection of these pathways via the use of QTL mapping and genome-wide association studies (GWAS) have further identified natural allelic variations in genes such as TaGS2-2A and TaAAP2-3B that are associated with superior NREM efficiency that serve as valuable markers for breeding (Du et al., 2024; Zhen et al., 2021). Similarly, the expression of the OsNAC42 transcription factor in rice results in the improvement of NUE through the enhancement of nitrate transporter expression and uptake (Zhang et al., 2021). The transcription of these transporters is regulated by a central regulatory network that involves phosphate starvation response 1 (PHR1) and its downstream targets that is activated in low-Pi conditions. The expression of high-affinity PHT1 transporters is often concentrated in root epidermal cells including root hairs thus providing a direct relationship between the morphological and molecular strategies of P acquisition.

Assimilation

Following proteolysis, the released amino acids have to be loaded into the vasculature for long distance transportation to the grain. This involves important family of transporters and a critical step of re-assimilation catalyzed by glutamine synthetase (GS). The GS2 isoform, which is localized in the chloroplast, is crucial for re-assimilating ammonia released during photorespiration and proteolysis and its activity in senescing flag leaves is an excellent predictor of grain protein content in wheat and maize (C. Li et al., 2025; Nehe et al., 2020).

Remobilization

A critical, yet often overlooked component of NUE is nitrogen remobilization (NREM), the process by which N from senescing vegetative tissues is hydrolyzed, transported and re-assimilated into developing grains. This senescence-associated recycling can account for more than 50-90% of the grain N content, especially if soil N availability decreases during the reproductive stage (Du et al., 2024; W. Wang et al., 2023). The efficiency of NREM is thus a major factor determining the protein content of the grain and the total NUE. The first and important step of NREM are the massive amounts of proteolysis in the senescing leaves that liberates amino acids for transport. This process is mediated by a suite of proteolytic enzymes with predominant roles being played by papain-like cysteine proteases and autophagy. In wheat expression of senescence-associated genes such as TaSAG2 and TaNAP is strongly correlated with the high expression of cysteine proteases (TaCEP1) which enable the degradation of chloro-plastic proteins, Rubisco being the main target (El Mazlouzi et al., 2022; Ma et al., 2025). Concurrently, autophagy is a catabolic pathway that is conserved in cellular degradation, which is indispensable for bulk degradation of cellular components. Knockdown of core autophagy-related (ATG) genes e.g. OsATG8, OsATG10 of rice cause delayed senescence and reduced protein degradation and significant reduction in grain yield and protein content, indicating the non-redundant role of autophagy in NREM (Hundleby & Harwood, 2022; Lv et al., 2020). The concerted activity of certain proteases and the auto-phagic machinery guarantees the effective release of amino acids in the main form of glutamine and asparagine, which are the basic transport forms of organic N. The amino acid transporters (AAPs) then help transport the glutamine and other amino acids into the phloem. For example, OsAAP3 in rice is up-regulated in the vascular system of leaves during senescence and overexpression of OsAAP3 in rice promotes NREM and grain protein content, while knockout of OsAAP3 has the opposite effect (Padhan et al., 2021).

Phosphorus Use Efficiency (PUE): Acquisition from the Rhizo-sphere

Root architecture modifications (cluster roots)

The first of the pillars of this strategy is the precise modification of the root system architecture (RSA) to produce a "foraging root" that may be more effective at exploiting heterogeneous soil P pools. Under Pi deficiency conditions, plants activate a series of developmental adaptations, such as greater lateral roots, greater root hair density and elongation and a shallower angle of growth to promote topsoil exploration. These morphological changes do not occur randomly but are regulated by elaborate signaling networks, hormones such as auxin and ethylene playing a pivotal role in this process as key regulators that spatially control the development of the cells in the roots in order to maximize the volume of soil exploited for every unit of carbon invested. The proliferation of lateral roots and elongation of root hairs increase dramatically the root surface area, which provide in turn a greater physical platform for deployment of high-affinity PHT1 transporters.

Exudation of organic acids and phosphatases

Manipulating genes concerned with the internal remobilization of P, like purple acid phosphatases (PAPs) and ribonucleases (RNAses) assist in rescue of P from organic compound during deficiency (Singh et al., 2021; Wani et al., 2021). For instance, in organic systems, the ability of a plant to efficiently mobilize P from the soil through root exudates (e.g. carbonates) or establish associations with arbuscular mycorrhizal fungi are of prime importance (Legzdiņa et al., 2022; Scandurra et al., 2024).

Phosphate transporter (PHT) families and their regulation

Concurrently with this, molecular physiology of Pi acquisition is determined by the activity of phosphate transporter (PHT) families, the expression and function of which are closely controlled by soil P availability. The PHT1 family in particular is responsible for the uptake of Pi from the rhizosphere followed by its distribution in the plant. The transcription of these transporters is regulated by a central regulatory network that involves PHOSPHate starvation response 1 (PHR1) and its downstream targets that is activated in low-Pi conditions. This regulation means that the biochemical machinery for the acquisition of Pi is turned on at just the right time and place it needs to be. Post-translational modifications and specific protein participation are further used to fine-tune PHT1 localization and activity preventing futile energy cycles in Pi-replete conditions and Pi scavenging during deficiency. The transcription factor PHR1 (PHOSPHATE STARVATION RESPONSE 1) is a key regulator similar to Dof1 for NUE which regulates a wide range of phosphate starvation-induced genes and its overexpression will enhance PUE in multiple plant species (Wani et al., 2021; Zhang et al., 2021). Overexpression of high-affinity phosphate transporters, e.g. OsPT6 in rice, improves phosphate uptake from the soil (Singh et al., 2021).

Water Use Efficiency (WUE): Physiology and Sensing Mechanisms

Genetic control of stomatal density and patterning.

The role of abscisic acid (ABA) signalling in drought response.

The DREB (Dehydration-Responsive Element Binding) sub-family of AP2/ERF transcription factors has become a leading candidate for this role, by binding directly to the dehydration-responsive element/C-repeat (DRE/CRT) cis-element in the promoters of an enormous number of stress-responsive genes (Kaya, 2025; Zhang et al., 2025). Figure 1 illustrates the mechanistic framework of DREB-mediated stress tolerance, depicting how DREB proteins activate downstream effector genes involved in osmoprotectant synthesis, oxidative stress protection, and hydraulic regulation. This first trigger initiates a coordinated cascade of transcriptional responses that is central to survival during drought and salinity. The mechanistic basis of tolerance conferred by DREB can be fragmented in three 'core' and interconnected physiological strategies, which are the activation of downstream effector genes, osmotic adjustment and mitigating oxidative damage. Concurrently, DREBs adjust osmotic adjustment, through up regulations of the biosynthesis of compatible osmolytes, proline, glycine betaine, and soluble sugars. These compounds act as molecular chaperones and osmo-protectants to preserve turgor pressure and protect the enzymatic function without interfering with the cell's metabolism (Z. Li et al., 2021; Zhou et al., 2020).

Deep root system genes and osmotic adjustment mechanism.

The genetic and physiological background of drought tolerance is significantly determined by the architecture of the root system (RSA), which is the "hidden half" of the plant and is responsible for the efficiency of water mining from the soil. A key characteristic which gives resilience is deep rooting which allows access to more stable water reservoirs in the subsoil horizons. Genetic control of deep rooting is associated with genes and quantitative trait loci

(QTLs) for the growth vigour of roots and responses to dry upper layers of soil. For example, in some cereals such as wheat and rice, particular QTLs are linked to deep roots development under drought, and this directly leads to a sustained canopy water status and yield stability (Uga, 2021). Complementing the rooting depth, the root growth angle is an important determinant of RSA spatial distribution directing root exploration either shallow or deep. A steeper and more vertical angle of root growth is facilitating a "steep, cheap, and deep" architecture, which is an efficient way of directing root growth downwards with a lower metabolic cost. Plants with narrower root angles have better drought avoidance ability due to their ability to quickly colonise deeper, wetter layers of soil ahead of competitors or surface drying (Uga, 2021). This architectural trait is under genetic control and there are some canonical examples of how a change in root angle can dramatically increase drought tolerance through deep rooting such as DRO1 in rice. Once water is extracted by the roots, its conduction to the shoot is controlled by xylem anatomy and root hydraulic conductivity which is a crucial physiological aspect of WUE. Xylem vessels with smaller diameter, while sometimes having a negative effect in reducing hydraulic conductivity, provide a very important advantage, giving higher resistance to drought-induced cavitation and embolism, thus ensuring hydraulic continuity under low water potentials (van der Bom et al., 2020). The genetic control of aquaporin's (water channels in cell membranes) enables plants to adapt hydraulic conductivity dynamically to changing soil moisture as a fine-tuning mechanism for water flow in plants.26,27 #75 This subterranean regulation of the hydraulic system is intrinsically related to the shoot-level estimates of WUE; roots with the ability to maintain the water supply from the deep soil layers, allow to continue carbon assimilation in the leaves, which improves the integrated whole plant WUE, biomass accumulation under drought conditions.

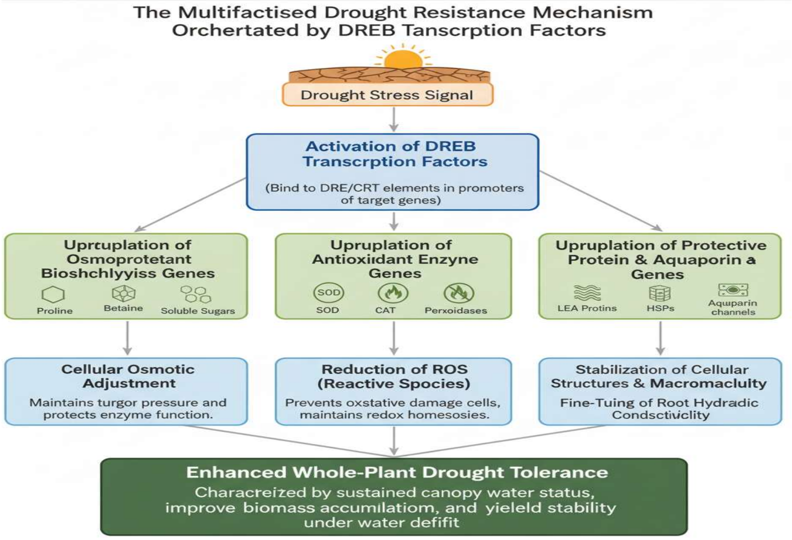

Fig. 1: Schematic representation of DREB-mediated activation of downstream effector genes leading to osmotic adjustment, oxidative stress protection, and cellular stabilization under drought and salinity stress. Stress-inducible promoters (Rd29A) enable targeted expression to circumvent pleiotropic growth penalties.

Enhancing Photosynthetic Efficiency

Overcoming photorespiration (C4 rice, synthetic photorespiration bypassing).

Of the many limitations imposed on photosynthetic efficiency in C3 crops, the process of photorespiration is a major fixed carbon and energy sink: potential yields are diminished by up to 40% in hot and dry conditions. This evolutionary relic is worsened by a rise in temperature and is a prime target of bioengineering. Initial attempts to bypass this expensive pathway have been to engineer synthetic 'photo respiratory' bypasses in which the metabolic flow of glycolate is diverted. Pioneering work involves inserting Escherichia coli glycolate catabolic pathways into plant chloroplasts, which showed that substitution of the native photo respiratory cycle by more efficient catabolic enzymes such as glycolate dehydrogenase and glyoxylate carboxyligase may give substantial improvement in biomass productivity in model plants such as Arabidopsis thaliana. However, having the ability to translate these successes to the staple crops has been difficult, leading either to chlorosis or stunted growth due to metabolic imbalance and the inability to accurately coordinate multiple transgenes in different cellular compartments. A more refined approach is the formation of intracellular metabolons, i.e., supramolecular complexes of sequential enzymes, to channel toxic intermediates, such as glyoxylate, and to avoid its release into the stroma. Recent developments within the field of synthetic protein scaffolds for co-localization of the bypass enzymes directly within the chloroplast have shown potential in reducing the metabolic crosstalk and enhancing catalytic efficiency, and can be considered a major step towards the robust implementation of this approach (Inckemann et al., 2024). A more ambitious and structurally complex approach is to engineer the entire C4 photosynthetic machinery into C3 plants; for example the international C4 Rice Project. The C4 pathway essentially is a carbon-concentrating mechanism which shuts down photorespiration by increasing CO2 levels near RuBisCO. This endeavor involves the concomitant installation of two interconnected components; the biochemical module and the anatomical module. Progress on the biochemical front has been very good with the successful reconstitution of key C4 enzymes, PEPC, PPDK and NADP-ME in rice, often using mesophyll-specific promoters. Some of the engineered lines show a C4 like metabolic flux; malate and aspartate is the primary carboxylation product. However, the development of the full potential of C4 efficiency is intrinsically related to the development of Kranz anatomy, which is a specialized leaf structure that spatially separates the carbon fixation (mesophyll cells) and assimilation (bundle sheath cells) cycles. The engineering of this complex three-dimensional trait de novo has been the more difficult task, as it requires coordinated changes in the density of veins, chloroplast differentiation and plasmo-desmatal connectivity (Q. Wang et al., 2023). A comprehensive summary of the key physiological mechanisms, their corresponding genetic targets, and engineering strategies for each resource-use efficiency trait is presented in Table 1. While the genetic screens have identified important regulators of leaf development contributing to these traits, and the genetic toolbox of inducing a reliable two-cell system in a C3 background is being studied, there is still room for discovery.

Table 1: Key Traits and Genetic Targets for Improving Nitrogen, Phosphorus, and Water Use Efficiency

Resource	Key Physiological Mechanism	Key Genetic / Molecular Components	Engineering Strategy & Observed Effect	References
Nitrogen	Uptake & Assimilation: Nitrate transport and incorporation into amino acids.	Transporters: NRT1/2, AMT1 families Enzymes: Glutamine Synthetase (GS) Transcription Factors (TFs): NLP, Dof1	Overexpression of *Dof1* rewires C/N metabolism, enhancing N uptake and biomass under low N.	(Cun et al., 2024; Islam et al., 2022)
	Remobilization (NREM): Proteolysis and autophagy for N recycling from senescing leaves to grains.	Proteases: Papain-like cysteine proteases (e.g., TaCEP1) Autophagy: ATG genes (e.g., OsATG8) Transporters: AAP family (e.g., OsAAP3)	Knockout of *OsNLP4* enhances N uptake. Overexpression of *OsAAP3* promotes NREM and grain protein content.	(Islam et al., 2022)
Phosphorus	Root Architecture: Modification for topsoil exploration (lateral roots, root hairs).	Hormonal Pathways: Auxin, Ethylene Genes: Regulators of root hair density and growth angle	Editing of auxin signaling components (AUX/IAA-ARF module) to develop shallow, branched roots for P foraging.	(Sharma et al., 2021; Zhang et al., 2023)
	Acquisition & Recycling: Phosphate transport and mobilization from organic sources.	Transporters: PHT1 family (e.g., OsPT6) TFs: PHR1 Enzymes: Purple Acid Phosphatases (PAPs)	Overexpression of *PHR1* or *OsPT6* enhances phosphate uptake. Manipulation of *PAPs* salvages P from organic pools.	(Sharma et al., 2021; Zhang et al., 2023)
Water	Stomatal Regulation: Control of gas exchange and transpiration.	Signaling: Abscisic Acid (ABA) pathway Developmental Genes: Regulators of stomatal density	Engineering stomatal density and sensitivity to reduce water loss without compromising CO_2 uptake.	(Giannoutsou et al., 2025; Kaur et al., 2021)
	Root System & Osmotic Adjustment: Deep water mining and cellular tolerance to dehydration.	Root Architecture: DRO1, QTLs for deep rooting TFs: DREB, NAC Osmoprotectants: P5CS (proline), betA (glycine betaine)	Inducible overexpression of *DREB* factors activates osmoprotectant synthesis and antioxidant systems, enhancing drought tolerance.	(Khan, 2025; Robertson et al., 2021)

Genetic Manipulation for Enhanced Resource-Use Efficiency

Contemporary Crop Improvement is increasingly characterized by sophisticated genetic interventions to optimise the use of resources and stress resistance. One of the main approaches is to engineer improved nutrient use efficiency and this can be realised by engineering of master transcription factors such as Dof1 to rewire nitrogen metabolism and the editing of negative regulators such as NLP genes to increase uptake. Parallel work on enhancing phosphorus uptake is aimed at overexpression of phosphate transporters as well as the central player PHR1. For abiotic stresses, the inducible expression of transcription factors, such as DREB, under inducible promoters leads to a multifaceted response, the induction of protection genes for the synthesis of osmolytes, antioxidant activity, and hydraulic control through aquaporins. Furthermore, precision breeding now also involves modification of root system architecture by manipulation of auxin signaling pathways and genes such as DRO1 for developing specialized root ideotypes for better soil foraging. Collectively, these methods of transgenic and genome editing open up a sophisticated toolbox to deconstruct complicated agronomic characters to pave a way for the development of high-yielding cultivars with less input needs.

Engineering Macro-Nutrient Efficiency: Transgenic and Editing Approaches

Case Study: NUE: Overexpression of ALMT (aluminum activated malate transporter) for better nitrogen uptake; manipulation of Dof1 transcription factor.

A key proof-of-concept for this strategy is the manipulation of transcription factors and the maize Dof1 protein is an excellent example. Heterologous expression of Dof1 in plants such as potato, wheat and Arabidopsis always leads to increased NUE through the coordinated expression of genes involved in organic acid metabolism, such as phosphor-enolpyruvate carboxylase (PEPC) (Fu, 2025). This transcriptional rewiring leads to the production of carbon skeleton for ammonia assimilation, which leads to better nitrogen uptake and biomass under low nitrogen conditions. The efficacious of Dof1 is due to its function as a master regulator of carbon flux towards the production of alpha-ketoglutarate, a critical acceptor of ammonium for integration into amino acids and thus optimizes carbon-nitrogen partitioning and core metabolism (Fu, 2025; Gupta et al., 2022). Beyond Dof1, other families of transcription factors have been targeted successfully to enhance NUE and this is often through manipulating the root architecture and nitrogen assimilation pathways. NLP (NIN-LIKE PROTEIN) transcription factors play an important role in activating genes induced by nitrate. NLP (NIN-LIKE PROTEIN) transcript factors are relevant for the activation of nitrate responsive genes and GRFs (GROWTH-Regulating FACTORS) interact with the GA and the cytokinin pathways to regulate root growth and nitrogen responsiveness (Sharma et al., 2023). The functional dissection of complex agronomic traits such as Nitrogen Use Efficiency (NUE) has been difficult in the past because of the complexity of genes that control the uptake, assimilation and signaling of nitrogen (N). The advent of the genome editing system, the cellular molecular scissors known as a Cas enzyme, in the form of the so-called genome editing technology known as the acronym for "Clustered Regularly Interspaced Short palindromic Repeats" has revolutionized this endeavor - offering a precise toolkit for the causal validation of genes and trait deconstruction. Knockout strategies, mostly based on the induction of frame shifts by the protein-editing technique of the clustered regularly interspaced short palindromic repeats (CRISPR) technology, have played a pivotal role in the characterization of negative regulators of NUE. For instance, editing of NIN-like protein (NLP) transcription factor genes OsNLP4 in rice and TaNLP2/3 in wheat produced knockout mutants with greatly enhanced N uptake and grain yield in low N conditions; thus, these genes were directly implicated as central repressors in the N signaling cascade (Liu et al., 2023). Similarly, knockout analysis of the ARE1 gene in rice suppressed N starvation-induced leaf senescence, confirming that ARE1 is a negative regulator of N remobilization (Karunarathne et al., 2022).

Case Study: PUE: Engineering secretion of phytases and overexpression of phosphate transporters.

For improvement in Phosphorus Use Efficiency (PUE), transgenic approaches have mainly been developed for the modulation of phosphate transporters and enzymes involved in phosphorus scavenging and recycling. Overexpression of the high-affinity phosphate transporters including OsPT6 in rice to boost phosphate uptake from soil (Singh et al., 2021). Furthermore, the manipulation of genes involved in the internal remobilization of phosphorus such as purple acid phosphatases (PAPs) and ribo-nucleases (RNAses) helps to salvage phosphate from organic compounds during deficiency (Singh et al., 2021; Wani et al., 2021). The transcription factor PHR1 (PHOSPHATE STARVATION RESPONSE 1) is a key regulator analogous to Dof1 for NUE that controls a wide range of phosphate starvation-induced genes, and its overexpression can enhance PUE in different plant species (Wani et al., 2021; Zhang et al., 2021).

Engineering Drought and Salinity Tolerance

Introduction of osmo-protectant genes (e.g., beta, P5CS).

Concurrently, DREBs regulate osmotic adjustment fine-tuning (up regulating biosynthesis of compatible osmolytes, such as proline, and glycine betaine and soluble sugars). These compounds serve as molecular chaperones and osmoprotectants to maintain turgor pressure and protect the function of enzymes without disturbing the cell metabolism (Z. Li et al., 2021; Zhou et al., 2020).

Overexpression of stress responsive transcription factors (DREB, NAC, and AREB).

Engineering complex abiotic stress tolerance in crops requires the existence of master regulatory switches orchestrating multifaceted physiological adaptations. The DREB (Dehydration-Responsive Element Binding) subfamily of AP2/ERF transcription factors has become a leading candidate for such a role, directly binding the dehydration-responsive element/C-repeat (DRE/CRT) cis-element located in the promoters of a large number of stress-responsive genes (Kaya, 2025; Zhang et al., 2025). This initial trigger initiates a coordinated cascade of transcriptional events underlying survival in drought and salinity. The major mechanism of DREB action is the transactivation of a protective network of downstream genes that encode proteins such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs) and aquaporin’s. This is a broad-spectrum response which assist with the stabilization of cellular structures, protection of macromolecules integrity and regulation of water transport under dehydrating conditions (Hrmova & Hussain, 2021; Kumar et al., 2022). A third arm of this defense, which is also critical, is enhancing the oxidative stress protection. Drought and salinity triggers the buildup of reactive oxygen species (ROS). DREB transcription factors help to reduce this effect by increasing the expression of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases to maintain redox homeostasis and prevent oxidative damage to cells (Haddoudi et al., 2025; Yang et al., 2025). A major translational challenge that is related to the constitutive overexpression of DREB genes is the frequent incidence of yield penalties under non-stress conditions, which can often be expressed as stunted growth and low fertility. This is attributed to the constant diversion of energy and resources towards the stress-response machinery (do Rego et al., 2021; Mei et al., 2022). To overcome this pleiotropic effect, the research efforts have shifted towards the fine-tuning of the DREB expression. The most promising strategy involves stress-inducible promoters, such as the Rd29A promoter that ensures high-level expression of the transgene only when stress is induced thereby uncoupling the high-level stress tolerance from stress-induced growth-penalties (Singh & Chandra, 2021; Wang et al., 2024).

Engineering hydraulic conductivity through aquaporin's.

Once water is pulled in by the roots, its movement to the shoot is dictated by xylem anatomy and root hydraulic conductivity which are important physiological components of WUE. The genetic control of the aquaporin's, which are water channels in cell membranes that enable plants to dynamically regulate hydraulic conductivity in response to soil moisture to fine-tune water flow has been reported by (C. Li et al., 2021). The main mechanism of DREB action has been transactivation of a protective system of downstream genes coding for proteins such as late embryogenesis abundant (LEA) proteins, heat-shock proteins (HSPs), and aquaporin's. This is a broad-spectrum response which assist with the stabilization of cellular structures, protection of macromolecules integrity and regulation of water transport under dehydrating conditions (Hrmova & Hussain, 2021; Kumar et al., 2022).

Modifying Root System Architecture for Better Resource Foraging

The search for crop improvement through the manipulation of the Root System Architecture (RSA) is ultimately based on the understanding of the genetic and hormonal networks that regulate root growth and development. Central to this control is the auxin signaling pathway and, in particular, the AUX/IAA-ARF module that plays the role of master regulator of RSA (Lombardi et al., 2021). This module, when coupled with other phytohormones such as cytokinins and brassinosteroids, provides an intricate way to control processes such as lateral root initiation, primary root elongation and gravitropic set point angles (GSA) which collectively determine a plant's strategy for exploring soil.5, 6 For example, transgenic manipulation of AUX/IAA genes or certain ARFs results in proliferation of lateral roots, developing a highly branched, shallower root system, which is ideal for topsoil foraging of immobile nutrients such as phosphorus (Panda et al., 2021). On the other hand, by modulating the crosstalk between auxin and cytokinin, one can promote a steeper, deeper rooting phenotype which is important as one wants to tap water as well as mobile nitrates from deeper soil strata (Kalra et al., 2023; Ranjan et al., 2022). Beyond hormonal transcription factors, small GTPases such as ROPs (Rho of Plants) are important signaling nodes that convert developmental signals into cytoskeletal remodeling and cell wall modification leading to direct root morphological changes (Kořínková et al., 2021). ROP proteins modulate polar auxin transport and are key factors in determining root hair developmental pattern and growth dynamics of root tip cells affecting root waving, skewing, and ultimately overall root architecture (Kořínková et al., 2022). Transgenic strategies involving the modification of ROP expression or activity have been shown to have an actual effect on resource uptake; e.g. overexpression of some ROP genes leads to an increase in root hair length and density, with substantial increase of the root-soil interface, thus enhancing water and nutrient uptake, especially when plants experience mild stress conditions (Y. Li et al., 2025). This makes ROPs important, downstream effectors that can tune RSA without entirely revamping the core hormonal signaling machinery. The functional benefits of manipulating these genetic nodes are being validated in controlled environments increasingly and, importantly, in soil-based systems. Transgenic lines in which the expression of auxin-related genes or transcription factors such as DEEPER Rooting 1 (DRO1) ortho-logs have been manipulated have repeatedly demonstrated the ability to modify the root growth angles thereby producing either a reduced or increased depth of the root system with demonstrable agronomic benefit (Farooq et al., 2024; Jan et al., 2024). Plants with an increased RSA are characterized by a better water extraction from subsoil (giving an increased drought tolerance), whereas plants with a more dense and superficial root network are characterized by a better efficiency of phosphorus and nitrogen uptake (Agrawal et al., 2023; Awasthi & Laxmi, 2021). These architectural changes are effective for plants to mine a larger volume of soil, thus mitigating abiotic stresses and decreasing the need for fertilizer and irrigation inputs (Hu et al., 2021; Jiang et al., 2024).

Advanced Genome Editing for Precision Breeding

Advanced genome editing is providing a revolution in precision breeding, opening up new sophisticated tools to engineer complex agronomic traits. Moving beyond single gene knockouts, the multiplexing of multiple alleles with the so-called multiplex editing enables the pyramiding of multiple alleles in parallel and thus speaks to the polygenic nature of traits such as nutrient use efficiency. This ability is fundamental to a "Green Revolution 2.0" where the ability to fine-tune plant architecture by editing a hormonal pathway at particular targets gives rise to optimized ideotypes, without trade-offs associated with traditional breeding. Furthermore, the accuracy of base and prime editing allows for the design of novel and improved alleles by installing specific nucleotide substitutions for the improvement of key transporters and enzymes. Collectively, these technologies pave the way to the deconstruction and holistic rewiring of plant genetics in order to produce newer crops with superior yield, resource efficiency and resilience.

Deconstructing Complex Traits and Multiplex Editing

Beyond validating the individual gene function, the pyramiding of multiple NUE-related alleles by multiplex editing is possible using the CRISPR-Cas strategy which addresses directly the polygenic nature of the trait. By targeting multiple significant regulators at the same time, researchers can develop novel genetic combinations that would be exceedingly difficult and time-consuming to achieve through conventional breeding. A prime example is the simultaneous editing of multiple NLP transcription factor genes in rice and wheat, which had a synergistic effect on N uptake and growth, which was greater than single gene knockouts.203 This multiplexing ability is extended to engineering whole pathways with co-editing of genes involved in N transport (e.g. AMT1 family), assimilation (e.g. GS/GOGAT cycle) and signaling (e.g. TGAs) being able to rewire the N economy of the plant in a holistic manner. The production of such multiplex-edited lines offers direct proof-of-concept for the cumulative and often epistatic effects of NUE alleles to show that the complexity of the trait can be systematically decoded and reconstructed for agronomic benefit 13, 14, 15

Editing for "Green Revolution" 2.0: Fine-Tuning Plant Architecture

The reliance on semi-dwarfing alleles, such as sd1 in rice and Rht in wheat, in the original Green Revolution was successful in averting lodging but entailed inherent trade-offs such as pleiotropic effects that had been induced in root architecture and nutrient efficiency that are now limiting further yield gains. The advent of accurate genome editing technologies promises a "Green Revolution 2.0" that allows progression from the use of random mutagenesis to the fine-tuning of specific genetic nodes that control plant architecture. This new paradigm is to surgically alter the hormonal pathways, in order to design crops with the desired, optimized height and tillering and root systems and without the yield penalties of their predecessors. By manipulating key regulators in gibberellin (GA), strigo-lactone (SL) and other hormone networks, researchers are now engineering plants with ideotypes adapted for better resource acquisition and efficiency (Huang et al., 2021). Central to this effort lies the sophisticated modulation of gibberellin pathway. Unlike traditional dwarfing which interferes with GA biosynthesis or signaling on a global level, editing enables tissue- and stage-specific control. For example, negative regulators of GA signaling, for example DELLA proteins, can be targeted to cause various dwarfing phenotypes suitable for various agro-ecosystems. More recently, by editing the GA inactivation gene GA2ox or the signaling repressor SLR1 in rice, researchers have found that they can precisely regulate the elongation of internodes to produce plants that are sturdy and resistant to lodging, without compromising the important role of GA in promoting grain development. This strategy escapes the root growth suppression that is often associated with the sd1 mutation, which preserves plant vigor and nutrient foraging capacity. The goal is no longer to simply make plants shorter, but to create the shape of their stature so that it will intercept light optimally and have mechanical strength. Simultaneously, genome editing of the strigolactone pathway is providing access to the strigo-lactone regulation of plant shoot branching and root symbiosis in a way that has never been achieved. SLs suppress the outgrowth of axillary buds, and the editing of key biosynthesis genes (such as CCD7 and CCD8) results in a massive enhancement of tillering or branching. However, the real value of editing is to develop weak or conditional alleles that will be useful by fine-tuning, rather than eliminating, the function of SL. This approach can be used to generate phenotypes that have moderately enhanced tiller numbers - thus aid the yield potential - without altering the function of SLs in promoting arbascular mycorrhizal associations which are important to phosphate acquisition (Qu et al., 2022). This decoupling of the architectural and symbiotic functionality of SL was not possible with traditional breeding and is an important step in the design of plants that are efficient in their use of both internal and external resources.

Creating Novel Alleles for Enhanced Function with Base and Prime Editing

Advanced genome editing techniques, specifically base editing and prime editing, have been wreaking havoc in the engineering of nutrient-efficient crops by allowing the engineering of precise changes in key genes that control nutrient uptake and utilization. Base editing makes it possible to directly and irreversibly convert base pair to base pair at a target locus without the need for double-stranded DNA breaks (DSB) and the use of donor templates. This technology is exceptionally adapted to the introduction of, or the recreation of, beneficial single nucleotide polymorphisms (SNPs), known to improve the activity of nutrient transporters and enzymes. For example, editors of base pairs have been used successfully to edit genes for nitrate transporters and phosphate transporters in cereals to generate new alleles that increase nitrogen and phosphorus uptake efficiency (Johnson et al., 2022). Similarly, targeted base editing has been employed in rice to develop optimized variants of genes involved in iron homeostasis, demonstrating the potential to improve micronutrient deficiencies (Khanna et al., 2023). This approach permits direct introduction of allelic variation coming from unadapted landraces into high-yielding elite cultivars and we can see that this approach allows to avoid the linkage drag associated with conventional breeding. Prime editing also broadens the range of precision genome engineering by providing a generic "search-and-replace" capability, where all 12 possible changes from one base to another can be made, as well as small changes where molecules are both inserted and deleted. This flexibility is important in order to reach a wider allelic space, as many useful traits are determined by targetable SNPs that are not amenable to base editing, or by particular combinations of mutations. The system, which involves a prime editing guide RNA (pegRNA) and a reverse transcriptase fused to a nickase Cas9, has been used for the precise editing of genes that are responsible for nutrient use efficiency. Research points to the use of in changing the key promoter or coding sequences of genes involved in nitrogen assimilation and zinc transport to fine-tune gene expression and the action of proteins to optimize the utilization of nutrient resources.12, 13 By offering a tool to simultaneously or sequentially install multiple edits, prime editing may help to recapitulate complex haplotypes that are found in wild relatives or even design superior optimised alleles that do not exist in nature. The application of these precision tools to enhance nutrient efficiency has been an example in the targeted use of these tools on certain gene families. One prominent target is the group of transporters for nutrients, for example, for nitrate (NRTs), phosphate (PHTs) and metal ions. Precision edits in these transporters can change their affinity, capacity to transport nutrients or their regulation and thus lead to improved nutrient capture from the soil. Beyond transporters, genes for enzymes in pathways of nutrient assimilation such as glutamine synthetase in nitrogen metabolism are being manipulated to decrease energy costs and to increase biomass yield 259. The fact that it is possible to make such precise changes in elite cultivars, without interfering with other desirable agronomic characteristics, is a major advantage over traditional mutagenesis. This strategy has the advantage of going beyond simple gene knockouts, which have been useful for functional genomics, but are limited to loss-of-function phenotypes, towards the creation of novel and gain-of-function alleles which can fine-tune complex metabolic and transport processes. Table 2 synthesizes the mechanisms, advantages, and specific RUE applications of these advanced genome editing technologies, including multiplex editing, base editing, and prime editing.

Table 2: Toolkit of Advanced Genome Editing Technologies for Engineering Resource-Use Efficiency

Technology	Mechanism	Key Advantage	Application Example in RUE	References
CRISPR-Cas9 Knockout	Induces double-strand breaks (DSBs), repaired by error-prone Non-Homologous End Joining (NHEJ), leading to frame shifts and gene knockouts.	Rapid functional validation of genes; effective for disrupting negative regulators.	Knockout of TaNLP2/3 in wheat and *OsNLP4* in rice enhanced N uptake and yield under low nitrogen conditions.	(Fernandes & Nascimento, 2022; Ruis et al., 2025)
Multiplex Editing	Simultaneous use of multiple guide RNAs with Cas9 to edit several genomic loci in one transformation.	Deconstructs polygenic traits; enables pyramiding of beneficial alleles and rewiring of entire pathways.	Co-editing of N transport (*AMT1*), assimilation (*GS/GOGAT*), and signaling (*TGA*) genes to holistically rewire plant N economy.	(Chen et al., 2025; Oh et al., 2020)
Base Editing	Uses a catalytically impaired Cas9 fused to a deaminase enzyme to directly convert one base pair to another (e.g., C•G to T•A) without DSBs.	Installs precise point mutations; creates novel gain-of-function alleles or recapitulates beneficial SNPs.	Converting specific nucleotides in nitrate (*NRT*) or phosphate (*PHT*) transporter genes to enhance their affinity or regulation.	(Azameti & Dauda, 2021; Choi et al., 2023)
Prime Editing	Uses a Cas9 nickase fused to a reverse transcriptase and a Prime Editing Guide RNA (pegRNA) to directly write new genetic information into a target site.	"Search-and-replace" functionality; enables all 12 possible base-to-base conversions, as well as small insertions and deletions.	Rewriting promoter or coding sequences of genes for zinc transport or nitrogen assimilation to fine-tune expression and protein activity.	(Aliciaslan et al., 2025; Zhao et al., 2023)

Integration with Sustainable Crop Management Practices

The frontier of sustainable agriculture is found in the powerful combination of the genetic improvement of crops and the use of advanced management systems. By combining resource efficient genotypes, specifically bred for improved Nitrogen and Water use, with the precision of the variable rate technologies we head beyond one size fits all to create a synergized system. This combination assures that not only is fertilizer and water applied more accurately, but are being utilized more by the plant itself in order to reduce the carbon and water footprint of production. Furthermore, the breeding for agro ecological systems is concerned about the development of resilient varieties, which can survive in low-input environment and the traits are focused on the low-input environment such as nutrient acquisition and intercrop compatibility. The collective effect of these strategies is profound and we have seen that in Life Cycle Assessments significant reductions in greenhouse gas emissions, water scarcity and eutrophication potential are possible, thus opening the way for a more productive and environmentally sustainable agri-food system.

SMS: "Without the ability to transmit data back to the farm, the value of the data needed to be inferred through retrospective analysis of additional datasets at the field or tractor level."

Synergy with Precision Agriculture

The synergy of the integration of genetically-improved, resource-efficient crop genotypes and precision agriculture (PA) management is a frontier in sustainable agronomy. Central to this synergy is the combination of genotypes with improved Nitrogen Use Efficiency (NUE) with technologies of variable rate applications of nitrogen (N). Modern NUE genotypes are designed or bred for higher nitrogen uptake, assimilation and remobilization, which is ideally suited for spatially-resolved, data-driven inputs of PA This combination is moving beyond the one-size-fits-all approach and is developing a system in which fertilizer is not only applied at the right place and time, but fertilizer is used in a more effective way by the plant itself. The genetic potential of these cultivars ensures that the marginal improvements in efficiency of precision N management are maximized resulting in greater yield stability and lessen environmental N losses than either of these innovations deployed in isolation. Efficacy of this synergy is best seen in the field of sensor-based fertilization. Proximal and remote sensing technologies have the potential to detect in-season spatial variability in crop N status, which is a source of information for variable rate prescriptions (Schwalbert et al., 2026; Singh et al., 2025). However, the agronomic interpretation of these sensor signals is highly genotype dependent on the inherent N responsiveness of the genotype. Standard varieties may have a muted response to corrective N applications, but NUE optimized genotypes are physiologically ready to take advantage of these supplemental inputs and translate the needs detected by the sensor into strong growth and yield more consistently. This creates a positive feedback loop, whereby the precision management uncovers the full genetic potential of, and the high performing genotype gives a clearer and more actionable signal for management changes. A critical layer of complexity and opportunity is injected by Genotype-by-Environment (GxE) interactions. The performance of a given NUE genotype is not constant but differs significantly over different soil types, climatic conditions and management histories. This requires change from static prescription maps to dynamic precision management systems. Such systems would combine real-time environmental data, soil sensor networks and crop growth models to adapt N rates and, prospectively, variety selection or planting density continuously. Figure 2 presents a conceptual framework illustrating how the convergence of resource-efficient genotypes, precision agriculture technologies, and agroecological principles creates a positive feedback loop that minimizes environmental footprints while maintaining productivity. Understanding GxE enables the creation of meta-models able to predict which combination of genotypes and management will be the best within a particular micro-environment in a field (taken to a spatial resolution to spatio-temporal one).

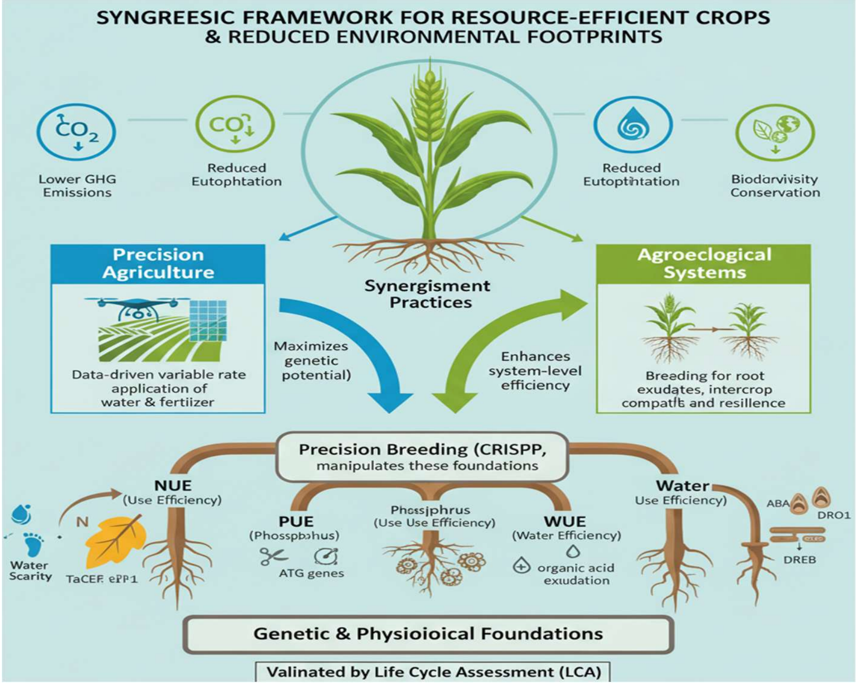

Fig. 2: Conceptual framework showing how resource-efficient genotypes (NUE, WUE, optimized root architecture) interact with precision agriculture technologies and agroecological principles to minimize environmental footprints. The integration enables dynamic management of Genotype-by-Environment (GxE) interactions and achieves measurable reductions in greenhouse gas emissions, water scarcity, and eutrophication potential through validated Life Cycle Assessment (LCA) outcomes.

Breeding for Agro ecological Systems

The principles of breeding for agro ecological systems, such as organic farming, constitute a basic re-orientation of the goals of crop improvement. Unlike the traditional approach to breeding where often yield under high input conditions is the primary objective, the objectives of agro ecological breeding are set to be achieved under low input and biologically complex environments. This requires attention to traits that will increase nutrient acquisition and cycling especially without synthetic fertilizers. For example, in the case of organic systems, the capacity of a plant to maintain the efficient mobilization of soil phosphorus via root exudates (such as carboxylates) or the establishment of associations with arbuscular mycorrhizal fungi are of prime importance (Legzdiņa et al., 2022; Scandurra et al., 2024). Breeding programs are also increasingly identifying and selecting for these root architectural and physiological traits to develop varieties that are less dependent on soluble nutrient inputs and these efforts are bringing crop genetics more closely in line with the nutrient conserving principles of agro ecology. Beyond nutrient efficiency, a fundamental concept in agro ecological breeding is building the resilience of crops to biotic and abiotic stresses without using synthetic chemicals. This includes selection for strong in-built defense mechanisms against weeds, pests and diseases. Research has proven that traits like rapid canopy closure for weed suppression, and production of allopathic compounds are very valuable in organic and conservation agriculture systems, (Caradonia et al., 2020; Thakur et al., 2023). Furthermore, resilience to abiotic stresses such as drought is of critical importance, because agro ecological systems often seek to buffer crop from climate variability through enhanced soil health. Breeding for these complex traits needs the adoption of a holistic approach, in which plants are not considered in isolation but in the context of their environment and the management practices in use (Rempelos et al., 2023). The integration of legumes via intercropping is a mainstay of many agro ecological systems so breeding needs to change to support this practice. This entails selection for varieties with greater "intercrop compatibility," in which the focus is more on the performance of individual plants rather than the productivity and stability of the crop mixture. Desirable traits are temporal complementarity in growth patterns, morphological traits that minimize competition for light and ability to thrive in the modified rhizosphere of a poly culture (Ambrogio et al., 2021). Breeding legumes and cereals specifically for these synergistic partnerships have the potential to release large amounts of system level nutrient use efficiency and overall yield resilience and leave the paradigm of breeding for solitary stand performance. The development in the breeding for agro ecological systems highlights the need for participatory approaches in the selection process in which farmers are directly involved. Farmers have invaluable knowledge about local growing conditions and can pinpoint traits of importance that may be lost in centralized breeding programs. Farmers have invaluable knowledge about local growing conditions and can identify traits of importance that may be lost in centralized breeding programs. This joint model of the development ensures new varieties are not only genetically adapted to low-input environments but are also practically and culturally fit to the different contexts of sustainable farming.

The Impact on Life Cycle Assessment (LCA)

The introduction of nitrogen-efficient crop varieties has the direct effect of lowering the carbon footprint of agricultural production by lowering the demand for synthetic fertilizers, the manufacture of which is extremely energy-intensive. Life Cycle Assessment (LCA) studies have shown that cultivars with improved Nitrogen Use Efficiency (NUE) have similar yields with much reduced application of nitrogen fertilizer. This reduction in fertilizer input directly reduces the cumulative energy demand and associated greenhouse gas emissions from the Haber-Bosch process and fertilizer transport which are dominant contributors of carbon footprint of crop systems (Kumar et al., 2024). As an example, expanding the global acreage of NUE cereals has the potential to reduce global greenhouse gas emissions from croplands by millions of tones' of carbon-dioxide equivalent per year, providing an example of how genetic advances in nutrient uptake efficiency have a sentimentally large impact at the food system level on climate change mitigation. Concurrently, the development of water efficient plant varieties is a powerful lever that enables the reduction of the water footprint of agriculture - a crucial topic in the context of growing hydrological scarcity. LCA methodologies that incorporate water stress indices show that drought tolerant or water use-efficient crops significantly decrease the blue water consumption - that is, the water being extracted from water sources for irrigation. By being productive with less water the varieties reduce the environmental impacts associated with water withdrawal, such as depletion of the water in the aquifer and energy consumption in pumping, reducing the overall footprint of water scarcity (Kumar et al., 2024). The effect on the entire system is great; modelling studies have shown that if such varieties are widely adopted, global freshwater resources will be significantly less under pressure, and agricultural systems will be better resilient and sustainable. Furthermore, the development and deployment of resource efficient crops is paramount to the curtailment of eutrophication potential, which is an environmental impact category that is dominated by agricultural nutrient runoff. LCA studies are consistent to demonstrate that nitrogen efficient genotypes, which enhance the synchrony between nutrient supply and crop demand, reduce the quantity of residual nitrogen in soil prone to leaching as a nitrate or volatizing as ammonia. This direct decrease in nitrogen losses per unit of yield is a major mechanism for the reduction of marine and freshwater eutrophication potentials (Ma et al., 2022). The quantifiable benefit can scale up to large geographical scales, where the additive effect of cultivation of these varieties can be seen to result in significant improvements in water quality and this provides a clear route from genetic gain to ecosystem service enhancement.

Challenges, Socio-Economic Considerations, and Future Directions

The challenge to engineer crops with better resource utilization efficiency is faced with many complex technical and biological issues, such as some of the most challenging cases of genotype-by-environment interactions and yield trade-offs, as well as a major pheno-typing bottleneck for key traits such as root architecture. These scientific challenges are even complicated by a patchwork of regulation around the world and various degrees of public acceptance which generate socio-economic barriers to innovation and access to markets. Consequently, future achievements depend on a two-pronged approach of promoting predictive breeding via integrated technological pipelines that integrate pan-genomics, phenomics and machine learning with constructing forward-thinking policy landscapes that balance economic incentives with sustainability outcomes for the successful development and adoption of these next-generation crops.

Technical and Biological Hurdles

Breeding crops for improved resource-use efficiency (RUE) is of prime importance to sustainable agriculture, but it is confronted with a great deal of biological complexities. A main challenge is the strong effect of Genotype-by-Environment (GxE) interactions that make the improvement of genotypes difficult. The dynamic nature of the field and often unpredictable nature of the conditions, especially the fluctuating nature of water and nutrient availability, has the disadvantage that a genotype that plays well in one environment may not work well in another location (Loc Nguyen et al., 2025). This heterogeneity of the environment presents a moving target to breeders as the genetic structure of traits such as nitrogen use efficiency (NUE) and water use efficiency (WUE) is very situationspecific. Consequently, the power of the quantitative trait loci (QTLs) and candidate genes identified in controlled environments often reduce when tested in a variety of real-world conditions in the field, highlighting the importance of multi-environmental testing frameworks to help dissect such intricate interactions. It was found that the effectiveness of the quantitative trait loci (QTLs) and candidate genes identified in control conditions often decrease when tested in diverse conditions of the field and it also demonstrated that the identification of QTLs for these traits is often highly correlated with yield. Further complicating the difficulty of the G x E interactions are deep-seated biological constraints, i.e. pleiotropic and yield trade-offs. The physiological pathways for nutrient and water uptake and utilization are intrinsically linked with yield formation and other agronomic traits which often lead to antagonistic genetic correlations. For example, selection for improved nutrient efficiency can have secondary consequences for the yield potential or harvest index due to an altered resource allocation strategy of the plant, essentially a reconfiguration of this plant's resource-use strategy (Li et al., 2022; Tiezzi & Maltecca, 2022). Similarly, pleiotropic effects, in which one gene affects many traits, can cause undesirable linkages in which improving especially RUE can come at the cost of other important traits, such as grain quality or phenology. These trade-offs point to the fact that RUE is not an isolated trait, but rather a complex syndrome for which a holistic, systems-level breeding approach is required. A key technical bottleneck in the advancement of RUE breeding is high-throughput phenotyping or more specifically root architecture as well as related physiological traits. The "hidden half" of the plant, the root system, is directly responsible for soil resource exploration, yet is notoriously difficult to measure within a field environment. While the methods that are based on laboratory data are useful for providing detailed morphological data, these methods tend to lack in terms of scalability and ecological relevance in breeding programmers (Gangashetty et al., 2023; Mbe et al., 2024). Although remote sensing and aerial phenomics have provided solutions for canopy level traits such as canopy temperature and normalized difference vegetation index (NDVI) as surrogate for water status, they provide only indirect measurement of root function and soil level resource capture. Developing reliable, non-destructive and high-throughput phenotyping platforms for root traits and in-situ resource uptake is currently a critical frontier for genetic improvement for RUE. The challenge is the phenotyping bottleneck; measuring deep rooting in addition to root angle and hydraulic traits accurately in large breeding populations is laborious and expensive (Uga, 2021).

Regulatory Frameworks and Public Acceptance

A central regulatory schism is present between those who regulate by the process by which a new plant variety is generated and those who focus on the characteristics of the final product. The United States, with its updated SECURE rule, is one example of the product-based approach where crops with genetic modifications that could have been achieved by traditional breeding and crop with no foreign DNA are generally exempt from pre-market regulatory oversight (Entine et al., 2021). This is supposed to stimulate innovation by making it less costly and time consuming to regulate them. The European Union, on the other hand, follows a stringent process-based approach where any organism produced by mutagenesis, including modern genome editing methods, falls under the strict risk assessment, traceability and labelling obligations of the GMO directive as laid down by the 8 ECJ ruling (Sprink et al., 2022). This de facto subjects genome-edited crops to the same level of regulation as transgenic GMOs, which this poses a major impediment to research and development in the EU. A hybrid model is taking shape in countries such as Japan, Argentina and Brazil, which use factors such as the presence of recombinant DNA in the end product to initiate a tiered risk assessment, which is meant to be more proportionate than the EU but more oversight than the US (Menz et al., 2020). This regulatory fragmentation has very real socio-economic impacts, especially in regards to international trade and research translation. The disparate frameworks embrace non-tariff trade barriers, where the crops that are approved in one jurisdiction can be illegal in another, thus disrupting global supply chains and creating uncertainty in the markets for developers (Sprink et al., 2022). This "regulatory patchwork" diminishes public and private investment in plant breeding research, particularly in minor crops or traits which solve regional problems because the route to market is unpredictable and expensive (Entine et al., 2021). The stringent European Union (EU) regulations, for example, have been suggested as a reason behind the shift of research and commercial investment away from Europe, which is one way that Europe is hindering their capacity to leverage these technologies in the interest of agricultural sustainability goals (Jones et al., 2022). This divergence not only chokes off innovation but also runs the risk of increasing the technological divide between countries that have enabling versus restrictive regulatory environments throughout the world. The public acceptance of genome-edited crops is inextricably related to these regulatory debates and quality of science communications around these debates. The conflation of genome-edited products with the older transgenic GMOs in the public discourse and in regulatory frameworks such as the EU's, contributes to consumer suspicion and mistrust (Menz et al., 2020) To combat this, transparent, proactive and scientific communication is critically required to explain the exact nature of the genetic changes and their safety profile distinguishing them from traditional GMOs where appropriate (Jones et al., 2022). Building public trust requires moving away from a deficit model of communication to open dialogues about the benefits, potential risks and ethical considerations, demystifying the technology and aligning its development with societal values and needs (Sprink et al., 2022).

Future Research and Policy Directions

The need to improve the food security of the world's population in the face of a changing climate requires a paradigm shift in crop improvement, away from yield-focused models towards a focus on resource use efficiency

(RUE). The future of genetic gain will therefore be in harnessing the power of integrated technological pipelines that incorporate the fusion of genomics, phonemics' and machine learning (ML) into a predictive breeding framework. High-throughput phonemics' using drones and spectral sensors provide massive and multidimensional datasets of plant physiology and performance in real-time (Robert et al., 2022). The real value of this data is unlocked by advanced machine learning (ML) algorithms such as deep learning and ensemble algorithms, which can detect complex, non-linear relationships between genotype and phenotype, enabling the accurate prediction of the performance of novel genetic combinations for traits such as water-use efficiency and nitrogen uptake. The true power of such data is unleashed by advanced machine learning (ML) algorithms, such as deep learning and ensemble algorithms, which can detect complex, non-linear relationships between genotype and phenotype and therefore accurately predict the performance of novel genetic combinations for traits such as water-use efficiency and nitrogen uptake. This synergy makes breeding a more proactive discipline, moving breeding from a reactive to a proactive discipline, allowing the rapid selection of improved genotypes with improved resource economy, months before they are even in the field. One key genomic basis for such an approach is the shift from single reference genomes to pan-genomes. The reference genome of one cultivar covers only a portion of the total genetic diversity of a species. Pan-genomes that include all sequence repertoire from many varieties and wild relatives are indispensable in the discovery of novel alleles and structural variants linked to RUE that are missing from elite germplasm. Integrating pan-genomic data with ML-driven predictive models enables breeders to tap into this untapped reservoir of genetic variation in a systematic way. Figure 3 outlines this comprehensive pipeline, from initial gene discovery through pan-genome analysis and precision genome editing to ML-driven phenotype prediction and eventual deployment of resource-efficient cultivars. This provides the ability to make the precise introgression of valuable alleles (that control deeper root systems, or greater photosynthetic efficiency) from exotic or wild accessions into high yielding backgrounds, thus breaking existing genetic plateaus to create crops inherently designed for a resource limited world.

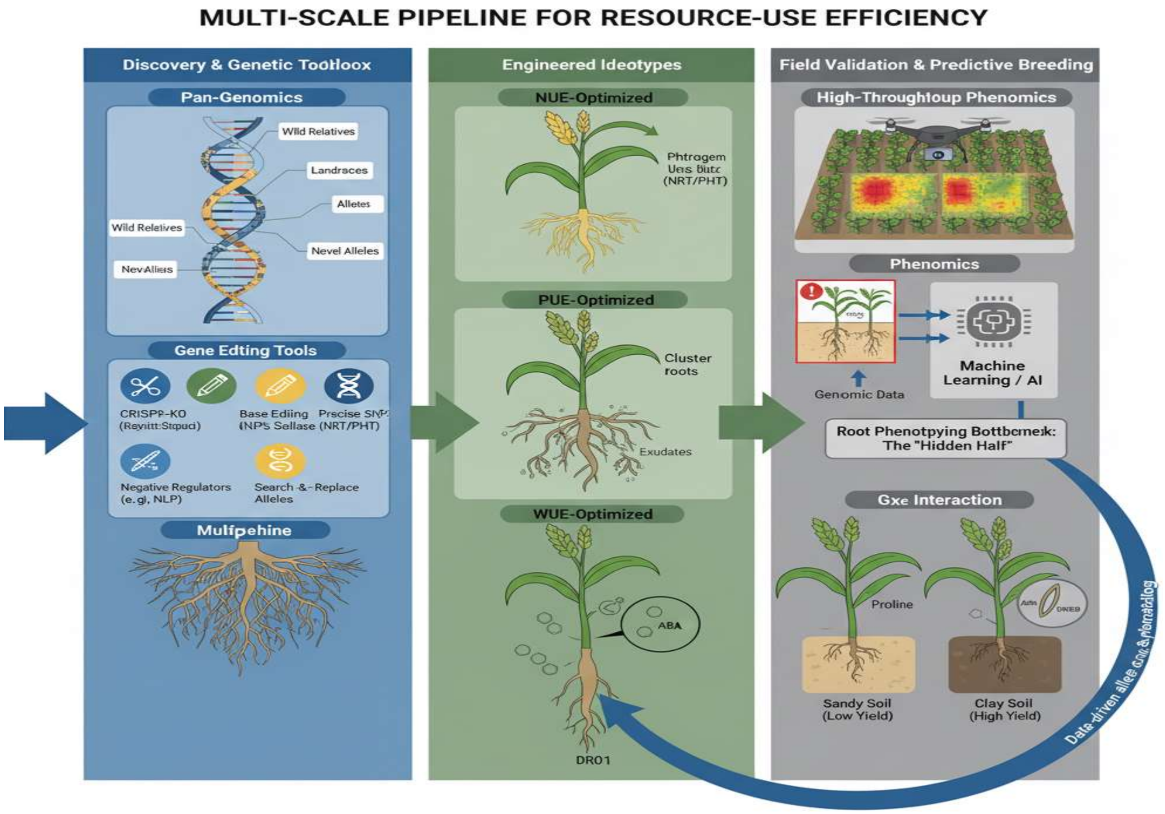

Fig. 3: A Pipeline for Developing Resource-Efficient Crops: From Gene Discovery to Predictive Breeding.

Conclusion

The search for elevated resource-use efficiency (RUE) is one of the pillars of the move towards a more productive and sustainable global agri-food system. As has been detailed in this review, the physiological and genetic basis of RUE are now being unravelled with unprecedented clarity to reveal the complexities but targetable pathways for the uptake, assimilation and remobilization of N, P, and water. Dissection of these traits from the proteo-lytic and autophagic machinery as the driving mechanism for nitrogen remobilization to the root architectural and transporter-based strategies for phosphorus mining to the hydraulic and osmotic adjustments for water optimization provides a blueprint for total improve in crops.

The development of sophisticated genetic tools, specifically precision genome editing, has completely revolutionized our capacity to act on this knowledge. We have come to an age of advanced genetic redesign in which the technology of gene knockouts has been replaced by an age of sophisticated, multiplex editing, in which we can deconstruct and pyramid alleles for polygenic, RUE traits; base and prime editing, by which we can create novel, enhanced gene variants; and targeted interventions, by which we can fine-tune plant architecture and hormonal pathways without the pleiotropic costs of the first Green Revolution. These technologies have enabled the complete engineering of the key processes, from photosynthetic efficiency through the use of synthetic bypasses, C4 pathway engineering, to engineering "ideal" root systems for a particular soil environment.

However, the potential benefits of these genetic improvements can only be achieved by the synergistic integration of these with sustainable crop management practices. The synergy between RUE-optimized genotypes and precision agriculture enables a powerful feedback loop to be created in which data-driven inputs can be used with maximum efficiencies by the plant. Furthermore, breeding for agro ecological systems implies that genetic improvements are relevant for low input and organic farming and focus on traits such as the ability to obtain nutrients from organic pools and intercrop compatibility. The positive effect of this integrated approach on the environment is unequivocal, with Life Cycle Assessments showing that there are significant reductions of greenhouse gas emissions, water scarcity footprints and eutrophication potential.

Despite the incredible progress, there are still grave challenges. The confounding effects of genotype-by-environment (G*E) interactions, the ongoing phenotyping bottleneck (particularly for root traits) and the complex trade-offs and their impact on yield highlight the biological complexity of these traits. These scientific challenges are further complicated by an incomplete regulatory system around the world and the importance of good public engagement and trust.

Therefore, the way forward requires a two-pronged strategy. Scientifically, we need to be open to integrate and predictive breeding pipelines incorporating the fusion of pan-genomics, high-throughput phonemics', and machine learning in the effort of accelerating the discovery and deployment of RUE alleles. Societally, we need to work on the creation of forward thinking policy frameworks that align economic incentives with sustainability outcomes to promote value chain alignment and create a market driven rewards for verifiable resource savings. The convergence of genetic innovation, digital agriculture and supporting policies is not only beneficial, but it is essential. By bringing together these strands, we can develop and implement systematically the next generation of crops that will be able to thrive with lower amounts of inputs, and can assure global food production within the planetary boundaries of a rapidly changing world.

DECLARATIONS

Funding: This study did not get any financial support from any organization.

Acknowledgement: Not applicable

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Not applicable

Ethics Statement: Not applicable

Author's Contributions: Ali Murtaza and Ayaz Haider Khan**:** writing, review, initial draft preparation of manuscript. Malaika Imtiaz, and Laiba Ansari**:** reviewed the manuscript. Muhammad Haris Shahid**:** writing, review and editing.

Generative AI Statements: The authors confirm that no generative artificial intelligence-based tools were employed for content generation or interpretation. All intellectual content, interpretations, and conclusions are the sole responsibility of the authors.

REFERENCES

Agrawal, R., Singh, A., Giri, J., Magyar, Z., & Thakur, J. K. (2023). MEDIATOR SUBUNIT17 is required for transcriptional optimization of root system architecture in Arabidopsis. *Plant Physiology*, *192*(2), 1548-1568. https://doi.org/10.1093/plphys/kiad129

Aliciaslan, M., Erbasan, E., Erendor, F., & Sanlioglu, S. (2025). Prime Editing: The Next Frontier in Precision Gene Therapy. *The Journal of Gene Medicine*, *27*(10), e70040. https://doi.org/10.1002/jgm.70040

Ambrogio, C., Charlotte, B., Dominic, A., & Andrew, T. (2021). A Participatory Wheat Variety Testing Programme With British Organic Farmers.

Awasthi, P., & Laxmi, A. (2021). Root Architectural Plasticity in Changing Nutrient Availability. *Signaling and Communication in Plants*, 25-37. https://doi.org/10.1007/978-3-030-84985-6_2

Azameti, M. K., & Dauda, W. P. (2021). Base Editing in Plants: Applications, Challenges, and Future Prospects [Review]. *Frontiers in Plant Science*, *Volume 12 - 2021*. https://doi.org/10.3389/fpls.2021.664997

Caradonia, F., Francia, E., Barbieri, R., Setti, L., Hagassou, D., & Ronga, D. (2020). Interspecific rootstock can enhance yield of processing tomatoes (<i>Solanum lycopersicum</i> L.) in organic farming. *Biological Agriculture & Horticulture*, *36*(3), 156-171. https://doi.org/10.1080/01448765.2020.1756407

Chen, K., Maimaitirexiati, G., Zhang, Q., Li, Y., Liu, X., Tang, H., Gao, X., Wang, B., Yu, T., & Guo, S. (2025). CRISPR-Cas9-based one-step multiplexed genome editing through optimizing guide RNA processing strategies in Pichia pastoris. *Synthetic and Systems Biotechnology*, *10*(2), 484-494. https://doi.org/10.1016/j.synbio.2025.01.005

Choi, E. H., Suh, S., Sears, A. E., Hołubowicz, R., Kedhar, S. R., Browne, A. W., & Palczewski, K. (2023). Genome editing in the treatment of ocular diseases. *Experimental & Molecular Medicine*, *55*(8), 1678-1690. https://doi.org/10.1038/s12276-023-01057-2

Cun, Z., Li, X., Zhang, J.-Y., Hong, J., Gao, L.-L., Yang, J., Ma, S.-Y., & Chen, J.-W. (2024). Identification of candidate genes and residues for improving nitrogen use efficiency in the N-sensitive medicinal plant Panax notoginseng. *BMC Plant Biology*, *24*(1), 105. https://doi.org/10.1186/s12870-024-04768-4

do Rego, T. F. C., Santos, M. P., Cabral, G. B., de Moura Cipriano, T., de Sousa, N. L., de Souza Neto, O. A., & Aragão, F. J. L. (2021). Expression of a DREB 5-A subgroup transcription factor gene from Ricinus communis (RcDREB1) enhanced growth, drought tolerance and pollen viability in tobacco. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *146*(3), 493-504. https://doi.org/10.1007/s11240-021-02082-7

Du, K., Zhao, W., Lv, Z., Xu, B., Hu, W., Zhou, Z., & Wang, Y. (2024). Optimal rate of nitrogen fertilizer improves maize grain yield by delaying the senescence of ear leaves and thereby altering their nitrogen remobilization. *Field Crops Research*, *310*, 109359. https://doi.org/10.1016/j.fcr.2024.109359

El Mazlouzi, M. E., Morel, C., Robert, T., Chesseron, C., Salon, C., Cornu, J.-Y., & Mollier, A. (2022). The Dynamics of Phosphorus Uptake and Remobilization during the Grain Development Period in Durum Wheat Plants. *Plants*, *11*(8), 1006. https://doi.org/10.3390/plants11081006

Entine, J., Felipe, M. S. S., Groenewald, J.-H., Kershen, D. L., Lema, M., McHughen, A., Nepomuceno, A. L., Ohsawa, R., Ordonio, R. L., Parrott, W. A., Quemada, H., Ramage, C., Slamet-Loedin, I., Smyth, S. J., & Wray-Cahen, D. (2021). Regulatory approaches for genome edited agricultural plants in select countries and jurisdictions around the world. *Transgenic Research*, *30*(4), 551-584. https://doi.org/10.1007/s11248-021-00257-8

Farooq, M., Rafique, S., Zahra, N., Rehman, A., & Siddique, Kadambot H. M. (2024). Root System Architecture and Salt Stress Responses in Cereal Crops. *Journal of Agronomy and Crop Science*, *210*(6). https://doi.org/10.1111/jac.12776

Fernandes, L. G. V., & Nascimento, A. L. T. O. (2022). A Novel Breakthrough in Leptospira spp. Mutagenesis: Knockout by Combination of CRISPR/Cas9 and Non-homologous End-Joining Systems [Original Research]. *Frontiers in Microbiology*, *Volume 13 - 2022*. https://doi.org/10.3389/fmicb.2022.915382

Fu, Z. (2025). Study on the Physiological Basis of Efficient Nitrogen Utilization and Green Fertilization Strategy of Rice. *Bioscience Methods*. https://doi.org/10.5376/bm.2025.16.0019

Gangashetty, P. I., Yadav, C. B., Riyazaddin, M., Vermula, A., Asungre, P. A., Angarawai, I., Mur, L. A. J., & Yadav, R. S. (2023). Genotype-by-environment interactions for starch, mineral, and agronomic traits in pearl millet hybrids evaluated across five locations in West Africa. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1171773

Giannoutsou, E., Adamakis, I.-D. S., & Samakovli, D. (2025). Guardians of Water and Gas Exchange: Adaptive Dynamics of Stomatal Development and Patterning. *Plants*, *14*(15), 2405. https://www.mdpi.com/2223-7747/14/15/2405

Gupta, S., Gupta, S. M., Gururani, K., Sinha, S., Gupta, R., & Kumar, A. (2022). Finger Millet as Input Use Efficient and Organic by Default Crop. *Compendium of Plant Genomes*, 201-223. https://doi.org/10.1007/978-3-031-00868-9_12

Haddoudi, L., Ayadi, M., Hdira, S., Hanana, M., Romero, I., Jouira, H. B., Djébali, N., Ndiko, L., Ballesta, M. T. S., Abdelly, C., & Badri, M. (2025). Genome-wide characterization of DREB transcription factors in Medicago truncatula: Insights into their roles in development and abiotic stress response. *Gene Reports*, *39*, 102170. https://doi.org/10.1016/j.genrep.2025.102170

Hrmova, M., & Hussain, S. S. (2021). Plant Transcription Factors Involved in Drought and Associated Stresses. *International Journal of Molecular Sciences*, *22*(11), 5662. https://doi.org/10.3390/ijms22115662

Hu, Q.-Q., Shu, J.-Q., Li, W.-M., & Wang, G.-Z. (2021). Role of Auxin and Nitrate Signaling in the Development of Root System Architecture. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.690363

Huang, X., Hilscher, J., Stoger, E., Christou, P., & Zhu, C. (2021). Modification of cereal plant architecture by genome editing to improve yields. *Plant Cell Reports*, *40*(6), 953-978. https://doi.org/10.1007/s00299-021-02668-7

Hundleby, P., & Harwood, W. (2022). Regulatory Constraints and Differences of Genome-Edited Crops Around the Globe. *Genome Editing*, 319-341. https://doi.org/10.1007/978-3-031-08072-2_17

Inckemann, R., Chotel, T., Brinkmann, C. K., Burgis, M., Andreas, L., Baumann, J., Sharma, P., Klose, M., Barrett, J., Ries, F., Paczia, N., Glatter, T., Willmund, F., Mackinder, L. C. M., & Erb, T. J. (2024). Advancing chloroplast synthetic biology through high-throughput plastome engineering of<i>Chlamydomonas reinhardtii</i>. https://doi.org/10.1101/2024.05.08.593163

Islam, S., Islam, R., Kandwal, P., Khanam, S., Proshad, R., Kormoker, T., & Tusher, T. R. (2022). Nitrate transport and assimilation in plants: a potential review. *Archives of Agronomy and Soil Science*, *68*(1), 133-150. https://doi.org/10.1080/03650340.2020.1826042

Jan, M., Muhammad, S., Jin, W., Zhong, W., Zhang, S., Lin, Y., Zhou, Y., Liu, J., Liu, H., Munir, R., Yue, Q., Afzal, M., & Wang, G. (2024). Modulating root system architecture: cross-talk between auxin and phytohormones. *Frontiers in Plant Science*, *15*. https://doi.org/10.3389/fpls.2024.1343928

Jiang, X., Zeng, X., Xu, M., Li, M., Zhang, F., He, F., Yang, T., Wang, C., Gao, T., Long, R., Yang, Q., & Kang, J. (2024). The whole-genome dissection of root system architecture provides new insights for the genetic improvement of alfalfa (<i>Medicago sativa</i> L.). *Horticulture Research*, *12*(1). https://doi.org/10.1093/hr/uhae271

Johnson, K. V. H., Krishna, T. P. A., Dash, M., Thiyageshwari, S., Ceasar, S. A., & Selvi, D. (2022). Food and Nutritional Security: Innovative Approaches for Improving Micronutrient Use Efficiency in Soybean (Glycine max (L.) Merrill) Under Hostile Soils. *Journal of Soil Science and Plant Nutrition*, *23*(1), 56-70. https://doi.org/10.1007/s42729-022-01025-1

Jones, M. G. K., Fosu-Nyarko, J., Iqbal, S., Adeel, M., Romero-Aldemita, R., Arujanan, M., Kasai, M., Wei, X., Prasetya, B., Nugroho, S., Mewett, O., Mansoor, S., Awan, M. J. A., Ordonio, R. L., Rao, S. R., Poddar, A., Hundleby, P., Iamsupasit, N., & Khoo, K. (2022). Enabling Trade in Gene-Edited Produce in Asia and Australasia: The Developing Regulatory Landscape and Future Perspectives. *Plants*, *11*(19), 2538. https://doi.org/10.3390/plants11192538

Kalra, A., Goel, S., & Elias, A. A. (2023). Understanding role of roots in plant response to drought: Way forward to climate-resilient crops. *The Plant Genome*, *17*(1). https://doi.org/10.1002/tpg2.20395

Karunarathne, S. D., Han, Y., Zhang, X. Q., & Li, C. (2022). CRISPR/Cas9 gene editing and natural variation analysis demonstrate the potential for <i>HvARE1</i> in improvement of nitrogen use efficiency in barley. *Journal of Integrative Plant Biology*, *64*(3), 756-770. https://doi.org/10.1111/jipb.13214

Kaur, H., Kohli, S. K., Khanna, K., & Bhardwaj, R. (2021). Scrutinizing the impact of water deficit in plants: Transcriptional regulation, signaling, photosynthetic efficacy, and management. *Physiologia Plantarum*, *172*(2), 935-962. https://doi.org/10.1111/ppl.13389

Kaya, C. (2025). Molecular crosstalk of transcription factors and hormones in drought tolerance genes in plants. *Turkish Journal of Botany*, *49*(3), 145-164. https://doi.org/10.55730/1300-008x.2852

Khan, N. (2025). Molecular Insights into ABA-Mediated Regulation of Stress Tolerance and Development in Plants. *International Journal of Molecular Sciences*, *26*(16), 7872. https://www.mdpi.com/1422-0067/26/16/7872

Khanna, K., Ohri, P., & Bhardwaj, R. (2023). Nanotechnology and CRISPR/Cas9 system for sustainable agriculture. *Environmental Science and Pollution Research*, *30*(56), 118049-118064. https://doi.org/10.1007/s11356-023-26482-8

Kořínková, N., Fontana, I. M., Nguyen, T., Pouramini, P., Bergougnoux, V., & Hensel, G. (2021). Enhancing crop productivity by CRISPR-mediated genetic improvement of root architecture: a focus on phytohormones. https://doi.org/10.22541/au.163310036.62797023/v1

Kořínková, N., Fontana, I. M., Nguyen, T. D., Pouramini, P., Bergougnoux, V., & Hensel, G. (2022). Enhancing cereal productivity by genetic modification of root architecture. *Biotechnology Journal*, *17*(7). https://doi.org/10.1002/biot.202100505

Kumar, S., Anusha, B. S., & Bhatia, A. (2024). Soil, Water, and Crop Management Practices to Mitigate Greenhouse Gases Emission. *Climate Change and Soil-Water-Plant Nexus*, 189-222. https://doi.org/10.1007/978-981-97-6635-2_7

Kumar, S., Muthuvel, J., Sadhukhan, A., Kobayashi, Y., Koyama, H., & Sahoo, L. (2022). Enhanced osmotic adjustment, antioxidant defense, and photosynthesis efficiency under drought and heat stress of transgenic cowpea overexpressing an engineered DREB transcription factor. *Plant Physiology and Biochemistry*, *193*, 1-13. https://doi.org/10.1016/j.plaphy.2022.09.028

Legzdiņa, L., Bleidere, M., Piliksere, D., & Ločmele, I. (2022). Agronomic Performance of Heterogeneous Spring Barley Populations Compared with Mixtures of Their Parents and Homogeneous Varieties. *Sustainability*, *14*(15), 9697. https://doi.org/10.3390/su14159697

Li, C., Li, L., Reynolds, M. P., Wang, J., Chang, X., Mao, X., & Jing, R. (2021). Recognizing the hidden half in wheat: root system attributes associated with drought tolerance. *Journal of Experimental Botany*, *72*(14), 5117-5133. https://doi.org/10.1093/jxb/erab124

Li, C., Shi, Y., Yu, Z., Zhang, Y., & Zhang, Z. (2025). Optimizing nitrogen application strategies can improve grain yield by increasing dry matter translocation, promoting grain filling, and improving harvest indices. *Frontiers in Plant Science*, *16*. https://doi.org/10.3389/fpls.2025.1565446

Li, D., Li, G., Wang, H., Guo, Y., Wang, M., Lu, X., Luo, Z., Zhu, X., Weiß, T. M., Roller, S., Chen, S., Yuan, L., Würschum, T., & Liu, W. (2022). Genetic Dissection of Phosphorus Use Efficiency and Genotype-by-Environment Interaction in Maize. *International Journal of Molecular Sciences*, *23*(22), 13943. https://doi.org/10.3390/ijms232213943

Li, Y., Ren, M., Wu, Y., Wang, L., Zhao, K., Gao, H., Li, M., Liu, Y., Zhu, J., Xu, J., Mo, X., Wu, Z., Lu, C., Zheng, S., & Mao, C. (2025). A root system architecture regulator modulates OsPIN2 polar localization in rice. *Nature Communications*, *16*(1). https://doi.org/10.1038/s41467-024-55324-5

Li, Z., Wang, G., Liu, X., Wang, Z., Zhang, M., & Zhang, J. (2021). Genome-wide identification and expression profiling of DREB genes in Saccharum spontaneum. *BMC Genomics*, *22*(1). https://doi.org/10.1186/s12864-021-07799-5

Liu, Y., Hu, B., & Chu, C. (2023). Toward improving nitrogen use efficiency in rice: Utilization, coordination, and availability. *Current Opinion in Plant Biology*, *71*, 102327. https://doi.org/10.1016/j.pbi.2022.102327

Loc Nguyen, V., Do, T. H., Phan, T. H. N., Nguyen, V. L., Chu, D. H., Bertero, D., Curti, N., Ta, V. T., McKeown, P. C., & Spillane, C. (2025). Impact of genotypes, environmental stresses, and genotype by environment interactions on growth and yield of quinoa at flowering stage. *PLOS ONE*, *20*(9), e0331652. https://doi.org/10.1371/journal.pone.0331652

Lombardi, M., De Gara, L., & Loreto, F. (2021). Determinants of root system architecture for future-ready, stress-resilient crops. *Physiologia Plantarum*, *172*(4), 2090-2097. https://doi.org/10.1111/ppl.13439

Lv, X., Zhang, Y., Zhang, Y., Fan, S., & Kong, L. (2020). Source-sink modifications affect leaf senescence and grain mass in wheat as revealed by proteomic analysis. *BMC Plant Biology*, *20*(1). https://doi.org/10.1186/s12870-020-02447-8

Ma, J., Zhang, K., Fang, B., Wang, X., Wang, S., Jiang, L., Wang, Z., & Hao, B. (2025). Optimization of nitrogen allocation and remobilization improves nitrogen use efficiency of winter wheat in the North China Plain. *European Journal of Agronomy*, *171*, 127782. https://doi.org/10.1016/j.eja.2025.127782

Ma, M., Li, H., Yan, D., Zhang, Y., Song, M., Wang, Y., Wang, H., Shao, R., Guo, J., & Yang, Q. (2022). Application of Blended Controlled-Release and Normal Urea with Suitable Maize Varieties to Achieve Integrated Agronomic and Environmental Impact in a High-Yielding Summer Maize System. *Agriculture*, *12*(8), 1247. https://doi.org/10.3390/agriculture12081247

Mbe, J. O., Dzidzienyo, D., Abah, S. P., Njoku, D. N., Aghogho, C. I., Onyeka, J., Tongoona, P., & Egesi, C. (2024). Genotype by environment interaction effect and fresh root yield stability of cassava genotypes under contrasting nitrogen regimes. *Scientific Reports*, *14*(1). https://doi.org/10.1038/s41598-024-71157-0

Mei, F., Chen, B., Du, L., Li, S., Zhu, D., Chen, N., Zhang, Y., Li, F., Wang, Z., Cheng, X., Ding, L., Kang, Z., & Mao, H. (2022). A gain-of-function allele of a DREB transcription factor gene ameliorates drought tolerance in wheat. *The Plant Cell*, *34*(11), 4472-4494. https://doi.org/10.1093/plcell/koac248

Menz, J., Modrzejewski, D., Hartung, F., Wilhelm, R., & Sprink, T. (2020). Genome Edited Crops Touch the Market: A View on the Global Development and Regulatory Environment. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.586027

Nehe, A. S., Misra, S., Murchie, E. H., Chinnathambi, K., Singh Tyagi, B., & Foulkes, M. J. (2020). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and protein concentration in Indian wheat cultivars. *Field Crops Research*, *251*, 107778. https://doi.org/10.1016/j.fcr.2020.107778

Oh, Y., Lee, B., Kim, H., & Kim, S.-G. (2020). A multiplex guide RNA expression system and its efficacy for plant genome engineering. *Plant Methods*, *16*(1), 37. https://doi.org/10.1186/s13007-020-00580-x

Padhan, B. K., Sathee, L., & Jain, V. (2021). Nitrogen remobilization and its importance in nitrogen use efficiency (NUE) of crops. *The Indian Journal of Agricultural Sciences*, *90*(12), 2251-2261. https://doi.org/10.56093/ijas.v90i12.110299

Panda, S., Majhi, P. K., Anandan, A., Mahender, A., Veludandi, S., Bastia, D., Guttala, S. B., Singh, S. K., Saha, S., & Ali, J. (2021). Proofing Direct-Seeded Rice with Better Root Plasticity and Architecture. *International Journal of Molecular Sciences*, *22*(11), 6058. https://doi.org/10.3390/ijms22116058

Qu, R., Zhang, P., Liu, Q., Wang, Y., Guo, W., Du, Z., Li, X., Yang, L., Yan, S., & Gu, X. (2022). Genome-edited ATP BINDING CASSETTE B1 transporter SD8 knockouts show optimized rice architecture without yield penalty. *Plant Communications*, *3*(5), 100347. https://doi.org/10.1016/j.xplc.2022.100347

Ranjan, A., Sinha, R., Singla-Pareek, S. L., Pareek, A., & Singh, A. K. (2022). Shaping the root system architecture in plants for adaptation to drought stress. *Physiologia Plantarum*, *174*(2). https://doi.org/10.1111/ppl.13651

Rempelos, L., Wang, J., Sufar, E. K., Almuayrifi, M. S. B., Knutt, D., Leifert, H., Leifert, A., Wilkinson, A., Shotton, P., Hasanaliyeva, G., Bilsborrow, P., Wilcockson, S., Volakakis, N., Markellou, E., Zhao, B., Jones, S., Iversen, P. O., & Leifert, C. (2023). Breeding Bread-Making Wheat Varieties for Organic Farming Systems: The Need to Target Productivity, Robustness, Resource Use Efficiency and Grain Quality Traits. *Foods*, *12*(6), 1209. https://doi.org/10.3390/foods12061209

Robert, P., Brault, C., Rincent, R., & Segura, V. (2022). Phenomic Selection: A New and Efficient Alternative to Genomic Selection. *Methods in Molecular Biology*, 397-420. https://doi.org/10.1007/978-1-0716-2205-6_14

Robertson, B. C., He, T., & Li, C. (2021). The Genetic Control of Stomatal Development in Barley: New Solutions for Enhanced Water-Use Efficiency in Drought-Prone Environments. *Agronomy*, *11*(8), 1670. https://www.mdpi.com/2073-4395/11/8/1670

Ruis, B. L., Bielinsky, A. K., & Hendrickson, E. A. (2025). Gene editing and CRISPR-dependent homology-mediated end joining. *Experimental & Molecular Medicine*, *57*(7), 1409-1418. https://doi.org/10.1038/s12276-025-01442-z

Scandurra, A., Corinzia, S. A., Caruso, P., Cosentino, S. L., & Testa, G. (2024). Productivity of Wheat Landraces in Rainfed and Irrigated Conditions under Conventional and Organic Input in a Semiarid Mediterranean Environment. *Agronomy*, *14*(10), 2338. https://doi.org/10.3390/agronomy14102338

Schwalbert, R. A., Parra, G., Sabino, M. J., Pott, L. P., Zbinden, F., Costanzi, J., & Di Mauro, G. (2026). Harnessing yield monitor technology to assess genotype yield stability at advanced on-farm stages of soybean breeding. *European Journal of Agronomy*, *172*, 127852. https://doi.org/10.1016/j.eja.2025.127852

Sharma, M., Singh, D., Saksena, H. B., Sharma, M., Tiwari, A., Awasthi, P., Botta, H. K., Shukla, B. N., & Laxmi, A. (2021). Understanding the Intricate Web of Phytohormone Signalling in Modulating Root System Architecture. *Int J Mol Sci*, *22*(11). https://doi.org/10.3390/ijms22115508

Sharma, N., Madan, B., Khan, M. S., Sandhu, K. S., & Raghuram, N. (2023). Weighted gene co-expression network analysis of nitrogen (N)-responsive genes and the putative role of G-quadruplexes in N use efficiency (NUE) in rice. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1135675

Singh, I., Kumar, K., Singh, P., Yadava, P., & Rakshit, S. (2021). Physiological and molecular interventions for improving nitrogen-use efficiency in maize. *Molecular breeding in wheat, maize and sorghum: strategies for improving abiotic stress tolerance and yield*, 325-339. https://doi.org/10.1079/9781789245431.0019

Singh, K., & Chandra, A. (2021). DREBs-potential transcription factors involve in combating abiotic stress tolerance in plants. *Biologia*, *76*(10), 3043-3055. https://doi.org/10.1007/s11756-021-00840-8

Singh, N. K., Singh, V., Harishankar, Panotra, N., Painkra, V. K., Sulochna, Manhas, S., Singh, S., M, R. G., & Singh, H. S. (2025). Next-Generation Nutrient Management Strategies for Wheat: Enhancing Productivity and Soil Quality. *Journal of Experimental Agriculture International*, *47*(5), 525-541. https://doi.org/10.9734/jeai/2025/v47i53441

Sprink, T., Wilhelm, R., & Hartung, F. (2022). Genome editing around the globe: An update on policies and perceptions. *Plant Physiology*, *190*(3), 1579-1587. https://doi.org/10.1093/plphys/kiac359

Thakur, N., Nigam, M., Mann, N. A., Gupta, S., Hussain, C. M., Shukla, S. K., Shah, A. A., Casini, R., Elansary, H. O., & Khan, S. A. (2023). Host-mediated gene engineering and microbiome-based technology optimization for sustainable agriculture and environment. *Functional & Integrative Genomics*, *23*(1). https://doi.org/10.1007/s10142-023-00982-9

Tiezzi, F., & Maltecca, C. (2022). Genotype by Environment Interactions in Livestock Farming. *Encyclopedia of Sustainability Science and Technology*, 1-21. https://doi.org/10.1007/978-1-4939-2493-6_1115-1

Uga, Y. (2021). Challenges to design-oriented breeding of root system architecture adapted to climate change. *Breeding Science*, *71*(1), 3-12. https://doi.org/10.1270/jsbbs.20118

van der Bom, F. J. T., Williams, A., & Bell, M. J. (2020). Root architecture for improved resource capture: trade-offs in complex environments. *Journal of Experimental Botany*, *71*(19), 5752-5763. https://doi.org/10.1093/jxb/eraa324

Wang, D., Zeng, Y., Yang, X., & Nie, S. (2024). Characterization of DREB family genes in Lotus japonicus and LjDREB2B overexpression increased drought tolerance in transgenic Arabidopsis. *BMC Plant Biology*, *24*(1). https://doi.org/10.1186/s12870-024-05225-y

Wang, Q., Zhang, J., Dai, Q., Cui, M., Yang, H., Cao, P., & Zhao, L. (2023). When green carbon plants meet synthetic biology. *Modern Agriculture*, *1*(2), 98-111. https://doi.org/10.1002/moda.17

Wang, W., Li, M.-Y., Zhou, R., Mo, F., Khan, A., Batool, A., Zhang, W., Lu, J.-S., Zhu, Y., Wang, B.-Z., Yang, Y.-M., Wang, J., Tao, X.-P., & Xiong, Y.-C. (2023). Leaf senescence, nitrogen remobilization, and productivity of maize in two semiarid intercropping systems. *European Journal of Agronomy*, *150*, 126943. https://doi.org/10.1016/j.eja.2023.126943

Wani, S. H., Vijayan, R., Choudhary, M., Kumar, A., Zaid, A., Singh, V., Kumar, P., & Yasin, J. K. (2021). Nitrogen use efficiency (NUE): elucidated mechanisms, mapped genes and gene networks in maize (Zea mays L.). *Physiology and Molecular Biology of Plants*, *27*(12), 2875-2891. https://doi.org/10.1007/s12298-021-01113-z

Yang, Y., Li, Y., Guo, Z., Zhao, Y., Zhou, X., Han, Y., & Lin, X. (2025). Identification of DREB gene family in foxtail millet (Setaria italica) and analysis of its expression pattern in response to abiotic stress. *Frontiers in Plant Science*, *16*. https://doi.org/10.3389/fpls.2025.1552120

Zhang, D., Yang, K., Kan, Z., Dang, H., Feng, S., Yang, Y., Li, L., Hou, N., Xu, L., Wang, X., Malnoy, M., Ma, F., Hao, Y., & Guan, Q. (2021). The regulatory module MdBT2–MdMYB88/MdMYB124–MdNRTs regulates nitrogen usage in apple. *Plant Physiology*, *185*(4), 1924-1942. https://doi.org/10.1093/plphys/kiaa118

Zhang, Y., Ma, Y., Zhao, D., Tang, Z., Zhang, T., Zhang, K., Dong, J., & Zhang, H. (2023). Genetic regulation of lateral root development. *Plant Signaling & Behavior*, *18*(1), 2081397. https://doi.org/10.1080/15592324.2022.2081397

Zhang, Y., Zhang, L., Chen, M., Wang, J., Dong, S., Yuan, X., & Li, X. (2025). Genome-wide identification and expression analysis of the DREB gene family in foxtail millet (Setaria Italica L.). *BMC Plant Biology*, *25*(1). https://doi.org/10.1186/s12870-025-06442-9

Zhao, Z., Shang, P., Mohanraju, P., & Geijsen, N. (2023). Prime editing: advances and therapeutic applications. *Trends in Biotechnology*, *41*(8), 1000-1012. https://doi.org/10.1016/j.tibtech.2023.03.004

Zhen, X., Zheng, N., Yu, J., Bi, C., & Xu, F. (2021). Autophagy mediates grain yield and nitrogen stress resistance by modulating nitrogen remobilization in rice. *PLOS ONE*, *16*(1), e0244996. https://doi.org/10.1371/journal. pone.0244996

Zhou, Y., Zhou, W., Liu, H., Liu, P., & Li, Z. (2020). Genome-wide analysis of the soybean DREB gene family: Identification, genomic organization and expression profiles in response to drought stress. *Plant Breeding*, *139*(6), 1158-1167. https://doi.org/10.1111/pbr.12867