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REVIEW

Decoding Sorghum Root System Architecture for Resource Use Efficiency and Climate Resilience Under Multifactorial Stress Conditions

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ABSTRACT

Climate-induced challenges, such as drought and nutrient depletion, are increasingly constraining global crop production, threatening food and nutritional security. *Sorghum bicolor* (L.), a climate-resilient cereal, demonstrates strong adaptive potential under resource-limited conditions due to its robust root system architecture (RSA). While above-ground improvements have received significant attention, the role of RSA in enhancing resource-use efficiency (RUE), particularly water use efficiency (WUE) and nitrogen use efficiency (NUE), remains underexploited in breeding programs. This review explores the physiological and molecular roles of sorghum RSA traits (e.g., root depth, density, branching pattern, and root angle) in improving RUE under abiotic stress. It highlights advances in multi-omics approaches, including transcriptomics, proteomics, and genome-wide association studies (GWAS), which provide insights into the genetic regulation of root development. High-throughput phenotyping platforms, including 2D, 3D, and emerging 4D imaging techniques, are evaluated for their effectiveness in capturing dynamic root traits and informing selection strategies. Sorghum's RSA offers a functional model for developing climate-resilient cultivars with improved WUE and NUE. The integration of modern phenotyping techniques with molecular insights and multi-omics strategies will expedite the identification of critical genetic and physiological determinants of RSA characteristics. This synthesis underscores the potential of RSA-targeted breeding strategies to enhance crop productivity and sustainability in water—and nutrient -constrained environments, aiding sustainable intensification and global food security in the face of climate change challenges.

1 | Introduction

Climate change is intensifying abiotic stresses such as drought and nutrient depletion, particularly in arid and semi-arid regions. These conditions directly affect crop performance by limiting resource availability. Drought and elevated temperatures, significant repercussions of climate change, among other abiotic stressors, adversely affect plant development and production by restricting the availability and absorption of vital resources, including water and nutrients (IPCC 2021; Pawar et al. 2023). Improving root system architecture (RSA) in climate-resilient crops, such as *Sorghum* *bicolor*, offers a promising strategy to enhance water and nitrogen uptake under these constraints. RSA defines the spatial organization of primary roots and root—and stem-derived branches (lateral roots), which play a fundamental role in plant adaptation to adverse soil conditions by improving plant growth and productivity (Smith and De Smet 2012).

In water-deficient conditions, nutrient mobility and uptake are impaired due to reduced mass flow and diffusion, directly affecting root absorption efficiency. Therefore, improving traits such as deeper rooting, increased root length density (RLD), and root angle plasticity is critical for maintaining resource use efficiency (RUE) under stress, RUE in sorghum refers to the plant's ability to maximize yield and productivity while minimizing the input of resources particularly water and other essential nutrients which are crucial for maintaining crop resilience and sustainable agricultural output in the context of climate change (Li, Zhu, et al. 2022).

The excessive application of nitrogen fertilisers in agriculture has been a longstanding global issue, as it leads to environmental deterioration, including soil acidification, groundwater pollution, and greenhouse gas emissions (Hakeem et al. 2016), and further contributes to human health concerns such as cancer and methemoglobinemia due to elevated nitrate levels (Rosales et al. 2020; Bijay-Singh and Craswell 2021). The inadequate nitrogen recovery by crops, along with diminished water availability, intensifies these problems, jeopardising the viability of intensive agricultural systems globally (Ranjan and Yadav 2019). Enhancing nitrogen use efficiency (NUE: Yield per unit nitrogen supplied) and water use efficiency (WUE: Yield produced per unit of water transpired) through genotypes with optimized root traits (e.g., deeper roots, higher root hair density) can reduce fertilizer dependence as well as scarcity of moisture stress during development stages, especially in crops like sorghum adapted to low-input systems (Fageria et al. 2008; Lynch 2013).

Sorghum (*Sorghum bicolor* L.), a fundamental food crop for millions in arid and semi-arid areas, serves as a paradigm for tackling these difficulties (Awio et al. 2024). Sorghum, indigenous to areas with little and unpredictable precipitation, has developed strong adaptive strategies to succeed in water-scarce environments; these adaptive features are attributed to its root architecture, which facilitates resource capture from deeper and drier soil layers (Prasad et al. 2021). Contemporary sorghum varieties possess a distinctive capacity to access water and nutrients from deeper soil strata, maintaining productivity during extended drought conditions. Sorghum provides substantial nutritional advantages, being a rich source of important proteins, fiber, minerals, and antioxidants, and thus constitutes a crucial crop for food security in resource-limited environments (Habyarimana et al. 2019; Xiong et al. 2021).

Globally, sorghum ranks among the top five cereal crops and serves as a major staple in arid and semi-arid regions due to its ability to thrive under water-limited and low-input conditions. It is cultivated across Africa, Asia, and the Americas, contributing significantly to food security, livestock feed, and industrial uses such as ethanol and biofuel production (Hariprasanna and Rakshit 2016). Additionally, global demand for sorghum has expanded due to its gluten-free nutritional profile and rising use in health-oriented food products. The versatility and adaptability of sorghum underscore its economic and agronomic importance in climate-resilient cropping systems (Dalton and Hodjo 2020). Sorghum's RSA is composed of root traits such as steep root angles, increased RLD, and extensive nodal rooting (Hostetler et al. 2023). These traits enable efficient exploitation of soil water and nitrogen, improving both WUE and NUE under variable climatic conditions (Tron et al. 2015; Dietrich 2018).

Moreover, the flexibility of RSA enables sorghum plants to adjust to abiotic stressors, modifying root development patterns to enhance resource acquisition in fluctuating environmental conditions (Orman-Ligeza et al. 2013; Adu et al. 2022). For instance, deeper roots enhance water absorption from subterranean layers during drought, whereas lateral roots maximize nutrient uptake from topsoil. Root hairs augment nutrient absorption by expanding root surface area, significantly contributing to the intake of nitrogen and other vital elements (Liao et al. 2006; Holz et al. 2017). Investigations on sorghum's RSA yield a significant understanding of the genetic and phenotypic characteristics linked to enhanced resource utilization efficiency.

Sorghum's natural drought resistance, enhanced by its effective root characteristics, establishes it as an exemplary crop for tackling the dual issues of water and nutrient deficiency in a changing environment (Borrell et al. 2014). Exploring the genetic basis of RSA traits through tools like Genome-Wide Association Studies (GWAS) and transcriptomics allows for the identification of root-related Quantitative Trait Loci (QTLs) and genes that contribute to drought resilience. These insights can guide sorghum breeding efforts toward developing root ideotypes tailored to low-input and stress-prone environments.

This review aims to deliver an exhaustive examination of the morphology and architecture of sorghum roots, emphasizing characteristics related to water and nitrogen use efficiency. This review critically evaluates the morphological and molecular characteristics of sorghum root architecture and their contribution to climate resilience. It studies the genetic and molecular pathways underlying RSA and its contribution to RUE. It synthesizes findings on root-related traits influencing WUE and NUE, highlights recent advances in high-throughput root phenotyping, and discusses integration strategies into sorghum breeding programs, providing a framework for harnessing sorghum's root capabilities to develop sustainable agricultural systems during climate change.

2 | Sorghum's Root System Unveiled for Resource Use Efficiency (RUE)

The root system of sorghum is similar in organization and development to that of maize (*Zea mays*) (Hostetler et al. 2023). The first root, or primary root, develops from the radicle (embryonic root) of the embryo. Shortly after the primary root begins elongating, seminal roots (seed roots) also develop from the embryo, emerging from the region above the radicle (primary root). The primary and seminal roots are temporary, eventually dying as their function is taken over by adventitious roots that develop from the stem. Sorghum develops two types of stem-borne roots (Demissie et al. 2023). Some adventitious roots develop near the base of the stem and are completely underground. Prop roots or brace roots begin development aboveground and grow into the soil. Prop roots help to support the aerial part of the plant; the aboveground parts of prop roots are green and conduct photosynthesis (Demissie et al. 2023; Earth@Home: Evolution 2023). During the 4–5 leaf stage, coleoptile nodal roots develop, increasing the complexity of the root system and improving the plant's capacity to access deeper soil layers (Singh et al. 2010; Bozhurin 2024).

Sorghum is distinctive among cereal crops because of its singular primary root and the prolific generation of root hairs, which enhance the surface area for the absorption of water and nutrients (Demissie et al. 2023). Research has demonstrated that root hairs are essential for the acquisition of immobile nutrients, including phosphorus, from the soil (Czarnota et al. 2003; Holz et al. 2017). The extensive and resilient root structure allows sorghum to access hard soil strata, tapping into previously unavailable water and nutrient reserves, rendering it particularly well-suited for cultivation in semi-arid and drought-prone areas. Notably, root hairs account for up to 80% of the total nutrient uptake capacity of roots, underscoring their critical functional role in resource acquisition (Jungk 2001). This trait is especially beneficial in situations when water supply is limited or inconsistently allocated during the growing season (Demissie et al. 2023).

Root plasticity, defined as the capacity of roots to alter their growth and function in reaction to nutritional gradients, is an essential characteristic. Sorghum roots exhibit plasticity by modulating architecture, prioritizing elongation into deeper profiles under drought, while adjusting lateral root formation in nutrient-rich zones; this dynamic allocation supports efficient soil exploration (Bollam et al. 2021; Lynch 2021). For example, plants may prioritise resource allocation to root extension (root penetration) over lateral root development, thereby enhancing their ability to reach subterranean moisture and nutrients (Singh et al. 2010). The role of sorghum's root system in its resilience can be demonstrated by comparing root biomass distribution in water-stressed and non-stressed conditions (Gonulal 2022). Sorghum plants under water stress demonstrate an increased root-to-shoot ratio, which is closely associated with their capacity to continue growth and preserve yields despite unfavourable conditions. Demissie et al. (2023) indicated that sorghum genotypes exhibiting greater RLD in deeper soil strata, displayed enhanced drought resistance and yield consistency. Incorporating both visual and quantitative data would highlight the essential function of sorghum's root system in RUE.

The RSA traits most relevant to RUE in sorghum include deeper root penetration, steep root angles, greater nodal root number, and higher root hair density. These traits improve both WUE and NUE by enhancing belowground resource capture, especially under drought or low-nutrient conditions (Borrell et al. 2014; Badigannavar et al. 2018). Understanding these adaptive mechanisms is essential for integrating root traits into breeding pipelines that aim to enhance climate resilience.

2.1 | Improving NUE

NUE is an essential characteristic in sustainable agriculture, affecting plant productivity and mitigating the environmental consequences of excessive nitrogen fertilisation. NUE is an agronomic parameter that relates plant growth to nitrogen availability in the soil (Moll et al. 1982). This parameter is essential for optimizing fertilizer inputs and minimizing environmental impacts in low-input and marginal farming systems. Notably, only 30%–40% of applied nitrogen fertilizer is typically taken up by crops, with the remainder lost to leaching, volatilization, and denitrification, contributing to environmental contamination and reduced nutrient recovery efficiency (Peinado-Torrubia et al. 2023). Recent evidence indicates that chloride nutrition can alleviate nitrogen deficiency symptoms and reduce plant nitrate requirement by enhancing the efficiency of nitrate assimilation pathways (Lucas et al. 2024). In *Sorghum bicolor*, NUE is governed by genotypic variation in root system traits such as RLD, surface area, and hair density, which influence nitrogen uptake from both surface and subsoil layers. These traits interact with root-expressed nitrogen transporters and assimilation genes to determine the plant's capacity for nitrogen acquisition and internal translocation (Garnett and Rebetzke 2013; Louvieaux et al. 2020).

The structure and architecture of sorghum roots affect its capacity to assimilate nitrogen. Variability in sorghum root architecture significantly affects NUE, with deeper and denser root systems enabling more effective nitrogen capture (Gastal and Lemaire 2002). Studies show that genotypes with greater RLD access residual soil nitrate efficiently under low-input conditions (Bollam et al. 2021) (Figure 1). Microscopic root hairs significantly enhance the effective root-soil contact, facilitating nitrate absorption in low-nitrogen conditions (Wang et al. 2006). Research indicates that genotypes possessing greater root biomass and volume during early growth stages demonstrate enhanced NUE. Anbessa and Juskiw (2012) showed in barley that augmented root dry weight and volume at the five-leaf stage resulted in markedly improved NUE, underscoring the significance of analogous traits in sorghum.

Enhancing NUE in sorghum by genetic and breeding methods is problematic due to the complexity of the trait and the difficulties associated with phenotyping root systems (Garnett et al. 2015). Bollam et al. (2021) evaluated 60 diverse sorghum genotypes and identified superior NUE performance under low nitrogen conditions. Genotypes with enhanced NUE showed differential expression of nitrogen-related genes, including *SbAMT* (ammonium transport), *SbNRT* (nitrate uptake), *SbGS* (glutamine synthesis), *SbNiR* (nitrite reduction), and *SbGDH* (glutamate metabolism), suggesting these are potential targets for marker-assisted selection.

Wild and landrace sorghum accessions represent untapped sources of allelic diversity for NUE traits. Massel et al. (2016) demonstrated that exotic germplasm possesses distinct RSA

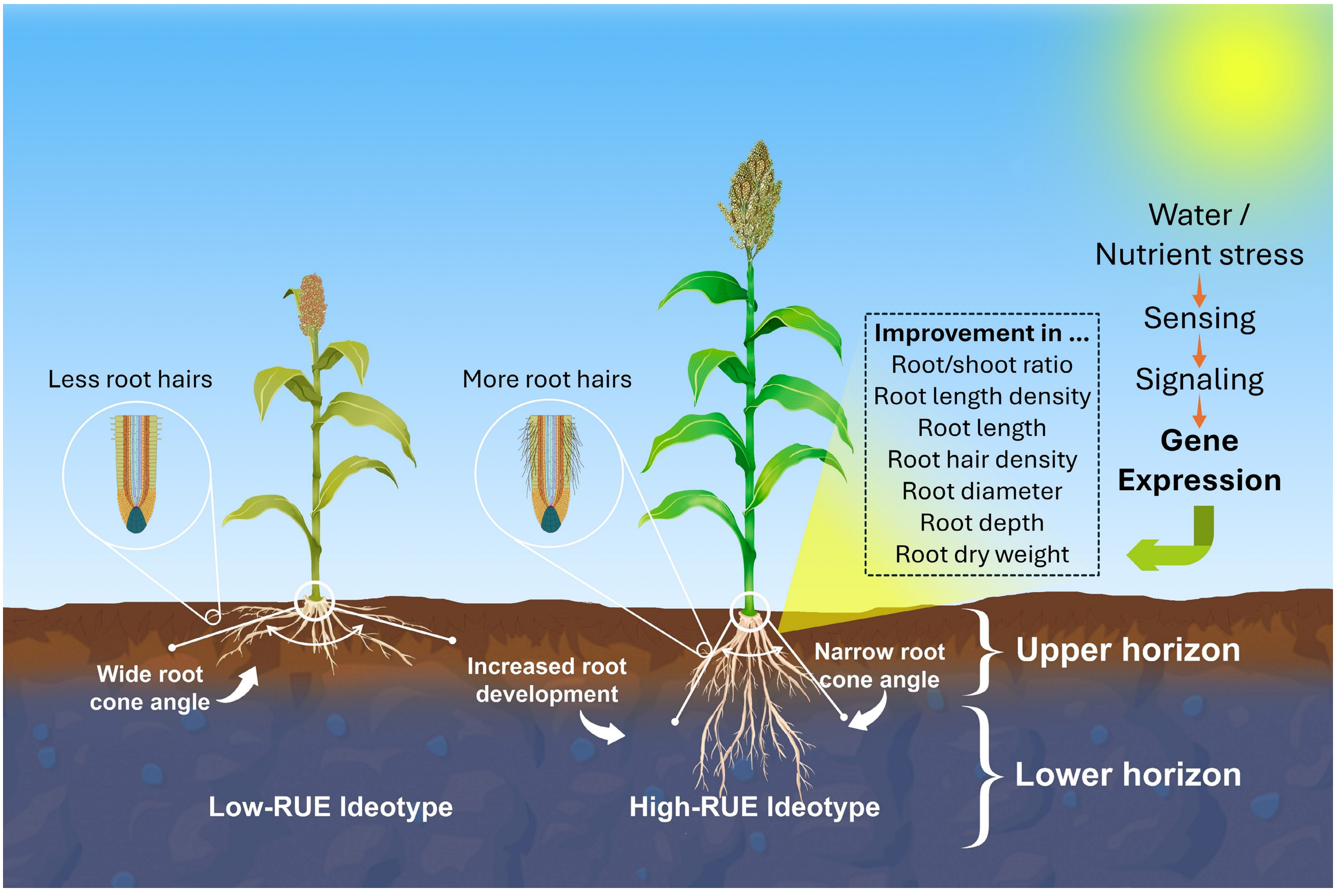

FIGURE 1 | Illustration of RSA ideotypes for enhanced RUE comparing low and high RUE Sorghum phenotypes to confer resilience to multifactorial stress. RUE, resource-use efficiency; RSA, root system architecture.

traits such as deeper rooting and prolific lateral branching, supporting improved nitrate acquisition in marginal soils. However, breeding for NUE remains constrained by phenotyping bottlenecks, especially for root traits in field environments. High-throughput proxy traits such as canopy NDVI, root-to-shoot ratio, and carbon allocation efficiency are being explored to facilitate indirect selection for NUE (Lopatin et al. 2019; Fiorani and Schurr 2013). The stay-green trait in sorghum prolongs photosynthetic activity under nitrogen stress, improving post-anthesis N remobilization and contributing to NUE (Nehe et al. 2018). This trait is associated with deeper root systems and improved shoot-to-root carbon allocation.

NUE enhancement in sorghum can be approached via (1) selection for RSA traits that improve late-season nitrogen uptake (Schneider et al. 2020), and (2) breeding for improved nitrogen remobilization efficiency (Ostmeyer et al. 2022). For instance, root depth and angle QTLs (Mace et al. 2011) can be combined with traits like delayed senescence to improve nitrogen retention and translocation efficiency.

Integration of GWAS and QTL mapping has enabled the identification of loci linked to NUE traits in sorghum, including root morphology and transporter genes. Marker-assisted selection targeting precise genes can accelerate the development of nitrogen-efficient cultivars (Massel et al. 2016). Speed breeding combined with high-throughput imaging offers a route to efficiently screen large populations. In summary, a holistic strategy that combines root trait optimisation, genetic enhancement, and sophisticated phenotyping techniques is essential for enhancing NUE in sorghum. These developments will augment productivity and promote sustainable agriculture methods in nitrogen-deficient conditions.

2.2 | Enhancing WUE

In sorghum, WUE is a vital trait for sustaining biomass production in rainfed and drought-prone environments. WUE is broadly defined as the amount of biomass or yield produced per unit of water consumed through transpiration (Stanhill 1986). Different forms of WUE are used in crop research and breeding, such as instantaneous WUE (WUEi), biomass-based WUE (WUEbio), yield-based WUE, and carbon isotope discrimination-based WUE (WUE^13C), each reflecting different physiological responses and time scales (Zabuloni et al. 2025; Franco-Navarro et al. 2025). Understanding these distinctions is essential for selecting the appropriate WUE metric under specific environmental and management conditions. WUE reflects the plant's capacity to balance water uptake and transpiration with carbon assimilation, particularly under variable soil moisture regimes (Narayanan et al. 2013). In reaction to soil moisture shortages, plants implement many adaptive strategies, including alterations in RSA and physiological changes such as osmolyte accumulation (FAO 2011; Raza et al. 2012). Root system characteristics are crucial for enhancing water

TABLE 1 | Key sorghum RSA traits contributing to WUE, their physiological roles.

Trait	Function in WUE	References
Root angle	Deep soil water access	Mace et al. (2011)
Root-to-shoot ratio	Drought adaptation	Abreha et al. (2021)
Stay green	Delayed senescence, longer uptake	McHenry et al. (2016)
Xylem vessel diameter	Hydraulic conductivity	Bijanzadeh et al. (2023)
RLD	Greater soil moisture exploration	Demissie et al. (2023)

Abbreviations: RLD, root length density; RSA, root system architecture; WUE, water-use efficiency.

absorption and guaranteeing crop viability and yield during water scarcity (Blum 2009; Palta et al. 2011).

Sorghum possesses a highly adaptable root system, rendering it an exemplary crop for research on drought tolerance and water efficiency (Verma et al. 2018). In drought situations, sorghum exhibits significant alterations in root shape to enhance its capacity for water absorption and conservation (Badigannavar et al. 2018). Drought-tolerant sorghum genotypes often exhibit deeper root systems with higher RLD, allowing access to subsoil water reserves during terminal droughts. This deep-rooting trait has been positively correlated with canopy temperature depression and increased grain yield under water stress (Li et al. 2019; Demissie et al. 2023).

Increased RLD and higher root-to-shoot ratios in water-stressed conditions contribute to greater soil exploration and sustained transpiration rates. These traits reduce midday stomatal closure and support continued photosynthesis, thereby enhancing biomass production per unit water (Lynch 2021; Djanaguiraman et al. 2023). The profundity of the root system facilitates access to subterranean water reserves, serving as a buffer against variations in surface soil moisture (Kou et al. 2022). This characteristic is especially beneficial in semi-arid areas, where precipitation is unpredictable and surface evaporation rates are elevated (Figure 1). The architectural structure of sorghum roots, particularly lateral root branching, augmented stele diameter and enhanced xylem vessel diameter, contribute to improved water extraction and hydraulic conductivity, hence supporting shoot growth during intermittent drought periods. (Ogbaga et al. 2014; Bijanzadeh et al. 2023).

The root angle is essential in influencing water availability, which is the angle between the trajectory of root growth and the vertical axis of the soil (Hodge et al. 2009). Sorghum genotypes with steeper root angles can penetrate deeper into the soil profile, hence reaching subterranean moisture stores inaccessible to genotypes with shallower root systems. Root angle QTLs have been mapped on *SBI-01* and *SBI-07* (Mace et al. 2011), indicating the potential for selection in drought-prone regions.

An elevated root-to-shoot ratio is an additional adaptation characteristic linked to improved WUE. Drought-resistant sorghum varieties dedicate a larger share of biomass to root growth, enhancing water absorption while sustaining aerial growth and yield (Abreha et al. 2021). This resource allocation technique improves resilience to water stress by emphasizing root development in challenging situations. The stay-green characteristic in sorghum is a recognized physiological adaptation linked to delayed leaf senescence and extended photosynthetic activity. This characteristic indirectly enhances root function by facilitating ongoing carbon allocation to roots in the later growth phases, therefore maintaining water and nitrogen absorption during grain filling (McHenry et al. 2016; Djanaguiraman et al. 2023).

Although vast root systems offer distinct benefits for water absorption, they may entail metabolic trade-offs, as sustaining a substantial root biomass demands considerable energy expenditure (Dunbabin et al. 2003). Achieving optimal WUE in sorghum necessitates a balance of root size, root activity, and the metabolic expenses related to root growth. Recent advancements in phenotyping instruments and root imaging technology provide the opportunity to analyse these trade-offs and direct breeding initiatives towards the creation of sorghum cultivars with enhanced root characteristics (Thorup-Kristensen and Kirkegaard 2016). Genetic variation in RSA traits such as root angle, RLD, and xylem diameter underlies differences in WUE among sorghum genotypes (Wasson et al. 2012; Lynch 2013). A summary of key sorghum root traits contributing to WUE, along with their physiological functions and supporting references, is presented in Table 1. These traits can serve as targets for root-focused breeding in water-limited environments.

Recent genomic research has identified QTLs linked to root architecture (Table 2) and water uptake efficiency, establishing a basis for marker-assisted selection (Li et al. 2014). Moreover, phenotypic linkages between root and shoot characteristics, including canopy temperature depression, present prospects for the development of selection approaches aimed at breeding water-efficient sorghum varieties. By harnessing natural variation in root traits and integrating high-throughput phenotyping, sorghum breeding can deliver cultivars with superior WUE. This will contribute to yield stability in drylands while promoting resource-efficient agriculture.

3 | Molecular Characterization of Sorghum Root Morphology

Understanding the impact of genetic factors on root characteristics in many sorghum varieties is challenging. Sorghum's extensive genetic diversity makes it an ideal model for investigating the molecular mechanisms governing root system development and adaptation that influence the development of the plant's root system (Tuberosa et al. 2021). An essential aspect of the research involves identifying and characterizing genetic

TABLE 2 | MTAs associated with different root traits in sorghum.

Trait	QTL name	Linkage group	Marker interval	Position	LOD score	References	R^2 (%)
Root length (cm)	*qRL4*	SBI-04	*Xsnp56-Xiabt194*	32.21	2.84	Rajkumar et al. (2013)	8.33
Root Volume	*qRV1*	SBI-01	*Xiabt210-Xiabt69*	183.51	2.01	Rajkumar et al. (2013)	13.96
	qRV4	SBI-04	*Xtxp51-Xtxp270*	258.01	3.62	Rajkumar et al. (2013)	13.09
Number of roots/plants	*qRN1*	SBI-01	*Xiabt210-Xiabt69*	181.51	2.62	Rajkumar et al. (2013)	17.87
Root to shoot ratio	*qRS10*	SBI-10	*Xiabt489-Xiabt364*	101.81	3.63	Rajkumar et al. (2013)	8.07
	qRS10.1	SBI-10	*Xiabt312-Xiabt178*	155.01	2.42	Rajkumar et al. (2013)	7.96
Nodal root angle	*qRA1_5*	SBI-05-II	*SPb-5892/SPb-4323*	51.8	3.69	Mace et al. (2011)	10.01
	qRA2_5	SBI-05-III	*SPb-6287/SPb-9490*	34	4.96	Mace et al. (2011)	29.78
	qRA1_8	SBI-08-II	*SPb-4767/SPb-4432*	25.4	2.68	Mace et al. (2011)	6.72
	qRA1_10	SBI-10	*SPb-1660/SPb-7058*	208.4	2.26	Mace et al. (2011)	11.65
Brace root	*qRT6*	SBI-6	*Xtxp127-Xtxp6*	97	9.1	Li et al. (2014)	7.0
	qRT7	SBI-7	*Dsenhsbm7-Xcup70*	79	47.8	Li et al. (2014)	52.2
Root fresh weight (g)	*qRF4*	SBI-04	*Xtxp51-Xtxp270*	258.01	2.6	Rajkumar et al. (2013)	9.21
Root dry weight (g)	*qRD4*	SBI-04	*Xtxp51-Xtxp270*	258.01	2.6	Rajkumar et al. (2013)	9.21
	qRDW1_2	SBI-02	*SPb-4366/SPb-5544*	216.8	2.82	Mace et al. (2011)	13.05
	qRDW1_5	SBI-05-II	*SPb-4086/SPb-6323*	101.7	3.97	Mace et al. (2011)	12.06
	qRDW1_8	SBI-08-II	*SPb-7889/SPb-6935*	123.3	2.63	Mace et al. (2011)	6.97

Abbreviations: LOD, logarithm of odds; QTL, quantitative trait locus; R^2, coefficient of determination; SBI, *Sorghum bicolor* chromosome.

variants across different sorghum varieties, each of which has unique root morphologies, lengths, and densities. Scientists employ advanced approaches such as GWAS and QTL mapping to examine the genetic structure that governs sorghum root characteristics. Table 2 is a compilation of several marker trait associations (MTAs) that have been discovered for various root traits in sorghum.

Gene expression profiling offers an in-depth view of how genetic factors respond to various environmental conditions (Figure 2B). In a recent study, Parra-Londono et al. (2018) utilized GWAS to analyze RSA of sorghum in environments with varying levels of phosphorus. The researchers elucidated the genetic foundation of root-system development and discovered noteworthy QTL on chromosomes 2, 3, 5, and 9. These QTL hotspots, located on chromosomes SBI-02 and SBI-03, exert significant control over multiple traits associated with root-system development. This study revealed that sorghum genotypes with compact and shallow root systems perform well under phosphorus-deficient conditions, while genotypes with exploratory root systems show superior adaptation under nitrogen or water-limited environments.

Transcriptome analyses offer valuable information about the regulatory networks governing the growth of sorghum roots. Zhang et al. (2019) conducted transcriptome profiling of drought-stressed sorghum leaves and roots using a seedling stage technique. The objective of the study was to identify genes

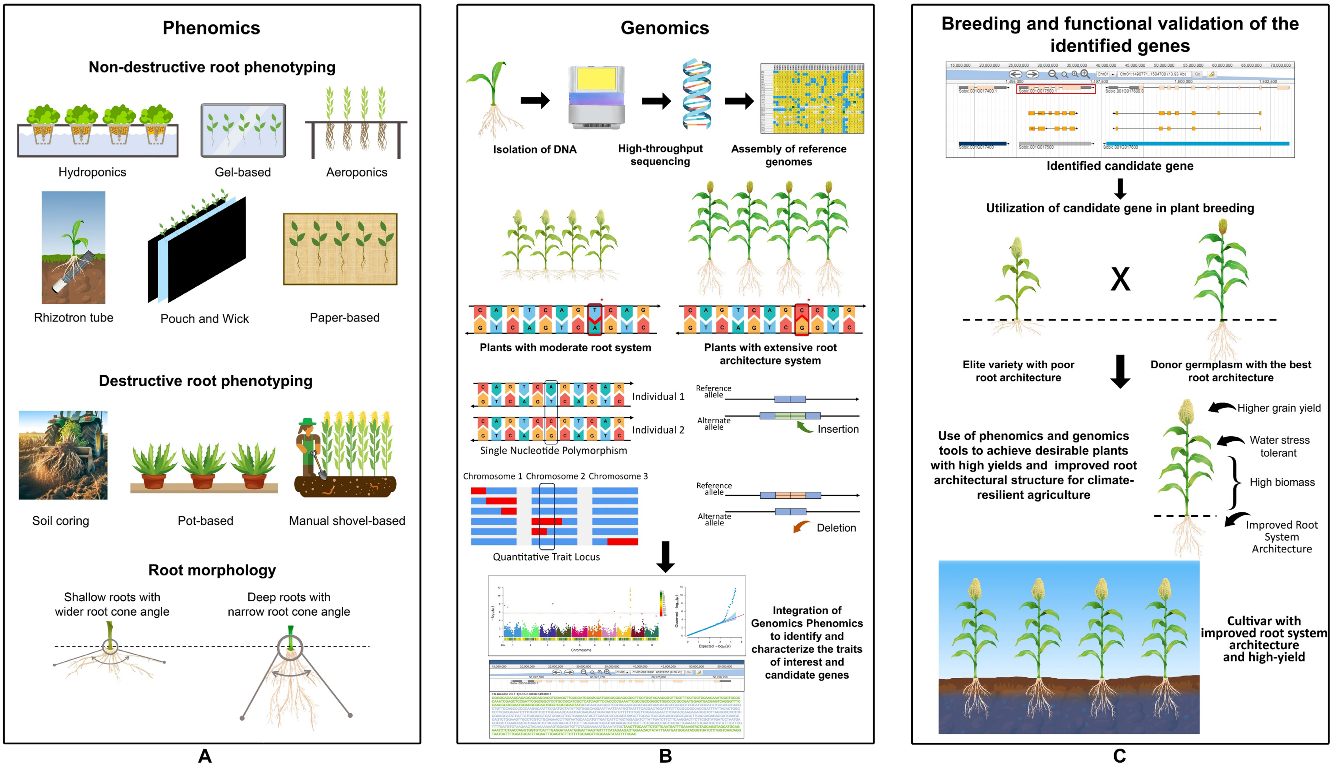

FIGURE 2 | Integrated breeding techniques to develop resource-efficient sorghum with enhanced root systems. (A) Various non-destructive and destructive methods are used to examine root system architecture. These methods are used to define root-related traits more precisely using high-throughput phenotyping. (B) Utilization of genomic tools to identify significant marker trait associations and candidate genes associated with RSA. (C) Enhancing Drought Adaptation in Sorghum through Improved Root Systems: Utilizing biotechnological tools to select genotypes with deeper, extensive roots for better water access. Improved root systems lead to increased resource use efficiency, ultimately resulting in higher yields of both grain and biomass.

that are differently expressed in response to drought stress and examine the molecular mechanisms that contribute to sorghum's ability to tolerate drought. Functional genomics tools, including gene deletion and overexpression assays, help validate the role of identified genes in shaping RSA traits under stress.

Proteomics, although still underutilized in sorghum, offers essential insights into the protein-level regulation of RSA traits through studies in model crops and cereals. Proteins function as essential regulators of RSA by influencing cellular processes, including root elongation, lateral root initiation, and responses to biotic and abiotic stressors. Numerous studies have emphasized the role of proteins and pathways in RSA. Auxin-responsive proteins, including *AUXIN-BINDING PROTEIN1* (*ABP1*), regulate root development by modulating cell division and elongation in response to auxin gradients (Sauer et al. 2013; Wang et al. 2010). Expansins, a class of proteins that loosen cell walls, promote root elongation by aiding cell wall expansion, allowing roots to penetrate deeper soil layers in search of water and nutrients under stress conditions (Cosgrove 2015).

While research directly connecting proteomics studies to sorghum RSA is sparse, insights can be gleaned from model crops like rice and Arabidopsis. *ROOT HAIR DEFECTIVE3* (*RHD3*) and its homologs are associated with root hair elongation and vesicle trafficking, both essential for effective water and nutrient absorption (Gendre et al. 2011). While direct evidence in sorghum is limited, these proteins have been characterized in Arabidopsis and rice, offering functional clues for sorghum research. Recent advancements in proteomics methods, including mass spectrometry and isotopic labelling, facilitate the identification of protein networks linked to RSA characteristics in response to environmental factors like drought or nitrogen deficiency (Li, Tan, et al. 2022). Nonetheless, tailored proteomics investigations in sorghum are necessary to discover critical proteins and their interactions pertinent to RSA. These efforts may connect genetic data with functional traits, facilitating the creation of resource-efficient sorghum cultivars via multi-omics methodologies (Gorthy et al. 2024).

The integration of omics technologies, like genomics, transcriptomics, and proteomics, provides a comprehensive understanding of the genetic landscape by linking molecular variation to phenotypic characteristics (Jadhav et al. 2024) (Figure 2B). GWAS on a large scale can help identify SNPs that are significant and tightly linked to traits of interest (Narkhede et al. 2022; Thakur, Gorthy, et al. 2024). Comprehending alterations in gene expression during development and in response to environmental stress contributes to our understanding of the mechanisms that drive root growth and adaptation (Somegowda et al. 2024). The study of the proteome enables scientists to enhance their comprehension of how certain proteins govern root growth and function (Somegowda et al. 2024). These integrated findings strengthen our understanding of genotype-to-phenotype relationships in sorghum RSA, guiding the development of resilient, resource-efficient cultivars.

4 | Advancing Root Phenotyping Capabilities: Addressing the Needs

Understanding plant responses to environmental stimuli and agronomic practices remains limited, primarily due to the challenges associated with monitoring root system behavior (Paez-Garcia et al. 2015). In sorghum, phenotyping of root traits plays a pivotal role in dissecting resource acquisition under stress conditions. These methods involve analyzing and evaluating the structural and physical traits of root systems under different conditions, with a specific emphasis on the efficiency of resource utilization.

Investigating factors that govern spatial expansion and temporal dynamics of root growth in sorghum can provide essential insights into optimizing RSA for efficient resource uptake across agroecological zones (Parra-Londono et al. 2018). Phenotyping enables the quantification of genotypic variability in root development and allocation strategies, especially under abiotic stress conditions such as drought and nutrient deficiency. An in-depth understanding of the internal regulatory mechanisms guiding root responses is necessary to design robust root phenomics strategies aimed at enhancing RUE.

RSA quantification in sorghum utilizes a range of methodologies under laboratory, glasshouse, and field conditions. Laboratory techniques typically involve destructive sampling, where plants are excavated, cleaned, and analyzed for parameters such as root length, diameter, and branching using microscopy-based methods (Lee et al. 2005). While such techniques enable high-resolution trait measurement, they often lead to the loss of fine root structures and provide only static snapshots of root systems.

Hydroponics, climate-controlled growth chambers, in vitro culture systems, and rhizotrons are increasingly being utilized as simulated environments to study root system development under controlled conditions (Dutta et al. 2023). Rhizotrons, both large and miniaturized formats, are non-invasive platforms that allow real-time visualization of root development under controlled conditions (Reni et al. 2025). In sorghum, mini-rhizotrons have proven useful in monitoring dynamic RSA traits such as elongation rate, root hair emergence, and depth progression under varying nutrient and moisture regimes (Clark et al. 2012; Hostetler et al. 2023). The integration of imaging software enables quantitative assessments of traits like root density and angle, essential for identifying genotypes suited for resource-limited conditions. Rhizotron-based phenotyping thus provides a bridge between controlled environment studies and field applications, supporting trait validation and selection. Apart from this, rhizotrons are typically fixed in place, making it difficult to move or reposition them once installed. This restricts flexibility in experimental design and site selection (Reni et al. 2025). While rhizotrons allow root observation, the visible area is restricted to the transparent panels, so roots growing away from these windows may not be captured, leading to incomplete data on total root architecture (Sartoni et al. 2015).

In field studies, techniques such as soil coring and augering are widely used to sample sorghum roots (Broad and Hammer 2004). Following the extraction of soil cores containing root systems using augers or coring devices, the cores are thoroughly cleaned and subsequently analyzed for various architectural parameters (Clark et al. 2012). Ground-penetrating radar is a geophysical technique that provides non-destructive data on root distribution in the field (Rocha et al. 2024; Wolfe et al. 2023). In addition, complete sorghum plants can be extracted from the field, their roots washed and then analyzed in a laboratory environment to facilitate comprehensive investigations of root structure under realistic conditions.

Past techniques for sampling roots have been progressively enhanced to aid in the study of root phenotyping. However, these methods, which involve destructive sampling, often lead to the loss of finer-scale root characteristics, such as smaller lateral roots and root hairs. Additionally, they only provide a single momentary measurement of root development (Bucksch et al. 2014). The labor-intensive and time-consuming nature of these techniques necessitates the development of rapid, high-throughput, and precise methods to assess RSA in situ, particularly for breeding programs aiming to improve resource-use efficiency in crops like sorghum (Bucksch et al. 2014; Li, Zhu, et al. 2022).

5 | Diversity of Root Phenotyping Techniques

Non-invasive and high-throughput methods for root phenotyping are essential to investigate the dynamic changes in root traits across developmental stages and environmental conditions (Figure 2A). Modern approaches aim to capture the spatial complexity and temporal dynamics of root systems with improved automation and resolution. In sorghum, the application of these evolving tools is critical for understanding root architectural responses to stress and guiding the selection of RSA traits that contribute to enhanced RUE.

5.1 | 2D Root Phenotyping

This widely used methodology investigates fundamental characteristics of roots by utilizing an advanced imaging apparatus, a controlled growth environment, and sophisticated software for analyzing images (Delory et al. 2022; Li, Zhu, et al. 2022). However, due to the limited nature of 2D methods, they can only provide limited information because of their single-plane imaging perspective. Consequently, they can measure only a small number of observable root traits (Wasaya et al. 2018). This platform comprises a growth system, imaging device, and image processing software.

Typically, the measurement of various aspects of RSA is done using digital cameras or scanners to capture images of the root system. Image acquisition is simple for plants that are not grown in soil, but for plants grown in mesocosms, they must either be physically separated from the soil or imaged through mini-rhizotron tubes. Once the images are obtained, it is necessary to measure the RSA traits to analyze the impact of various factors (such as genotypes, mutations, nutrient conditions, etc.) on the growth of the root system (Zhu et al. 2011). Selecting relevant RSA traits for evaluation remains a critical and challenging step in 2D phenotyping.

In the past, there has been an emphasis on studying RSA traits that are relatively simple, mainly due to the limited availability of tools to assess and describe complex RSA. In the last 10 years, multiple advanced image analysis programs have been created to enhance the range and intricacy of RSA traits that can be examined, as well as to improve the speed and precision of RSA trait measurements (Zhu et al. 2011). Joshi et al. (2017) developed a phenotyping platform that allows for rapid, non-destructive, and digital measurement of the nodal root angle of sorghum during the seedling stage. Soil-less methods, such as aeroponics, hydroponics, pouch-and-wick systems, and agar gel-based platforms, provide a way to easily see the roots and efficiently control the environment (Kuijken et al. 2015). Aeroponics is primarily employed for the examination of root architecture in vegetables, allowing for the customization of its parameters as needed (Tiwari et al. 2020). In sorghum, its application remains limited and mostly exploratory. Hydroponics allows for the efficient characterization of root morphological traits in different crops. However, it is primarily suitable for short-term observations and may not be ideal for studying root hairs (Ranjan and Yadav 2019; Chen et al. 2020). The pouch-and-wick system is a cost-effective and uncomplicated technique for assessing root morphology, but it is only suitable for examining roots at the seedling stage (Adu et al. 2014; Wu et al. 2018). Agar gel-based phenotyping systems have restrictions in that they can only be used to analyze the roots of seedlings. Additionally, the root traits observed in seedlings may not accurately reflect those of mature plants. Nonetheless, such early-stage traits may offer predictive value for later-stage root system development (Bengough et al. 2004; McPhee 2005).

Soil-based root phenotyping platforms, such as *RhizoPot* and the system developed by Bontpart et al. (2020), offer the ability to observe roots in their natural environment. However, these platforms often have restrictions in terms of depth and the number of samples that can be processed simultaneously. Transparent soil has been suggested to overcome the constraints of soil-based platforms, enabling the study of root characteristics in living organisms. However, there are still some drawbacks that require attention (Ma et al. 2019).

Several software programs have been developed to analyze the kinematics or morphology of root growth and gravitropism. These methods include *RootTrace* (French et al. 2009), *Relative Elemental Growth Rate* (REGR) analysis (Walter et al. 2002; Nagel et al. 2009), Kine-Root (Basu et al. 2007), and *RootFlowRT* (Van Der Weele et al. 2003). These programs specifically concentrate on the analysis of root growth using a sequence of images taken over a period. To simplify the process of implementing this approach, integrated platforms have been created to automate both the acquisition and analysis of images. *Phytomorph* (Brooks et al. 2009) is a comprehensive initiative designed to create advanced imaging and analysis technologies for the study of plant growth. *PlaRoM* (Yazdanbakhsh and Fisahn 2009) is a robotic growth system that is fully automated and self-contained. It is designed to image and analyze the growth of Arabidopsis roots on agar plates that are oriented vertically.

The image analysis methods have proven to be highly valuable in accurately measuring the impact of temperature, genotype, and nutrient availability on root growth with great precision in terms of both space and time. However, their usefulness is limited when it comes to analyzing RSA on a larger scale. In response to this requirement, a second category of programs has been created to measure RSA characteristics throughout the entire root system. The following software tools include *WinRhizo* (www.regentinstruments.com), Delta-T-Scan (www.delta-t.co.uk), *WR-RIPL* (http://rootimage.msu.edu), RMS (Ingram and Leers 2001), *RooTracker* (www.biology.duke.edu/rootracker), *EZ-Rhizo* (Armengaud et al. 2009), and *DART* (Bot et al. 2009). These programs measure various advanced features of RSA, such as branching, density, angles, total area, and root order. Certain programs, such as *DART*, incorporate temporal analysis of measurements that can be utilized to monitor the emergence of lateral roots. Each of these programs possesses distinct advantages and disadvantages concerning their suitability for various systems, the degree of automation, and the quantity and intricacy of RSA characteristics analyzed.

5.2 | 3D Root Phenotyping

Investigations on RSA have been carried out using two-dimensional images, resulting in the omission of vital information as roots develop in a three-dimensional environment (Hargreaves et al. 2008; Lafond et al. 2015). Although 2D root phenotyping methods offer ease and simplicity, their effectiveness is constrained by their inability to capture the spatial complexity of root architecture as they only offer a single-point perspective, which restricts the range of traits that can be measured. Therefore, the use of 3D root phenotyping is increasing in popularity because of technological advancements that allow for digital reconstruction and the extraction of features on a large scale (Li, Zhu, et al. 2022).

3D root phenotyping in sorghum provides a more holistic understanding of root system structure and function, enabling deeper insights into their responses to water and nutrient-related stresses relevant to RUE. Xiang et al. (2019) have devised a proactive approach for 3D-based plant phenotyping that utilizes external light sources to directly capture the 3D arrangement of plant canopies. These platforms can precisely quantify nodal root angles and other key RSA traits in a non-destructive manner, while allowing parallel processing of multiple samples.

X-ray computed tomography (CT) enables the reconstruction of root architecture in three dimensions by analyzing the attenuation of X-rays (Heeraman et al. 1997). Although CT technology originated in medical diagnostics, its adaptation to plant phenotyping initially faced limitations in resolution and scan duration. Recent advancements in CT, such as increased voltage and improved computing technology, enable faster scans while minimizing radiation exposure (Li et al. 2014). Magnetic resonance imaging (MRI), a three-dimensional technique, employs magnetic fields to produce datasets of roots, with the quality of the images being influenced by the substrate (Van Dusschoten et al. 2016). Both computed tomography and magnetic resonance imaging have significant expenses associated with their equipment and space requirements, which restrict their extensive utilization (Zappala et al. 2013). Ground Penetrating Radar (GPR) is a 3D imaging tool that can be used in the field, although its application in sorghum is still exploratory and affected by soil texture, moisture, and root size. (Liu et al. 2018). Gel-based growth systems provide alternative methods, such as 3D laser scanning or digital camera imaging, for efficiently measuring RSA traits (Fang et al. 2009; Iyer-Pascuzzi et al. 2010). Electrical capacitance (EC), which measures the dielectric properties of plant tissues (Dalton 1995), has been explored for estimating root biomass, though its accuracy and reproducibility remain under debate.

Additional 3D techniques, such as electrical resistivity tomography (ERT), electrical impedance tomography (EIT), neutron radiography (NR), positron emission tomography (PET), thermoacoustic tomography (TT), electrical current source density (ECSD), and neutron tomography (NT), provide alternative options, each with distinct principles and applications. However, it is worth noting that some of these methods can be expensive or have limited resolution capabilities (Li, Zhu, et al. 2022). Software tools like *RootReader3D* provide efficient means for reconstructing and analyzing root systems in three dimensions, supporting high-throughput analysis of RSA traits. Although these methods have demonstrated potential, they are still constrained by drawbacks such as lengthy scanning durations and limited imaging capabilities (Fang et al. 2009; Iyer-Pascuzzi et al. 2010). Continual efforts are being made to enhance the 3D reconstruction and analysis pipelines to achieve a thorough characterization of RSA.

5.3 | 4D Root Phenotyping

4D root phenotyping integrates non-invasive imaging methods such as MRI, PET, and X-ray CT to facilitate comprehensive temporal and spatial analysis of root traits under soil conditions (Herrero-Huerta et al. 2022). By utilizing 4D root phenotyping, scientists can analyze the progression of root structure and activity over time, enabling a dynamic understanding of root system responses to fluctuating environmental conditions, particularly those related to resource limitations. Specialized algorithms and software are employed to analyze the 4D imaging data and extract pertinent characteristics of the roots (Herrero-Huerta et al. 2022). Techniques such as curve-skeleton computation and cylindrical fitting allow for the accurate modeling of root architecture from the digital twin. This enables the quantification of key architectural traits such as root length, volume, branching density, and growth angles (Herrero-Huerta et al. 2022). Recently, Pflugfelder et al. (2021) employed 4D MRI to examine the structure of wheat roots in their natural soil environment. Their analysis revealed substantial diversity in root characteristics across different genotypes, highlighting the significance of root phenotyping in enhancing plant productivity. In sorghum, 4D root phenotyping is particularly valuable for examining spatio-temporal changes in root architecture in response to water and nutrient availability, thus offering insights into resource-use efficiency mechanisms. This may facilitate the development of sorghum cultivars with enhanced resilience and optimized resource utilization.

6 | Breeding Strategies for Improving RUE

Breeding for RUE in *Sorghum bicolor* integrates phenomic and genomic approaches targeting RSA and its physiological interactions with nutrient and water uptake efficiency. Despite progress in dissecting WUE and NUE traits individually, targeted breeding programs specifically focused on integrated RUE improvement remain underdeveloped.

Root ideotype breeding follows a sequential approach, starting with the identification of the traits associated with water and nitrogen use efficiency within the roots as well as the correlative above-ground traits. This is followed by characterizing the genes underlying these traits and integrating them into breeding programs to enhance crop productivity. Extensive efforts have been made to identify root and shoot traits associated with drought resilience in sorghum. Specifically, sorghum varieties with deeper roots, extensive biomass, quick root growth, a larger root angle and wider vessels exhibit greater RUE. Parallel studies also focused on traits other than the structure of the root system and its impact on the plants' ability to tolerate drought (Mwamahonje et al. 2021; Demissie et al. 2023). Previous research has firmly established a correlation between canopy temperature and high normalized difference vegetation index (NDVI) with deeper roots, making them reliable indirect selection tools for deeper rooting in sorghum breeding pipelines, particularly under low-input environments (Pask et al. 2014; Li et al. 2019).

The determination of NUE is significantly complicated by the extensive reliance on grain yield as a major trait. The complexity of NUE further highlights the need to combine root trait phenotyping with genotypic data. This is because grain yield is influenced by the prevailing environmental conditions and the complex interactions between genotypes and the environment (Nguyen and Kant 2018). Thus, this calls for extensive phenotyping techniques to be integrated into the breeding programs with respect to cost efficiency, precision and throughput. It is crucial to prioritize the development of a universally applicable method for phenotyping in all crop species. This method will facilitate RUE research, aid breeding programs worldwide, and enable effective communication and knowledge transfer across different crops. The discovery of various landraces or wild accessions could expedite the improvement of sorghum's RUE. This phenomenon has primarily been observed in crop pre-breeding research (Sargar et al. 2024), where the introduction of alien segments responsible for root traits into the elite cultivars could confer an advantage (Thakur, Ingle, et al. 2024).

Recently, Rambla et al. (2022) showed that it is possible to introduce root characteristics into high-quality plant material while maintaining similar above-ground characteristics as the original parent plant. The researchers proposed a technique that entails choosing a solitary plant based on its root characteristics. The shoot and root biomass exhibit a significant positive association, suggesting that the selection of plants with high shoot biomass will likely lead to the selection of plants with high root biomass as well. This phenomenon is widely recognized because of plant algometry and reflects the effects of natural selection on early growth and strength. According to Ober et al. (2021), creating elite introgression lines that possess target root traits and exhibit similar above-ground behavior would offer valuable resources for assessing the efficacy of the new trait in various environments or manufacturing environments.

Various above and belowground characteristics can influence these properties, and because of recombination in breeding, an increase in a proxy may be attributed to the enhancement of one characteristic while another characteristic regresses. Hence, the primary objective should be to pursue root ideotype breeding by employing high-throughput root phenotyping (Bernardo et al. 2025). The biomass correlation can also be quantified and integrated into multi-trait selection indices, facilitating high-throughput screening of RSA indirectly via above-ground growth metrics (Watt et al. 2013; Zurek et al. 2015). In the future of plant breeding for nutrient use efficiency, it is expected that multi-objective optimization approaches and genetic selection will play a significant role (York 2018).

The direct selection of promising RSA to adapt to the target environments is extremely challenging due to the phenotyping bottleneck. One can indirectly choose the desired RSA by considering the above-ground candidate traits linked to RSA through direct visual selection or by examining the QTL/genes that support RSA (Maqbool et al. 2022). Identification of MTAs linked to root-related traits enables the identification of specific regions of the sorghum genome, providing direction for further investigation and use in breeding. Genomic tools could further help characterize the trait by adding information on the function, its specific gene location, pathway involved and its network specific to deficient conditions. Thus, the identified alleles could be utilized through speed breeding and marker-assisted selection to accelerate the development of cultivars with improved root traits for enhanced RUE. By harnessing sorghum's genetic diversity and using its high heritability for root traits, breeders may develop new varieties that effectively adapt to dynamic environmental conditions (Mathew and Shimelis 2022) (Figure 2).

7 | Conclusions and Future Perspectives

Global agricultural productivity is increasingly constrained by biotic and abiotic stresses, nutrient depletion, and climate variability. Addressing these requires a shift toward crop improvement strategies that integrate RSA into selection frameworks, especially for RUE in crops like sorghum. In this regard, developing an intensive breeding program preliminarily requires the identification of root traits associated with water and nitrogen use efficiency, as well as their correlative above-ground traits. While there has been progress in studying plant roots over the past few decades, many of the findings and techniques have not yet been effectively applied to improve the genetic efficiency of resource utilization. Future research must integrate RSA traits, especially those linked to WUE and NUE, into crop simulation models and breeding pipelines for predicting performance under varying environmental conditions.

While controlled-environment screening platforms and multi-omics approaches have greatly advanced our understanding of RSA, these findings must be validated across multi-environment field trials to confirm their consistency and agronomic value (Figure 2C). Many RSA traits exhibit strong environmental interactions, and their performance under greenhouse or hydroponic conditions may not directly translate to complex field conditions. Therefore, linking high-throughput phenotyping outputs with field-based performance data is critical to ensure that selected RSA traits genuinely contribute to improved WUE and NUE across diverse production environments. This validation pipeline strengthens the translational impact of phenotyping and genomic selection, supporting the deployment of climate-resilient sorghum varieties in real-world agricultural systems. Hence, the recognition of significant root system structures and their application in the development of enhanced crop varieties could contribute to crop improvement initiatives. Advancements in software-driven root phenotyping, particularly high-throughput 3D and 4D imaging, will enable more precise mapping of RSA traits in sorghum under realistic field conditions. This will help enhance the ability of plants to absorb water and nitrogen, as well as resist diseases.

Image-based root phenotyping platforms have provided new opportunities to evaluate genetic diversity in the structure of root systems. Efforts are required to enhance the method for characterizing the ability of roots to adapt to different soil conditions. This involves simulating natural conditions in a controlled environment or using accurate tools for measuring root characteristics in actual field conditions. The ability of a plant to efficiently grow roots in areas of the soil that are rich in resources is an intriguing characteristic to investigate to enhance resource utilization efficiency. Utilizing 2D, 3D, and 4D imaging techniques in root phenotyping is crucial for understanding the response of plant roots to drought. These methods, in addition to canopy temperature depression and high-throughput phenotyping, provide valuable insights into the mechanisms that drive plant RUE. This knowledge helps researchers develop crop varieties that are more resilient and efficient in their use of resources. Substituting or adding traditional destructive methods with noninvasive techniques can speed up efforts to enhance crops by developing a more efficient root system. Although the advanced imaging platforms, multi-omics methods, and computational tools discussed here provide powerful opportunities to characterize RSA and its contribution to WUE and NUE, most of these techniques are still constrained by limited field validation. Differences in soil heterogeneity, climatic environments, and management conditions can influence the reproducibility of results across locations and genetic backgrounds. Therefore, integrating controlled-environment phenotyping with multi-location field trials and long-term evaluation remains essential to confirm the functional relevance of RSA-linked traits and support their deployment in breeding pipelines. Improving RSA can reduce reliance on external inputs by enhancing the intrinsic capacity of sorghum roots to explore and exploit soil resources efficiently. This not only supports farmer profitability in low-input systems but also contributes to long-term agroecosystem sustainability.

Author Contributions

P.S. and E.H. conceived the review, developed the framework, and led the writing and critical revision of the manuscript. P.J., S.M., H.K., G.P., and S.C. contributed to literature assessment, drafting of specific sections, and refinement of the manuscript. N.T. prepared the diagrams and contributed to visualization. All authors reviewed and approved the final manuscript.

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Disclosure

Generative AI tools were not used in the preparation of the manuscript text or for data analysis. A small part of Figure 2 is generated using Adobe Photoshop AI-assisted features to create a minor schematic component for the root phenotyping methods and did not involve any biological data or scientific interpretation. All AI-assisted visuals were reviewed and verified by the authors.

Data Availability Statement

Data sharing is not applicable to this article, as no new data were created or analyzed in this study.

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