Great Britain
Journals Press

RESEARCH FINGERPRINT

IDENTIFIER
LJRS-226762

PEER REVIEW
Double Blind

SIMILARITY CHECK
Perplexity AI and iThenticate

ACCESS
Open Access

LANGUAGE
English

PRINT ISSN
2631-8490

ONLINE ISSN
2631-8504

EDITION

ABBREVIATION
LJRS

VOLUME
26

ISSUE
6

YEAR
2026

KEY DATES

RECEIVED
2026-04-05

ACCEPTED
2026-04-13

CATALOGING

CROSSMARK DOI
10.34257/LJRS226762UK

AGRIS CLASS
F30

PACS CLASS
87.18.Ed

INSPEC CLASS
A8710

CAS CLASS
10-5

ACCESS ONLINE

London Journal of Research In Science: Natural and Formal
General Section

Article Record

Variation in Root Architectural Traits Among EMS-Induced Mutants of the Drought-Tolerant Upland Rice Cultivar Nagina 22 and Their Association with Vegetative Biomass

CORRESPONDENCE →

AUTHORS & AFFILIATIONS

Dr. Prakash K ¶*

S. Suma ¶

H. G. Praveen ‖
Plant Physiologist

M. S.P. Kanavi §

K. S. Shashidhara ‡

Manjunatha H. §

S. T. Bhairappanavar ¥

K. V. Shivakumar ⁜

¶ Department of Crop Physiology, College of Agriculture, Hassan, India

‖ Plant Physiologist, Enlight Agrotech India Pvt Ltd. Agri Innovation Centre, University of Agricultural Sciences, Bangalore-560065, India (OA)

§ Department of Genetics and Plant Breeding, College of Agriculture, Hassan, India

‡ Department of Genetics and Plant Breeding, UAS, VC, Farm Mandya-571405, India

+2 additional affiliations and +1 additional authors listed on the metadata continuation page.

ABSTRACT

Background: Root system architecture (RSA) plays a critical role in drought adaptation by enhancing water and nutrient uptake from deeper soil layers. However, limited genetic variability for root traits in elite rice germplasm constrains breeding progress for drought-prone rainfed ecosystems.

Methods: This study characterized 187 EMS-induced mutants derived from the drought-tolerant upland rice cultivar Nagina 22 (N-22) for key root architectural traits under controlled rootstructure conditions during summer 2023 at the University of Agricultural Sciences, Bengaluru. Root length, root volume, root biomass, and total vegetative biomass were measured at 45- 50 days after sowing. Statistical analyses included ANOVA, frequency distribution analysis, Pearson's correlation, and regression modeling.

Results: Significant genetic variability left(mathrm p < 0 . 0 5 right) was observed...

Full abstract continues on the metadata continuation sheet.

Index Terms: Oryza sativa L • root system architecture • EMS mutagenesis • drought tolerance • root biomass • genetic variability • Nagina 22

FUNDING
No external funding was declared for this work.

CONFLICTS
The authors declare no conflict of interest.

AI USAGE
No generative AI was used for analysis or results.

HOW TO CITE
K et al. (2026). Variation in Root Architectural Traits Among EMS-Induced Mutants of the Drought-Tolerant Upland

METADATA CONTINUATION

AUTHOR CONTACT QR LEDGER

Dr. Prakash K¶*

COMPLETE AUTHOR LEDGER

01. Dr. Prakash K ¶* | Department of Crop Physiology, College of Agriculture, Hassan, India
02. S. Suma ¶ | Department of Crop Physiology, College of Agriculture, Hassan, India
03. H. G. Praveen ‖ | ∐ Plant Physiologist | Plant Physiologist, Enlight Agrotech India Pvt Ltd. Agri Innovation Centre, University of Agricultural Sciences, Bangalore-560065, India
04. M. S.P. Kanavi § | Department of Genetics and Plant Breeding, College of Agriculture, Hassan, India
05. K. S. Shashidhara ‡ | Department of Genetics and Plant Breeding, UAS, VC, Farm Mandya-571405, India
06. Manjunatha H. § | Department of Plant Pathology, College of Agriculture, Hassan, India
07. S. T. Bhairappanavar ¥ | Department of Agronomy, College of Agriculture, Hassan, India
08. K. V. Shivakumar ※ | Department of Crop Physiology, University of Agricultural Science, Mandya, India
09. M. S. Sheshshayee ⁋ | Department of Crop Physiology, University of Agricultural Sciences, Bangalore, Bengaluru, India

FULL ABSTRACT

Background:

Root system architecture (RSA) plays a critical role in drought adaptation by enhancing water and nutrient uptake from deeper soil layers. However, limited genetic variability for root traits in elite rice germplasm constrains breeding progress for drought-prone rainfed ecosystems.

Methods:

This study characterized 187 EMS-induced mutants derived from the drought-tolerant upland rice cultivar Nagina 22 (N-22) for key root architectural traits under controlled rootstructure conditions during summer 2023 at the University of Agricultural Sciences, Bengaluru. Root length, root volume, root biomass, and total vegetative biomass were measured at 45- 50 days after sowing. Statistical analyses included ANOVA, frequency distribution analysis, Pearson's correlation, and regression modeling.

Results:

Significant genetic variability left(mathrm p < 0 . 0 5 right) was observed across all traits. Root length ranged from 8.0 to 6 0 . 2 5 ~ mathrm c m (mean: 17.79 cm), root volume from 2.33 to 7 3 . 3 3 ~ mathrm c m ^ 3 (mean: 1 1 . 4 0 ~ mathrm c m ^ 3), and root biomass from 0.09 to 7.01 g plant ^{1} (mean: 1.12 g plant ^{1}). Total vegetative biomass ¯ ¯ ranged from 1.28 to 34.89 g plant ^{1}. Regression analysis revealed strong positive associations ¯ between vegetative biomass and root volume (mathrm R ^ 2 = 0 . 5 6 4 , mathrm p < 0 . 0 1), followed by root length mathrm R ^ 2 = 0.217, mathrm p < 0 . 0 1) and root biomass mathrm R ^ 2 = 0 . 1 7 2 , mathrm p < 0 . 0 5). Root volume showed moderate correlation with root length mathrm Delta R ^ 2 = 0 . 2 5 2 , mathbf p 4 5 mathrm c m ^ 3 .

Conclusion:

The substantial genetic variability in root architectural traits among EMS-induced N-22 mutants provides valuable pre-breeding material for drought tolerance improvement. Mutants with enhanced root volume and biomass represent promising donors for marker-assisted breeding programs targeting water-mining capacity in aerobic and upland rice production systems.

ARCHIVAL RECORD

LJRS · Vol 26 · Issue 6 · 2026

Article ID LJRS-226762 · DOI 10.34257/LJRS226762UK

Print ISSN 2631-8490 · Online ISSN 2631-8504

FULL-LENGTH RESEARCH ARTICLE

Variation in Root Architectural Traits Among EMS-Induced Mutants of the Drought-Tolerant Upland Rice Cultivar Nagina 22 and Their Association with Vegetative Biomass

Dr. Prakash K[¶*], S. Suma[¶], H. G. Praveen[‖∐], M. S.P. Kanavi[§], K. S. Shashidhara[‡], Manjunatha H.[§], S. T. Bhairappanavar[¥], K. V. Shivakumar[※], and M. S. Sheshshayee[ℙ]

QUALIFICATIONS / ROLES

∐ Plant Physiologist

AFFILIATIONS

¶ Department of Crop Physiology, College of Agriculture, Hassan, India
‖ Plant Physiologist, Enlight Agrotech India Pvt Ltd. Agri Innovation Centre, University of Agricultural Sciences, Bangalore-560065, India (**OA**)
§ Department of Genetics and Plant Breeding, College of Agriculture, Hassan, India
‡ Department of Genetics and Plant Breeding, UAS, VC, Farm Mandya-571405, India

+3 additional affiliations listed on metadata continuation page.

Abstract

Background: Root system architecture (RSA) plays a critical role in drought adaptation by enhancing water and nutrient uptake from deeper soil layers. However, limited genetic variability for root traits in elite rice germplasm constrains breeding progress for drought-prone rainfed ecosystems.

Methods: This study characterized 187 EMS-induced mutants derived from the drought-tolerant upland rice cultivar Nagina 22 (N-22) for key root architectural traits under controlled rootstructure conditions during summer 2023 at the University of Agricultural Sciences, Bengaluru. Root length, root volume, root biomass, and total vegetative biomass were measured at 45- 50 days after sowing. Statistical analyses included ANOVA, frequency distribution analysis, Pearson's correlation, and regression modeling.

Results: Significant genetic variability $left(mathrmp < 0.05right)$ was observed across all traits. Root length ranged from 8.0 to $60.25\ mathrmcm$ (mean: 17.79 cm), root volume from 2.33 to $73.33\ mathrmcm^3$ (mean: $11.40\ mathrmcm^3$), and root biomass from 0.09 to 7.01 g plant ^{1} (mean: 1.12 g plant ^{1}). Total vegetative biomass $^{-}$ $^{-}$ ranged from 1.28 to 34.89 g plant ^{1}. Regression analysis revealed strong positive associations $^{-}$ between vegetative biomass and root volume ($mathrmR^2 = 0.564$, $mathrmp < 0.01$), followed by root length $mathrmR^2$ = 0.217, $mathrmp < 0.01$) and root biomass $mathrmR^2 = 0.172$, $mathrmp < 0.05$). Root volume showed moderate correlation with root length $mathrmDeltaR^2 = 0.252$, $mathbf p45mathrmcm^3$.

Conclusion: The substantial genetic variability in root architectural traits among EMS-induced N-22 mutants provides valuable pre-breeding material for drought tolerance improvement. Mutants with enhanced root volume and biomass represent promising donors for marker-assisted breeding programs targeting water-mining capacity in aerobic and upland rice production systems.

Keywords: *Oryza sativa L, root system architecture, EMS mutagenesis, drought tolerance, root biomass, genetic variability, Nagina 22*

Correspondence: Dr. Prakash K

1 Introduction

Rice (*Oryza sativa* L.) serves as the primary staple food for more than half of the global population, with Asia accounting for approximately 90% of global rice production and consumption (Khush, 2005; FAO, 2023). However, rice productivity is increasingly threatened by water scarcity, exacerbated by climate change, declining freshwater availability for agriculture, and competing demands from industrial and domestic sectors (Bouman *et al.*, 2007; Kumar *et al.*, 2022). In rainfed upland and lowland ecosystems, which together constitute nearly 50% of the global rice area, intermittent drought episodes can reduce grain yield by up to 50- 70%, depending on stress severity and timing (Serraj *et al*., 2011; Pandey *et al*., 2022).

Root system architecture (RSA)- the three-dimensional configuration of the root system in soil- is a critical determinant of plant performance under water-limited conditions (Lynch, 2019; Uga *et al*., 2013). RSA traits, including root length, root volume, root biomass, root branching intensity, and deep root proportion, collectively govern a plant's capacity to extract water and nutrients from the soil profile (Courtois *et al*., 2013; Niones *et al*., 2015). Under drought stress, genotypes with deeper and more voluminous root systems maintain higher tissue water status through enhanced water mining from subsurface soil layers, thereby sustaining photosynthetic activity and biomass accumulation (Henry *et al*., 2011; Kano-Nakata *et al*., 2013).

In rice, significant genotypic variation exists for root architectural traits, with upland and aerobic-adapted cultivars generally exhibiting deeper and more extensive root systems compared to lowland varieties (Kamoshita *et al*., 2008; Uga *et al*., 2011). The upland cultivar *Nagina 22* (N-22) is widely recognized as a valuable genetic resource for drought tolerance, attributed in part to its robust root system, efficient water-use strategies, and heat tolerance (Kumar *et al*., 2021; Reddy *et al*., 2020). N-22 has been extensively used in physiological breeding programs aimed at dissecting drought adaptation mechanisms (Sheshshayee *et al*., 2018).

Despite the adaptive value of favorable RSA traits, narrow genetic variability in root characteristics within elite rice germplasm has constrained breeding progress for drought-prone environments (Gamuyao *et al*., 2012; Uga, 2021). To address this limitation, induced mutagenesis using chemical mutagens such as ethyl methanesulfonate (EMS) has emerged as a powerful approach to create novel genetic variation (Parry *et al*., 2009; Sikora *et al*., 2011). EMS induces primarily single nucleotide substitutions, generating stable, heritable mutations that can produce gain- or loss-of-function alleles for target traits (Till *et al*., 2007).

Large-scale EMS mutant populations have been developed in several crop species, including rice (Suzuki *et al*., 2008; Wu *et al*., 2017). In the N-22 background, a comprehensive EMS mutant resource comprising approximately 87,000 M_2 lines was developed through a national initiative (Mohapatra *et al*., 2014; Mithra *et al*., 2016). A subset of these mutants has been advanced to homozygosity (M_8 generation) and screened for various morpho-physiological, biochemical, and yield-associated traits (Mithra *et al*., 2018; Reddy *et al*., 2020). Previous studies have demonstrated that EMS mutagenesis can generate novel alleles for root architectural traits, offering opportunities for gene discovery and marker-assisted selection (Ashokkumar *et al*., 2015; Anandan *et al*., 2022).

Understanding the quantitative relationships between root architectural traits and whole-plant biomass accumulation is essential for designing effective selection strategies (Zhao *et al*., 2021; Chagas *et al*., 2025). Several genome-wide association studies (GWAS) in diverse rice panels have identified quantitative trait loci (QTLs) for root length, root thickness, and root biomass that co-localize with biomass accumulation under both well-watered and drought-stressed conditions (Li *et al*., 2017; Zhao *et al*., 2021). Positive associations between root volume and shoot biomass have been reported in multiple studies (Courtois *et al*., 2013; Niones *et al*., 2015), suggesting that root system size directly influences above-ground productivity through enhanced resource acquisition.

However, systematic phenotyping of root traits in EMS mutant populations of elite drought-tolerant cultivars like N-22 remains limited, particularly under field-like root-structure systems that enable precise, minimally invasive root extraction. Most previous studies have relied on hydroponic or pot-based phenotyping, which may not fully represent root growth dynamics under actual soil conditions (Wasson *et al*., 2012; Atkinson *et al*., 2019).

Given the critical importance of root traits for drought adaptation in upland rice ecosystems, and the availability of a well-characterized EMS mutant population in the drought-tolerant N-22 background, there is an urgent need to: (1) systematically characterize the extent of genetic variability in key root architectural traits among stabilized N-22 mutants, (2) identify superior mutants with enhanced root traits for use as donors in breeding programs, and (3) elucidate the quantitative associations between root traits and vegetative biomass to guide trait-based selection strategies.

Therefore, the present study was undertaken with the following objectives:

1. To characterize genetic variability in root length, root volume, and root biomass among 187 EMS-induced N-22 mutants under controlled root-structure conditions.
2. To identify superior mutants with enhanced root traits compared to the wild-type N-22 and standard checks.
3. To quantify the associations between root architectural traits and total vegetative biomass using correlation and regression analyses.

2 Materials and methods

2.1 Plant Material

A subset of 187 stabilized, homozygous EMS-induced mutants (M_8 generation) derived from the drought-tolerant upland rice cultivar *Nagina 22* (N-22) was used in this study. The parental N-22 seeds were treated with 0.8% ethyl methanesulfonate (EMS) to induce random point mutations (Mohapatra *et al*., 2014). The generated M_0 seeds were advanced to M_2 generation, and a set of M_2 seeds was procured from the National Institute for Plant Biotechnology (ICAR-NIPB), New Delhi. The mutants were screened in early generations for yield, biomass, and carbon isotope discrimination ($\Delta^{13}C$) as an indicator of water use efficiency to capture the entire variability in a subset of M_4 generation mutants (Reddy *et al*., 2019). The complete list of 187 mutants used in this study is provided in Table S1.

Two standard checks were included: IR-64 (lowland, high-yielding variety with relatively shallow root system) and Dhaksha (aerobic-adapted, drought-tolerant variety with robust root system). The wild-type N-22 served as the recurrent parent control.

2.2 Experimental Site and conditions

The experiment was conducted during the summer (Rabi) season of 2023 at the root structure facility of the Department of Crop Physiology, University of Agricultural Sciences, Bengaluru, India (13°05′N, 77°34′E). The site is characterized by a tropical savanna climate with red loamy soil type (pH 6.8). During the experimental period, the mean maximum temperature was 32.4 ± 2.1°C, mean minimum temperature was 19.6 ± 1.8°C, and average relative humidity was 65 ± 8%.

2.3 Root Structure design and Preparation

Two identical root structures were constructed for precise root phenotyping. Each structure measured 2.0 m in height, 3.0 m in width, and 20 m in length, constructed with cement blocks and raised 0.9 m above ground level (Figure S1). An additional 1.5 m tall wall was built along the longitudinal center to create two halves, each 150 cm wide. The root structures were filled with red loamy soil mixed with farmyard manure at a ratio of 3:1 (soil:FYM) to ensure uniform fertility and physical properties. The soil was allowed to settle for 15 days before sowing, with periodic irrigation to achieve uniform compaction.

2.4 Experimental design and Crop Establishment

The experiment was laid out in an augmented completely randomized design (augmented CRD) with the wild-type N-22 as the recurrent parent and IR-64 and Dhaksha as checks. Each mutant was represented by 4- 6 plants per root structure, with 187 mutants distributed across both structures. Seeds were sown directly in the root structures at a spacing of 20 cm between rows and 15 cm between plants within rows (Figure S2). Five replicates were maintained for each mutant.

Fertilizers were applied at the recommended dose of 100:50:50 kg $N:P_2O_5:K_2O$ ha^{-1}. Nitrogen was applied in three split doses: 50% as basal, 25% at tillering, and 25% at panicle initiation. Phosphorus and potassium were applied as basal. Iron deficiency was corrected by application of Fe-EDDHA 6% at 80 g per 20 L of water. MS medium (Murashige and Skoog basal medium) at 4.4 g L^{-1} was applied as a foliar spray to supplement micronutrients. Plant protection measures were followed as per standard recommendations for upland rice (Figure S3).

2.5 Irrigation management

Irrigation was provided to maintain field capacity until 30 days after sowing (DAS) to ensure uniform crop establishment. Subsequently, a mild water deficit was imposed by extending the irrigation interval to simulate realistic upland conditions. Soil moisture content was monitored gravimetrically at weekly intervals.

2.6 Measurement of Root Traits

At 45- 50 days after sowing (peak vegetative stage), the root structures were carefully dismantled (Figure S4). Roots were gently extracted from the soil using a pressurized water jet to remove adhering soil without damaging the root system. Care was taken to preserve the integrity of the entire root system, including fine roots.

2.6.1 Root Length (cm) Root length was measured from the base of the stem (root- shoot junction) to the tip of the longest root using a graduated ruler (precision: ±0.5 cm). Roots longer than 30 cm were separated and recorded to quantify deep root biomass potential.

2.6.2 Root Volume (cm^3) Root volume was determined using the water displacement method. Cleaned, intact roots were submerged in a graduated cylinder (250 mL capacity) filled with a known volume of water. The volume of water displaced after complete immersion of the root system was recorded as root volume (precision: ±0.5 cm^3).

2.6.3 Root Biomass (g $plant^{-1}$) After measurement of length and volume, root samples were washed with distilled water to remove any residual soil particles. The roots were then oven-dried at 70°C for 72 hours until constant weight was achieved. Dry root weight was recorded using an electronic balance (precision: ±0.01 g).

2.7 Measurement of Vegetative Biomass

Total vegetative biomass (g $plant^{-1}$) was determined as the sum of shoot dry weight, stubble (stem) dry weight, and leaf dry weight. Plant samples were harvested at the same stage as root sampling (45- 50 DAS). Shoots were separated into leaf blades and stubble (culms and leaf sheaths). All samples were oven-dried at 70°C for 72 hours and weighed individually.

2.8 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GraphPad version 8.0. Descriptive statistics (range, mean, standard error, coefficient of variation) were calculated for each trait. Frequency distributions were constructed to visualize the pattern of trait variation across the mutant population.

Pearson's correlation coefficients were calculated to assess pairwise associations among root traits and between root traits and vegetative biomass. Simple linear regression analysis was performed to quantify the strength of associations, with coefficient of determination (R^2) reported as a measure of explained variance. Significance of differences was tested at p ▯ 0.05, p ▯ 0.01, and p ▯ 0.001 levels. The critical difference (CD) at 5% probability was calculated for comparing mutant means.

3 Results

3.1 Genetic Variability in Root Traits

Significant genetic variability ($p < 0.05$) was observed among the 187 EMS-induced N-22 mutants for all three root architectural traits measured: root length, root volume, and root biomass (Table 1; Figure 1).

Root length exhibited a broad range from 8.0 cm (mutant MG262) to 60.25 cm (mutant MG153), with a population mean of 17.79 cm. The coefficient of variation (CV) for root length was 18.51%, indicating moderate genetic variability. Wild-type N-22 had a root length of 20.0 ± 9.17 cm,

Table 1. Genetic variability in root traits among Nagina 22 mutant lines grown during summer 2023.

Parameter	**Mean**	**Minimum**	**Maximum**	**CD (5%)**	**SE(m)**	**CV (%)**
Root length (cm)	17.79	8.00	60.25	11.23	7.11	18.51
Root volume (cm^3 plant^{-1})	11.40	2.33	73.33	22.31	11.21	11.51
Root biomass (g plant^{-1})	1.12	0.09	7.01	5.36	6.21	10.32
Total vegetative biomass (g plant^{-1})	6.55	1.28	34.89	11.32	5.32	14.20

Source: Domain Author

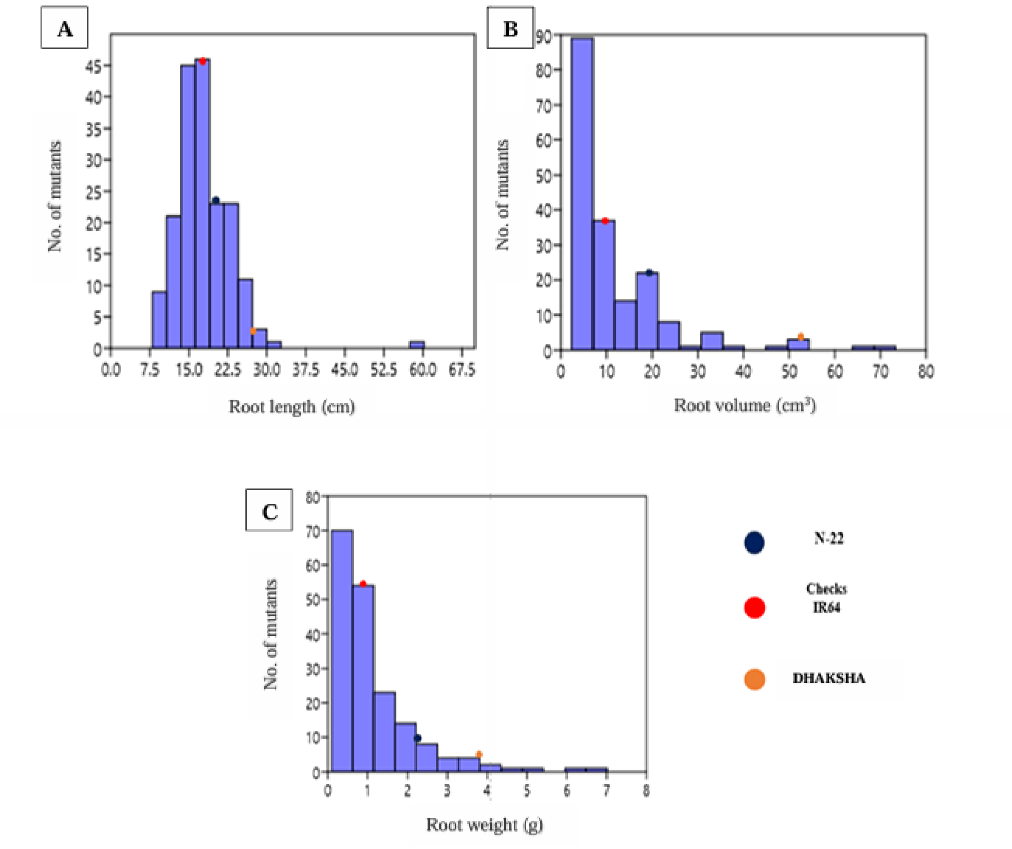

Figure 1. Distribution of root traits among mutants and checks. (A) Frequency distribution of root length (cm), (B) root volume (cm^3), and (C) root weight (g) among mutant lines. Histograms represent the number of mutants across trait classes. Colored dots indicate check varieties: N-22 (blue), IR64 (red), and Dhaksha (orange). The distributions reveal substantial variability in root architectural traits among the evaluated population, with a few extreme transgressive segregants.

placing it within the upper range of the mutant distribution. The aerobic check Dhaksha showed root length comparable to the best-performing mutants (27.33 ± 9.87 cm), while the lowland check IR-64 exhibited lower root length (18.33 ± 11.02 cm). Approximately 15% of mutants showed root length exceeding that of wild-type N-22.

Root volume ranged from 2.33 cm^3 (MG262) to 73.33 cm^3 (MG353), with a population mean of 11.40 cm^3. The CV for root volume was 11.51%. Wild-type N-22 had a root volume of 18.33 ± 18.93 cm^3. The aerobic check Dhaksha exhibited substantially higher root volume (53.33 ± 37.86 cm^3) compared to both N-22 and IR-64 (7.33 ± 4.62 cm^3). Notably, several mutants—including MG353 (73.33 cm^3), MG382 (45.0 cm^3), MG387 (50.0 cm^3), and MG471 (53.33 cm^3)—displayed root volume values comparable to or exceeding Dhaksha, indicating successful generation of transgressive variation through EMS mutagenesis.

Root biomass ranged from 0.09 g plant^{-1} (MG308) to 7.01 g plant^{-1} (MG353), with a population mean of 1.12 g plant^{-1}. The CV for root biomass was 10.32%. Wild-type N-22 had root biomass of 2.30 ± 2.49 g plant^{-1}. Dhaksha exhibited superior root biomass (3.77 ± 2.40 g plant^{-1}), while IR-64 had lower biomass (0.68 ± 0.51 g plant^{-1}). Mutants such as MG353 (7.01 g), MG382 (5.11 g), MG387 (3.08 g), and MG508 (3.44 g) showed root biomass values significantly higher than wild-type N-22 and comparable to or exceeding Dhaksha.

3.2 Frequency distribution of Root Traits

Frequency distribution analysis revealed the pattern of trait variation across the mutant population (Figure 1A–C). Root length distribution showed a positive skew, with the majority of mutants (approximately 65%) exhibiting root length in the range of 10–20 cm, while a small proportion (<5%)

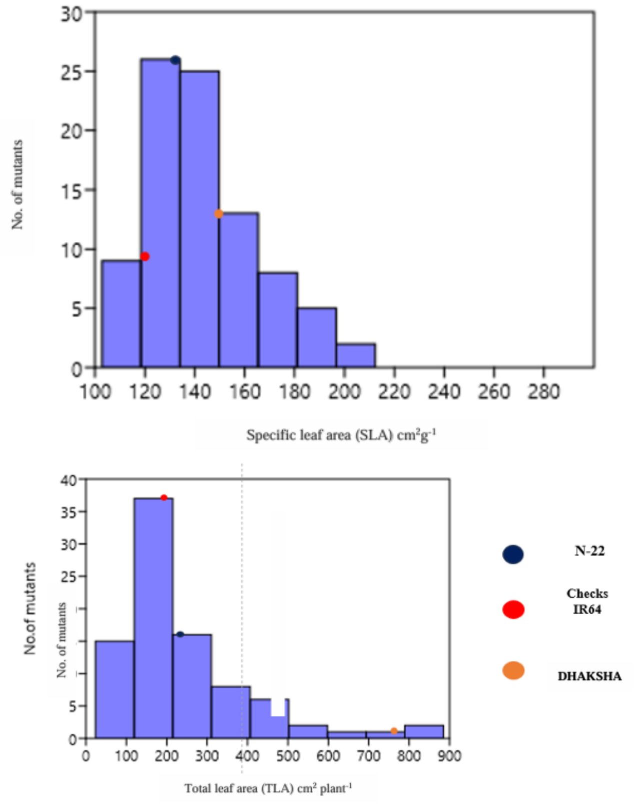

Figure 2. Distribution of leaf traits among mutants and checks. (A) Frequency distribution of specific leaf area (SLA; cm² g^{-1}) and (B) total leaf area (TLA; cm² plant^{-1}) among mutant lines. Bars represent the number of mutants within each class interval. Colored markers denote check varieties (N-22, IR64, and Dhaksha). The skewed distribution of TLA suggests the presence of high leaf area mutants compared to checks.

Trait	Root volume	Root biomass	Vegetative biomass
Root length	0.502**	0.415*	0.466**
Root volume	-	0.751***	0.751***
Root biomass	-	-	0.542**

Table 2. Pearson's correlation coefficients among root traits and vegetative biomass.

**Note: *p < 0.05; **p < 0.01; ***p < 0.001*

showed root length exceeding 30 cm. This indicates that while most EMS-induced mutations did not dramatically alter root length, a subset of mutants achieved substantial increases through potentially favorable mutations.

Root volume distribution was more dispersed, with a tendency toward lower values. Approximately 55% of mutants had root volume below 10 cm^3, while about 10% exceeded 30 cm^3. The presence of mutants with root volume > 50 cm^3 (MG353, MG471, MG382, MG387) demonstrates the effectiveness of EMS mutagenesis in generating extreme phenotypes for this trait.

Root biomass distribution followed a pattern similar to root volume, with most mutants (⍰60%) having root biomass below 1.0 g plant^{-1}. However, approximately 8% of mutants exceeded 2.0 g plant^{-1}, and a few (MG353, MG382) reached values above 5.0 g plant^{-1}, representing more than a 2-fold increase over wild-type N-22.

3.3 Genetic Variability in Total Vegetative Biomass

Total vegetative biomass ranged from 1.28 g plant^{-1} (MG262) to 34.89 g plant^{-1} (MG353), with a population mean of 6.55 g plant^{-1} (Table 1; Figure 1C). The CV for total vegetative biomass was 14.20%, indicating significant variation among the mutants. The wild-type N-22 had a total vegetative biomass of 2.82 ± 1.15 g plant^{-1}, while Dhaksha (aerobic check) had 8.33 ± 3.12 g plant^{-1} and IR-64 had 4.23 ± 2.01 g plant^{-1}. Several mutants showed substantial transgressive variation for vegetative biomass, with MG353 showing the highest value (34.89 g plant^{-1}).

3.4 Correlation and Regression Analysis

Pearson's correlation analysis revealed significant positive associations among root traits and between root traits and vegetative biomass (Table 2; Figures 3A–D, and Figure 4).

A moderate positive correlation ($r = 0.502$, $p < 0.01$) was observed between root length and root volume, with regression analysis indicating that root length explained 25.2% of the variation in root volume ($R^2 = 0.252$, $p < 0.01$; Figure 3A). This suggests that longer roots generally occupy greater soil volume, although other factors (root branching, root thickness) also contribute to volume.

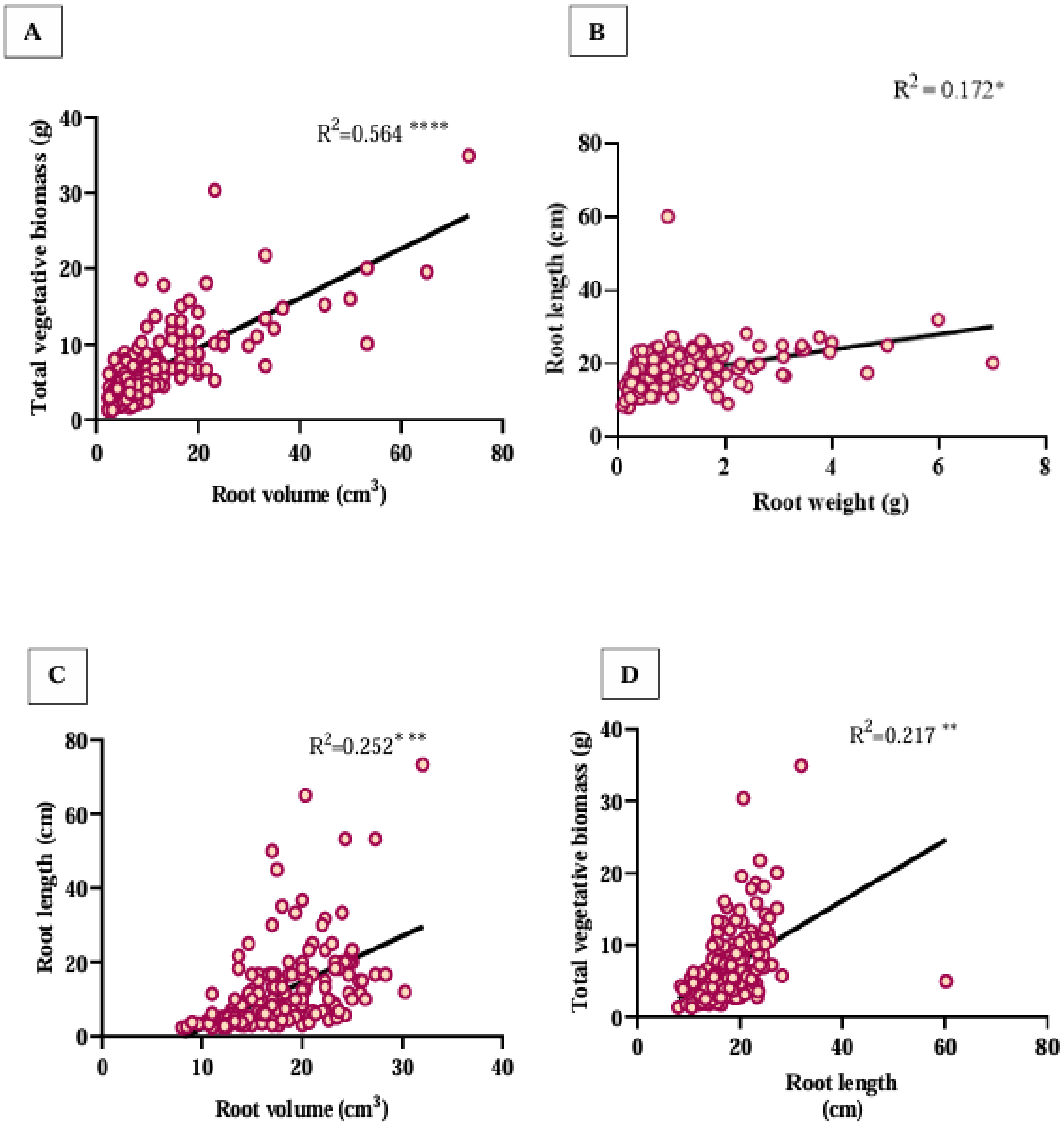

Figure 3. Relationships among root traits and biomass accumulation. (A) Relationship between total vegetative biomass (g) and root volume (cm^3), (B) root length (cm) versus root weight (g), (C) root length (cm) versus root volume (cm^3), and (D) total vegetative biomass (g) versus root length (cm). Each point represents an individual genotype. Solid lines indicate linear regression fits with corresponding R^2 values and significance levels (*, **, ***). Results indicate moderate to strong positive associations between root traits and biomass.

A significant positive correlation ($r = 0.415$, $p < 0.05$) was found between root length and root biomass, with root length explaining 17.2% of the variation in root biomass ($R^2 = 0.172$, $p < 0.05$; Figure 3B). This indicates that root length is a moderate predictor of root biomass accumulation.

The strongest association was observed between root volume and total vegetative biomass ($r = 0.751$, $p < 0.001$), with root volume explaining 56.4% of the variation in biomass ($R^2 = 0.564$, $p < 0.001$; Figure 3C). This strong positive relationship suggests that plants with more voluminous root systems consistently produce greater above-ground biomass. A significant positive correlation ($r = 0.466$, $p < 0.01$) was observed between root length and vegetative biomass, with root length explaining 21.7% of biomass variation ($R^2 = 0.217$, $p < 0.01$; Figure 3D).

Root biomass showed a strong positive correlation with vegetative biomass ($r = 0.542$, $p < 0.01$; Figure 4A), further supporting the functional link between root system size and above-ground productivity. As expected, total leaf area exhibited a strong positive association with vegetative biomass ($r = 0.724$, $p < 0.001$; Figure 4B), reflecting the role of photosynthetic capacity in biomass accumulation.

3.5 Identification of Superior Mutants

Based on the combined assessment of root traits and vegetative biomass, several mutants were identified as superior donors for drought tolerance breeding (Table 3).

Mutant MG353 emerged as the most promising genotype, exhibiting exceptional root volume (73.33 cm^3), root biomass (7.01 g), and vegetative biomass (34.89 g)—values approximately 4-fold, 3-fold, and 12-fold higher than wild-type N-22, respectively. Mutant MG153 exhibited extraordinary root length (60.25 cm), representing a 3-fold increase over the population mean, though its root volume and biomass were moderate.

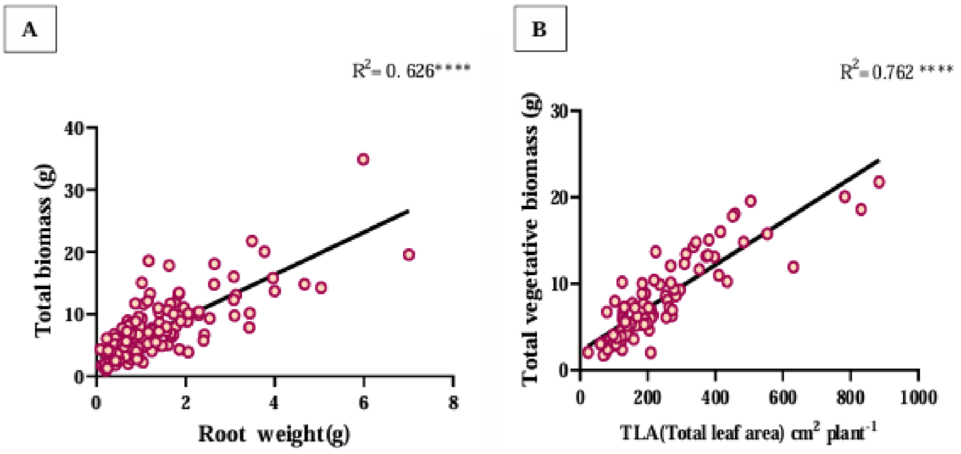

Fig. 7: Graphs showing regression for (A) Root weight and total vegetative biomass (B)TLA (total leaf area) and total vegetative biomass. R^2 represents Pearson's correlation. ($p \geq 0.254$****).

Figure 4. Association of biomass with root weight and leaf area. (A) Relationship between total biomass (g) and root weight (g), and (B) total vegetative biomass (g) versus total leaf area (TLA; cm² plant⁻¹). Each point represents a genotype, and solid lines represent fitted regression models. The high R^2 values and significance levels (****) indicate strong positive relationships, highlighting the contribution of both root biomass and leaf area to overall plant biomass accumulation.

Table 3. Superior mutants with enhanced root traits and vegetative biomass.

Mutant	Root length (cm)	Root volume (cm³)	Root biomass (g)	Vegetative biomass (g)	Remarks
MG382	17.50	45.00	5.11	4.23	High root volume and biomass
MG387	17.00	50.00	3.08	4.12	High root volume
MG471	24.33	53.33	2.06	1.46	Long root length, high volume
MG508	25.00	23.33	3.44	3.95	Long root length, high biomass
MG153	60.25	11.00	1.27	2.41	Extremely long root length
MG353	20.33	73.33	7.01	34.89	Superior for all traits
Wild-type N-22	20.00	18.33	2.30	2.82	Recurrent parent
Dhaksha (check)	27.33	53.33	3.77	8.33	Aerobic check

Source: Domain Author

Mutants MG382, MG387, MG471, and MG508 also showed consistently superior root traits, making them valuable genetic resources for trait introgression.

4 Discussion

4.1 Ems Mutagenesis Creates Substantial Genetic Variability in Root Traits

A primary finding of this study is the demonstration that EMS-induced mutagenesis in the N-22 background generated extensive genetic variability for root architectural traits, including several mutants with trait values substantially exceeding both the wild-type parent and standard checks. The ranges observed- root length: 8- 60 cm, root volume: 2.3- 73.3 cm^3, root biomass: 0.09- 7.01 g- represent 7.5-fold, 31.5-fold, and 78-fold variation, respectively. This magnitude of variability far exceeds that typically found in natural germplasm collections or biparental mapping populations (Courtois *et al.*, 2013; Uga *et al.*, 2013).

The coefficient of variation values (10- 18%) indicate moderate to high genetic variability, consistent with previous reports on EMS-induced mutant populations in rice (Mohapatra *et al.*, 2014; Mithra *et al.*, 2018). The presence of transgressive segregants- mutants with extreme phenotypes beyond the parental range- is particularly noteworthy. Mutants such as MG353 (73.3 cm^3 root volume, 7.01 g root biomass) and MG153 (60.25 cm root length) represent transgressive variation that could not have been predicted from the parental phenotype.

These findings align with previous studies demonstrating that EMS mutagenesis effectively generates novel alleles for root traits. In barley, Maluszynski *et al.* (2001) reported EMS-induced mutants with altered root morphology, including both enhanced and reduced root growth phenotypes. In rice, Ashokkumar *et al.* (2015) identified EMS mutants with improved root length and root density under drought stress. More recently, Anandan *et al.* (2022) characterized root QTLs using EMS-derived mutants, validating the utility of this approach for dissecting root trait genetics.

The random nature of EMS-induced point mutations (primarily G/C to A/T transitions) (Till *et al.*, 2007) likely generated mutations in multiple genes regulating root development, including those involved in auxin signaling, cell wall modification, and nutrient sensing. The phenotypic diversity observed- ranging from extremely short roots (MG262: 8 cm) to exceptionally long roots (MG153: 60 cm)- suggests mutations in different genetic pathways affecting root elongation, branching, and biomass allocation.

4.2 Root Volume as the Primary Determinant of Vegetative Biomass

The strong positive association between root volume and vegetative biomass ($r = 0.751$, $R^2 = 0.564$) is among the highest reported in rice root studies. This finding has important implications for trait-based breeding, as it suggests that selection for increased root volume could indirectly improve above-ground biomass accumulation- a key determinant of grain yield under both optimal and stress conditions.

The mechanistic basis for this relationship likely involves enhanced resource acquisition. Greater root volume- achieved through a combination of longer roots, thicker roots, and more extensive branching- increases the soil volume explored by the root system, thereby improving access to water and nutrients (Lynch, 2019; Kano-Nakata *et al.*, 2013). Under the mild water deficit imposed in this study (post-30 DAS), mutants with larger root volumes would have maintained higher tissue water status, sustaining photosynthetic rates and carbon assimilation. This interpretation is consistent with studies showing that root system size positively correlates with canopy development and biomass accumulation under water-limited conditions (Henry *et al.*, 2011; Niones *et al.*, 2015).

The finding that root volume explained more than twice the variation in biomass compared to root length (56.4% vs. 21.7%) suggests that root volume integrates multiple aspects of root system architecture- including length, thickness, and branching- that collectively determine resource capture capacity. A long but thin root system with minimal branching (e.g., MG153) may not achieve the same resource acquisition efficiency as a moderately long but highly branched and voluminous root system (e.g., MG353). This observation aligns with the concept of "root system efficiency" proposed by Lynch (2019), where the spatial configuration of roots, rather than absolute length alone, determines resource capture.

4.3 Comparison with Checks and Wild-Type

The inclusion of two standard checks- lowland IR-64 and aerobic Dhaksha- provided valuable benchmarks for evaluating mutant performance. Dhaksha, which has been specifically bred for aerobic conditions, exhibited superior root traits (27.3 cm root length, 53.3 cm^3 root volume, 3.77 g root biomass) consistent with its drought-adapted phenotype (Sheshshayee *et al.*, 2018). Several mutants, particularly MG353, MG382, MG387, and MG471, matched or exceeded Dhaksha for key root traits, demonstrating that EMS mutagenesis successfully generated alleles conferring root system architecture comparable to or better than elite aerobic varieties.

The lowland check IR-64, which is adapted to puddled conditions where deep rooting is not advantageous, showed lower root volume and biomass (7.33 cm^3, 0.68 g), consistent with its shallow root system. Wild-type N-22 showed intermediate root traits (18.3 cm^3 volume, 2.30 g biomass), confirming its status as a moderately deep-rooted upland cultivar (Kamoshita *et al.*, 2008; Kumar *et al.*, 2021).

Importantly, the identification of mutants with root traits exceeding Dhaksha indicates that N-22 contains latent genetic variation for root architecture that can be unlocked through mutagenesis. This finding has practical implications: the superior mutants identified here can serve as donors for introgressing favorable root traits into elite but shallow-rooted rice varieties.

4.4 Implications for Drought Tolerance Breeding

The results of this study have direct application for breeding drought-tolerant rice varieties suitable for rainfed upland and aerobic production systems. The strong association between root volume and biomass suggests that indirect selection for increased root volume- which can be phenotyped relatively easily using the water displacement method- could effectively improve biomass accumulation. Given that grain yield under drought stress is strongly correlated with biomass at flowering (Blum, 2009; Sheshshayee *et al.*, 2018), these root trait improvements are likely to translate into yield benefits.

The superior mutants identified (MG353, MG382, MG387, MG471, MG508, MG153) represent valuable genetic resources for pre-breeding programs. Mutant MG353, with its exceptional root volume and biomass, is particularly promising as a donor for enhancing water mining capacity. The extreme root length of MG153 (60.25 cm) may be valuable for accessing deep soil water in environments with pronounced dry spells. These mutants can be crossed with high-yielding but drought-sensitive varieties, followed by marker-assisted selection once the underlying mutations are characterized.

Future research should focus on: (1) genetic mapping of the mutations responsible for superior root traits using next-generation sequencing approaches (MutMap or whole-genome resequencing), (2) validation of drought tolerance of selected mutants under field conditions across multiple seasons and locations, and (3) assessment of grain yield and yield stability of introgression lines developed using these superior donors.

4.5 Limitations and Future Directions

While this study provides comprehensive phenotyping of root traits under controlled root-structure conditions, several limitations should be acknowledged. First, the root structure system, while enabling precise root extraction, does not fully replicate field conditions with heterogeneous soil profiles and biotic interactions. Second, the mild water deficit imposed may not represent severe drought scenarios typical of some rainfed environments. Third, root traits were measured at a single time point (peak vegetative stage); temporal dynamics of root growth were not captured.

Future studies should address these limitations by: (1) validating the performance of selected mutants under multiple field environments with varying drought intensities, (2) characterizing root system architecture across multiple growth stages using non-invasive phenotyping platforms (e.g., rhizotrons, X-ray CT), and (3) assessing the stability of root trait expression across seasons and soil types.

Additionally, the molecular characterization of superior mutants- through whole-genome sequencing and identification of causative mutations- would enable the development of diagnostic markers for marker-assisted breeding. The identification of genes underlying enhanced root volume and biomass could also provide insights into the genetic control of root system architecture in rice.

5 Conclusion

This study systematically characterized root architectural variation among 187 EMS-induced mutants derived from the drought-tolerant upland rice cultivar Nagina 22. Substantial genetic variability exists for root length (8–60 cm), root volume (2.3–73.3 cm^3), and root biomass (0.09–7.01 g), with several mutants showing transgressive segregation exceeding the wild-type and standard checks. Root volume showed the strongest association with vegetative biomass ($R^2 = 0.564$, $p < 0.001$), explaining more than twice the variation compared to root length ($R^2 = 0.217$), suggesting that root volume is the primary root architectural determinant of above-ground productivity. Superior mutants—notably MG353 (73.3 cm^3 root volume, 7.01 g root biomass, 34.9 g vegetative biomass), MG382, MG387, MG471, MG508, and MG153 (60.25 cm root length)—were identified as promising donors for drought tolerance breeding programs. The EMS mutant population in N-22 background represents a valuable genetic resource for dissecting the molecular basis of root system architecture and for developing climate-resilient rice varieties with enhanced water mining capacity. The identified superior mutants can be directly utilized in pre-breeding programs aimed at improving drought tolerance in rainfed and aerobic rice production systems. The strong empirical relationship between root volume and biomass provides a basis for trait-based selection strategies. Future research should focus on molecular characterization of causative mutations and field validation of drought tolerance and yield performance.

REFERENCES

[1] Anandan, A., Anumalla, M., Pradhan, S.K., & Ali, J. (2022). Population structure, genetic diversity and GWAS mapping for root traits in rice (*Oryza sativa* L.) using EMS-induced mutants. *Scientific Reports*, 12, 14258.

[2] Ashokkumar, S., Jaganathan, D., Ramanathan, A., & Muthurajan, R. (2015). Creation of novel alleles for root traits through EMS mutagenesis in rice. *Plant Genetic Resources*, 13(3), 256-264.

[3] Atkinson, J.A., Pound, M.P., Bennett, M.J., & Wells, D.M. (2019). Uncovering the hidden half of plants using new advances in root phenotyping. *Current Opinion in Biotechnology*, 55, 1-8.

[4] Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research*, 112(2-3), 119-123.

[5] Bouman, B.A.M., Humphreys, E., Tuong, T.P., & Barker, R. (2007). Rice and water. *Advances in Agronomy*, 92, 187-237.

[6] Chagas, J.C.G., Rocha, M.A., & Silva, A.J. (2025). Genome-wide association mapping of root traits and biomass accumulation in rice under drought stress. *Plant Genome*, 18(1), e20512.

[7] Courtois, B., Audebert, A., Dardou, A., Roques, S., Ghneim-Herrera, T., Droc, G., Frouin, J., Rouan, L., Gozé, E., Kilian, A., & Ahmadi, N. (2013). Genome-wide association mapping of root traits in a japonica rice panel. *PLoS ONE*, 8(11), e78037.

[8] FAO. (2023). *The State of Food Security and Nutrition in the World 2023.* Food and Agriculture Organization of the United Nations, Rome.

[9] Gamuyao, R., Chin, J.H., Pariasca-Tanaka, J., Pesaresi, P., Catausan, S., Dalid, C., Slamet-Loedin, I., Tecson-Mendoza, E.M., Wissuwa, M., & Heuer, S. (2012). The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature*, 488, 535-539.

[10] Henry, A., Gowda, V.R.P., Torres, R.O., McNally, K.L., & Serraj, R. (2011). Variation in root system architecture and drought response in rice (*Oryza sativa* L.): Phenotyping of the OryzaSNP panel in rainfed lowland fields. *Field Crops Research*, 120(2), 205-214.

[11] Kamoshita, A., Babu, R.C., Boopathi, N.M., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Research*, 109(1-3), 1-23.

[12] Kano-Nakata, M., Gowda, V.R.P., Henry, A., & Serraj, R. (2013). Root system architecture and water use efficiency in rice. In *Improving Water and Nutrient-Use Efficiency in Rice* (pp. 45-62). IRRI, Los Baños.

[13] Khush, G.S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, 59, 1-6.

[14] Kumar, A., Dixit, S., & Henry, A. (2022). Rice breeding for drought tolerance: Progress and future prospects. *Frontiers in Plant Science*, 13, 812456.

[15] Kumar, S., Dwivedi, S.K., & Singh, S.K. (2021). Nagina 22: A donor for multiple abiotic stress tolerance in rice. *Indian Journal of Plant Physiology*, 26, 245-258.

[16] Li, Y., Li, H., Li, Y., & Zhang, S. (2017). Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. *Crop Journal*, 5(3), 231-239.

[17] Lynch, J.P. (2019). Root phenotypes for improved nutrient capture: An underexploited opportunity for global agriculture. *New Phytologist*, 223(2), 548-564.

[18] Maluszynski, M., Ahlowalia, B.S., & Sigurbjornsson, B. (2001). Application of in vivo and in vitro mutation techniques for crop improvement. *Euphytica*, 118(2), 167-176.

[19] Mithra, S.A., Kandwal, P., Kale, P.B., Prakash, C., Ramkumar, M.K., Yadav, N., & Jeyaparakash, P. (2018). Whole genome characterization of a few EMS-induced mutants of upland rice variety Nagina 22 reveals a staggeringly high frequency of SNPs which show high phenotypic plasticity towards the wild-type. *Frontiers in Plant Science*, 9, 1179.

[20] Mithra, S.A., Kar, M.K., Mohapatra, T., Robin, S., Sarla, N., Sheshashayee, M., & Sharma, R.P. (2016). DBT propelled national effort in creating mutant resource for functional genomics in rice. *Current Science*, 110(4), 543-548.

[21] Mohapatra, T., Robin, S., Sarla, N., Sheshashayee, M., Singh, A.K., Singh, K., & Sharma, R.P. (2014). EMS induced mutants of upland rice variety Nagina 22: Generation and characterization. *Proceedings of the Indian National Science Academy*, 80(1), 163-172.

[22] Niones, J.M., Inukai, Y., Suralta, R.R., & Yamauchi, A. (2015). Field evaluation on functional root plasticity for maintaining productivity in rice under water stress conditions. *Plant Production Science*, 18(2), 165-177.

[23] Pandey, S., Bhandari, H., & Hardy, B. (2022). Economic costs of drought and rice farmers' coping mechanisms: A review. *Field Crops Research*, 275, 108345.

[24] Parry, M.A.J., Madgwick, P.J., Bayon, C., Tearall, K., Hernandez-Lopez, A., Baudo, M., & Labhilili, M. (2009). Mutation discovery for crop improvement. *Journal of Experimental Botany*, 60(10), 2817-2825.

[25] Reddy, S.H., Singhal, R.K., Dacosta, M.V.J., Kambalimath, S.K., Rajanna, M.P., Muthurajan, R., Sevanthi, A.M., Mohapatra, T., Sarla, N., Chinnusamy, V., & Singh, A.K. (2020). Leaf mass area determines water use efficiency through its influence on carbon gain in rice mutants. *Physiologia Plantarum*, 169(2), 194-213.

[26] Serraj, R., McNally, K.L., Slamet-Loedin, I., Kohli, A., Haefele, S.M., Atlin, G., & Kumar, A. (2011). Drought resistance improvement in rice: An integrated genetic and resource management strategy. *Plant Production Science*, 14(1), 1-14.

[27] Sheshshayee, M.S., Vijayaraghavareddy, P., Sreevathsa, R., Rajendrareddy, S., Arakesh, S., & Bharti, P. (2018). Introgression of physiological traits for a comprehensive improvement of drought adaptation in crop plants. *Frontiers in Chemistry*, 6, 92.

[28] Sikora, P., Chawade, A., Larsson, M., Olsson, J., & Olsson, O. (2011). Mutagenesis as a tool in plant genetics, functional genomics, and breeding. *International Journal of Plant Genomics*, 2011, 314829.

[29] Suzuki, T., Eiguchi, M., Kumamaru, T., Satoh, H., Matsusaka, H., Moriguchi, K., & Kurata, N. (2008). MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. *Molecular Genetics and Genomics*, 279(3), 213-223.

[30] Till, B.J., Cooper, J., Tai, T.H., Colowit, P., Greene, E.A., Henikoff, S., & Comai, L. (2007). Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biology*, 7, 19.

[31] Uga, Y. (2021). Challenges to design-oriented breeding for root system architecture in rice. *Breeding Science*, 71(1), 3-12.

[32] Uga, Y., Okuno, K., & Yano, M. (2011). Dro1, a major QTL involved in deep rooting of rice, and its potential for drought tolerance breeding. *JIRCAS Working Report*, 70, 55-62.

[33] Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., & Yano, M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, 45, 1097-1102.

[34] Wasson, A.P., Richards, R.A., Chatrath, R., Misra, S.C., Prasad, S.V.S., Rebetzke, G.J., & Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, 63(9), 3485-3498.

[35] Wu, J.L., Lei, C.L., & Wang, J. (2017). Creation of a rice mutant population and its application in functional genomics. *Journal of Integrative Agriculture*, 16(12), 2685-2694.

[36] Zhao, Y., Yin, Z., Wang, X., Jiang, C., Aslam, M.M., Gao, F., & Pan, Y. (2021). Genetic basis and network underlying synergistic roots and shoots biomass accumulation revealed by genome-wide association studies in rice. *Scientific Reports*, 11, 13769.