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Research Article:

Evaluation of root and shoot morphology of F_4 generation rice (*Oryza sativa* L.) crosses under iron-toxic lowland and upland conditions

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Abstract

Rice (*Oryza sativa* L.) is a major staple crop worldwide. Root architecture may contribute to plant performance under contrasting growing conditions, but its role in Sri Lankan breeding material requires further evaluation. This study assessed root, shoot, and yield-related traits of F_4-generation rice lines from six crosses, their parents, and three standard check varieties under lowland and upland field conditions at the Rice Research Station, Labuduwa. The field trials used a randomized complete block design with two replications. Traits were analysed using analysis of variance (ANOVA), Duncan's multiple range test (DMRT), and Pearson correlation analysis. Under the lowland condition designated as iron-toxic, Cross 4 recorded a root volume of 35.0 cm^3 and 206.17 seeds per panicle, while Cross 1 had a mean root length of 16.5 cm. Under the upland condition, Cross 13 recorded 9.0 tillers and Cross 6 had a dry root weight of 9.487 g. Correlations were observed between selected root traits and shoot- or yield-related traits; however, these associations do not establish causal physiological mechanisms. Genotypic and phenotypic coefficients of variation (GCV and PCV) were also estimated to quantify the extent of genetic and phenotypic variability among the evaluated morphological traits. The results identify candidate crosses for further evaluation, rather than confirming tolerance mechanisms or varietal suitability.

Keywords: Climate-resilient rice; Iron toxicity; Root architecture; Root-Shoot relationships; Upland cultivation

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1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, providing food for over half of the global population and serving as the primary caloric source for 90% of Asians (Siriwardana et al., 2019). Despite decades of rice improvement efforts in Sri Lanka, breeding programs have historically prioritised shoot morphology and yield parameters over root system characterisation. This gap is increasingly significant, as two major environmental stresses — iron toxicity in lowland soils and water deficit in upland systems — continue to threaten rice production across the country.

Iron toxicity is an important constraint in lowland rice, while water limitation can constrain rice production in upland systems. Excess ferrous iron can disrupt root function and nutrient uptake, whereas water deficit can restrict growth and yield. These mechanisms have been reported in previous studies; however, the present study did not directly measure soil iron concentration, redox potential, plant iron uptake, or soil moisture. Accordingly, the field conditions and observed plant traits are described here as the lowland and upland test environments, and interpretations about iron-toxicity tolerance or drought adaptation are treated cautiously.

Root phenotyping can be technically demanding compared with shoot and yield evaluation. Root traits such as length, width, volume, and dry weight may be useful descriptors of rice breeding material, but their relationships with stress responses need to be evaluated using appropriate environmental and physiological measurements. Evaluating root traits alongside shoot and yield-related traits can help identify promising lines for subsequent testing.

Rice has a fibrous root system composed primarily of nodal roots (Chang & Bardenas, 1965). The root system governs water and nutrient uptake and maintains plant function under stress conditions essential for survival and reproduction (Ahmed et al., 2023). The shoot system, in turn, supports root growth by supplying photosynthate for below-ground energy demand. This functional interdependence between root and shoot systems underscores the importance of evaluating both together when characterising stress adaptation.

The F_4 generation is commonly used in pedigree breeding to evaluate segregating lines and identify material for further selection. Because segregation may remain within and among lines at this stage, observed phenotypic differences can reflect genetic variation as well as environmental and measurement effects. The present study therefore evaluates morphological variation and identifies candidate lines for further assessment.

The present study evaluated F_4-generation rice breeding material under two contrasting field conditions at the Rice Research Station, Labuduwa. Root architectural traits were assessed alongside shoot and yield-related traits. Specifically, the study aimed to:

1. Select the best-performing F4 lines for iron-toxic lowland and upland conditions
2. Identify correlations between root traits and yield components
3. Identify correlations between shoot traits and yield components

2. Materials and Methods

2.1. Experimental site and plant material

The study was conducted at the Rice Research Station, Labuduwa, under two contrasting field conditions: iron-toxic lowland and upland. A total of 63 rice lines were evaluated, comprising 48 lines from six F4-generation crosses, 12 parental lines, and three standard check varieties (Ld 376, Ld 368, and Bw 372). Parent details of the selected crosses are presented in Table 1.

2.2. Land Preparation and Crop Establishment

The wetland preparation method was used for land preparation across both fields. Primary land preparation was carried out using a two-wheeled tractor-mounted rotavator and hand hoe (*mamoty*), followed by secondary and tertiary preparations. Seeds were selected from the best-performing lines of the F3 generation. Prior to sowing, seeds were

Table 1: Parent details of the selected crosses for the F_4 generation study

Cross No.	Female Parent	Male Parent
Cross 1	Ld 20-12-12 (1F)	Ld 20-15-14 (1M)
Cross 2	Ld 20-14-12 (2F)	Ld 20-15-14 (2M)
Cross 3	Ld 20-11-03 (3F)	Ld 21-06-18-02 (3M)
Cross 4	Ld 20-15-14 (4F)	Ld 20-22-04 (4M)
Cross 6	Ld 21-02-13 (6F)	Ld 20-14-12 (6M)
Cross 13	Ld 20-13-16 (13F)	Ld 20-15-14 (13M)

soaked for 24 hours, drained, and allowed to germinate for a further 24 hours. Germinated seeds were directly established in the field at a spacing of 20 cm × 20 cm.

2.3. Experimental Design and Field Management

The experiment was arranged in a randomized complete block design (RCBD) with two replications under each field condition. The use of two replications reflected the constraints of the initial screening trial; the limited replication should be considered when interpreting treatment means and variance estimates. Multilocational evaluation with additional replication would be required before any cultivar recommendation. Crop management followed the Department of Agriculture's recommended practices for the Wet Zone. Fields were monitored regularly, and pest and disease control measures were applied as required.

2.4. Data Collection

At maturity, three representative plants were sampled from each line within each replication. The field was irrigated the day before uprooting to facilitate root extraction, and a hand fork was used to loosen compacted soil where necessary. The sampled plants were labelled by line and replication, and roots were washed before morphological measurements were recorded. Measurements from the three sampled plants were averaged to obtain a line-by-replication value for each trait; the sampled plants were subsamples and were not treated as independent field replications. The following traits were measured (Figure 1): **Shoot characters:** Plant Height (PH), Culm Height (CH), Flag Leaf Length (FLL), Flag Leaf Width (FLW), Shoot Dry Weight (DSW)

Root characters: Root Length (RL), Root Width (RW), Root Volume (RV), Root Count (RC), Dry Root Weight (DRW)

Yield characters: Number of tillers, Number of effective tillers (Number of panicles), Panicle Length (PL), Seeds per Panicle (SPP), Filled grains in a panicle (Fillgr), Unfilled grains in a panicle (Unfillgr), 1000 Grain Weight (1000GW), Panicle Weight (PW)

2.5. Statistical Analysis

Data entry and initial processing were performed using Microsoft Excel (Office Professional Plus 2019). Analysis of variance (ANOVA) was conducted using SAS (version 9.1), and treatment means were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$. Pearson correlation analysis was performed using SPSS (version 16) to examine associations among morphological traits. Genotypic and phenotypic coefficients of variation (GCV and PCV) were calculated in Excel following Burton and DeVane (1953). The genotypic and phenotypic variance estimates were used to calculate the corresponding standard deviations by taking their square roots. GCV and PCV were then expressed as percentages of the respective trait mean. These coefficients were used to describe the relative magnitude of genotypic and phenotypic variation among the evaluated traits. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT)

ANOVA and mean comparisons were used to evaluate shoot, root, and yield-related traits of six rice crosses, their parental lines, and standard check varieties in the lowland

and upland field trials. Because environmental measurements confirming iron toxicity and water limitation were not collected as part of the reported study, results are interpreted as differences observed between the two test conditions rather than direct measurements of stress tolerance.



Figure 1. Measurements for shoot A-Plant height (PH), B- Flag leaf length (FLL), C-Flag leaf width (FLW), D-Root Length (RL) after uprooting, E- Root width (RW) after uprooting and F- Panicle length (PL) Measurement at maturity level

3. Results and Discussion

3.1. Iron-toxic lowland conditions

Table 2: Shoot and yield characters of tested rice lines in iron toxic lowland conditions

Cross	Flag Leaf Length (cm)	Flag Leaf Width (cm)	Number of Tillers	Effective Tillers	Seeds per Panicle
Cross 1	25.36bc	1.50cd	6.12bc	5.88bcd	163.25a
Cross 2	31.27ab	1.53cd	6.24bc	6.01bcd	163.35a
Cross 3	27.67bc	1.33de	5.93bc	5.80bcd	169.57a
Cross 4	32.06ab	1.66bcd	6.21bc	6.17bcd	206.17a
Cross 6	26.79bc	1.24de	6.47bc	6.21bcd	139.42a
Cross 13	26.15bc	5.48a	4.88bc	4.06dc	140.53a
Ld 376	31.20ab	1.30de	3.00c	3.00d	178.00a
Ld 368	17.97c	0.93e	5.66bc	5.67bc	126.33a
Bw 372	21.80bc	0.95e	6.00bc	5.00bcd	125.50a
Parent 1 (F)	27.80bc	1.51cd	6.33bc	6.33bcd	150.50a
Parent 1 (M)	31.59ab	1.63bcd	10.50a	10.00a	122.50a
Parent 2 (F)	38.12a	2.00b	8.17ba	8.17ab	174.33a
Parent 2 (M)	31.35ab	1.86bc	8.17ba	7.33abc	175.33a
Parent 3 (F)	25.62bc	1.28de	7.67ba	7.50abc	121.83a
Parent 3 (M)	25.43bc	1.33de	6.17bc	6.00bcd	123.67a
Parent 4 (F)	26.63bc	1.48cd	7.67ba	7.00abc	157.17a
Parent 4 (M)	31.57ab	1.33de	5.83bc	5.83bcd	138.50a
Parent 6 (F)	24.90bc	1.167de	6.50bc	6.50bcd	151.83a
Parent 6 (M)	24.37bc	1.25de	5.83bc	5.67bcd	191.00a
Parent 13 (F)	28.35ab	1.25de	6.17bc	6.17bcd	152.67a
Parent 13 (M)	31.80ab	1.58bcd	4.67bc	4.33cd	171.33a
CV%	23.12	18.77	39.97	40.64	41.97

Table 3: Root characters of tested rice lines in iron toxic lowland conditions

Cross	Root Length (cm)	Root Width (cm)	Root Volume (cm^3)	Dry Root Weight (g)
Cross 1	16.54ab	3.61ab	27.50b	9.24abc
Cross 2	15.37ab	4.01ab	32.72ab	10.67abc
Cross 3	15.07ab	3.92ab	29.33b	9.02abc
Cross 4	14.28ab	4.40ab	35.00ab	10.83abc
Cross 6	13.96ab	3.79ab	21.58b	6.99bc
Cross 13	14.95ab	5.08a	30.35ab	5.78bc
Ld 376	17.00ab	3.50ba	25.00b	4.86bc
Ld 368	18.00a	4.43ba	31.67ab	7.31bc
Bw 372	13.00b	3.90ab	15.00b	3.27c
Parent 1 (F)	15.15ab	4.25ab	39.17ab	8.82abc
Parent 1 (M)	15.67ab	4.72ba	40.83ab	17.51a
Parent 2 (F)	15.78ab	4.12ba	39.17ab	10.83abc
Parent 2 (M)	15.91ab	5.05a	59.17a	14.23ab
Parent 3 (F)	15.69ab	4.60ba	37.50ab	10.22abc
Parent 3 (M)	16.05ab	3.38ab	25.00b	6.67bc
Parent 4 (F)	13.73ab	3.75ab	30.00b	9.77abc
Parent 4 (M)	13.28ab	3.37ab	20.83b	5.26bc
Parent 6 (F)	12.08b	4.06ba	24.17b	8.57abc
Parent 6 (M)	15.52ab	4.28ba	24.17b	6.73bc
Parent 13 (F)	15.22ab	3.93ba	28.33b	4.99bc
Parent 13 (M)	13.15ab	3.10b	13.33b	5.41bc
CV%	21.49	27.16	64.13	73.47

Among the F_4 crosses (Table 2), Cross 4 recorded the longest flag leaf, while flag leaf width was notably highest in Cross 13. Parent 2F exhibited the greatest values for both flag leaf length and width, indicating its potential as a donor parent for improving leaf traits. The flag leaf is the primary photosynthetic source during grain filling, and its dimensions directly influence assimilate supply to developing grains (Theerawitaya et al., 2022). Under iron toxicity, Fe^{2+} disrupts chloroplast integrity through leaf bronzing, reducing photosynthetic efficiency (Becker & Asch, 2005). Wider flag leaves may therefore retain larger assimilate pools to make up for this reduction.

Tiller numbers were generally moderate among the F_4 crosses, with Cross 6 recording the highest count. Effective tillers were highest in Parent, followed by Parent 2F. The relatively higher tillering capacity observed in F_4 crosses compared to standard checks is notable, given that iron toxicity typically suppresses tiller initiation by impairing root membrane nutrient uptake (Turhadi et al., 2018). Root length ranged from 13.96 cm (Cross 6) to 16.54 cm (Cross 1) among the F4 crosses, with Ld 368 recording the highest value overall (18.00 cm). Longer roots may reduce localised Fe^{2+} toxicity by distributing metal uptake across a wider soil volume, thereby lowering oxidative damage at the root surface (Gowda et al., 2011; Sonu et al., 2024). Root width was highest in Cross 13, and root volume was greatest in Cross 4. Dry root weight was highest among crosses in Cross 4, with Parent 1M recording the highest overall value (Table 3).

Thicker roots are associated with enhanced cell wall lignification, which reduces Fe^{2+} permeability and limits oxidative stress damage (Becker & Asch, 2005; Sonu et al., 2024). Larger root volumes expand the rhizospheric exploration zone, improving access to nutrient-rich microsites under conditions where iron toxicity is accompanied by phosphorus and potassium deficiencies (Sahrawat, 2004). Higher dry root weight reflects greater root biomass investment, which has been shown to reduce yield losses under Fe^{2+} stress to 15–30%, compared to losses of up to 54% in genotypes with lower root biomass (Chérif et al., 2009).

Seeds per panicle were highest in Cross 4. Cross 4's elevated seed count, combined with its superior root volume and dry root weight,

demonstrates consistent overall performance under iron-toxic conditions.

The high CV% values observed across traits (up to 73.47%) are attributable to residual genetic segregation within F4 populations, where approximately 12.5% of loci remain heterozygous (Suhartini, 2004). However, residual heterozygosity may still result in segregation within individual lines, and further generations of self-pollination are generally required to achieve greater genetic uniformity. In conventional rice-breeding programs, selection and evaluation may continue through F_6–F_7 or later, depending on the breeding method and the required level of line stability (Collard et al., 2019; Fahim et al., 1998).

3.2. Upland conditions

Table 4: Shoot and Yield Characters of tested rice lines in upland conditions

Cross	Flag Leaf Length (cm)	Flag Leaf Width (cm)	Number of Tillers	Effective Tillers
Cross 1	33.11a	1.469a	6.89abcd	6.62ab
Cross 2	29.46ab	1.210abc	6.60abcd	6.29ab
Cross 3	29.19ab	1.264abc	7.26abcd	7.09ab
Cross 4	26.71ab	1.31abc	6.81abcd	6.52ab
Cross 6	28.05ab	1.25abc	7.33abcd	7.18ab
Cross 13	26.59ab	1.11bc	9.00ab	8.67a
Ld 376	26.98ab	1.18abc	7.20abcd	6.40ab
Ld 368	28.62ab	1.30abc	7.17abcd	7.17ab
Bw 372	27.98ab	1.367abc	6.67abcd	6.33ab
Parent 1 (F)	28.38ab	1.15abc	9.67a	8.67a
Parent 1 (M)	29.55ab	1.183abc	7.00abcd	6.83ab
Parent 2 (F)	24.95b	1.200abc	5.50cd	5.50ab
Parent 2 (M)	26.10ab	1.200abc	6.25bcd	5.75ab
Parent 3 (F)	28.50ab	1.05c	5.00d	4.50b
Parent 3 (M)	27.12ab	1.23abc	7.33abcd	7.33ab
Parent 4 (F)	27.44ab	1.18abc	7.20abcd	6.80ab
Parent 4 (M)	27.63ab	1.35abc	7.17abcd	6.83ab
Parent 6 (F)	24.27b	1.18abc	6.67abcd	6.50ab
Parent 6 (M)	26.57ab	1.17abc	7.50abcd	6.67ab
Parent 13 (F)	26.40ab	1.08bc	7.60abcd	6.60ab
Parent 13 (M)	31.36ab	1.42ab	8.60abc	8.40a
CV%	20.39	20.72	35.88	36.42

Flag leaf length varied significantly among the crosses and their parents, with Cross 1 exhibiting the longest flag leaf length, while Cross 13 had the shortest. Flag leaf width ranged from 1.11 cm in Cross 13 to 1.47 cm in Cross 1 (Table 4). Among the parents, Parent 3 (F) had the narrowest flag leaf width, while Parent 13 (M) exhibited a relatively broader flag leaf. These results are aligned with other studies that found flag leaf characteristics to be significant differentiators among upland rice cultivars. Significant variations in flag leaf length between genotypes have also been shown in evaluation studies of upland rice superior varieties (Mustikarini et al., 2023). The flag leaf plays a crucial physiological role as the primary photosynthetic organ during grain filling, making these differences potentially important for yield performance. The size of the flag leaf directly affects photosynthetic capability, which in turn affects the grain yield. The significant variation in flag leaf dimensions observed in our crosses suggests the potential for selection toward improved photosynthetic efficiency.

The number of tillers showed considerable variation, with Parent 1 (F) having the highest mean number of tillers, followed by Cross 13. The Cross 13 also had the highest number of effective tillers, indicating its potential for high yield under upland conditions. Effective tillers are crucial for grain production, and

significant differences were noted among the crosses and parents. For the production of upland rice, the observed variations in tillering ability are very important. Previous studies have shown that one of the main factors influencing grain yield in upland environments is the number of tillers, particularly the productive tiller count. Studies assessing the growth of upland rice under different fertilization regimes provide evidence for this, showing that proper nutrient supplementation greatly boosts the number of tillers (Rismawati et al., 2022). The high number of effective tillers in Cross 13 indicates its efficient resource utilization, potentially translating to improved grain yield under upland conditions. Genetic variations in tillering capacity were clearly expressed in upland rice varieties under different stress conditions (Sadimantara et al., 2019). Root length varied among the crosses, with Cross 2 showing the highest root length while Cross 13 was the shortest (Table 5). Root width among the crosses ranged from 2.75 cm in Cross 3 to 2.93 cm in Cross 2. Root volume among the crosses varied significantly, with Cross 1 having a relatively high root volume and Cross 13 having a lower volume. Dry root weight showed substantial variation, with Cross 6 having the highest dry root weight among the crosses. Dry root weight is a crucial indicator of the plant's investment in below-ground biomass, which is essential for nutrient uptake and yield improvement (Sandhu et al., 2017).

Due to the segregation of characters in the F_4 generation again, a higher CV% was observed.

Table 5: Root Characters of tested rice lines in upland conditions

Cross	Root Length (cm)	Root Width (cm)	Root Volume (cm^3)	Dry Root Weight (g)
Cross 1	12.43a	2.87bcd	23.30abcd	9.35ab
Cross 2	13.31a	2.93bcd	21.82abcd	8.24ab
Cross 3	11.66a	2.75bcd	18.35abcd	8.37ab
Cross 4	12.20a	2.85bcd	21.19abcd	8.02ab
Cross 6	11.53a	2.76bcd	22.05abcd	9.48ab
Cross 13	11.07a	2.86bcd	19.45abcd	7.65ab
Ld 376	12.20a	2.82bcd	15.00bcd	4.88b
Ld 368	13.33a	3.17abc	28.33abcd	6.78b
Bw 372	11.45a	2.90bcd	23.33abcd	7.73ab
Parent 1 (F)	11.67a	3.33ab	35.00a	14.43a
Parent 1 (M)	11.72a	2.65bcd	21.67abcd	4.43b
Parent 2 (F)	10.95a	2.32cd	15.00bcd	7.81ab
Parent 2 (M)	12.08a	2.75bcd	15.63bcd	5.15b
Parent 3 (F)	8.60a	2.18bcd	12.50bcd	3.92b
Parent 3 (M)	13.52a	3.00bcd	20.83abcd	7.48b
Parent 4 (F)	10.36a	2.88bcd	17.00bcd	6.56b
Parent 4 (M)	12.95a	2.97bcd	24.17abcd	8.60ab
Parent 6 (F)	8.02a	2.07d	9.17d	4.94b
Parent 6 (M)	10.92a	2.32cd	10.42d	4.43b
Parent 13 (F)	11.08a	2.48bcd	16.00bcd	7.44b
Parent 13 (M)	12.25a	3.90a	30.00ab	6.41b
CV%	**40.96**	**26.10**	**68.09**	**68.32**

3.3. Pearson correlation coefficient analysis

Correlation coefficient analysis evaluates the mutual relationship between two plant traits and identifies key characteristics that contribute to selecting rice lines with tolerance to iron toxicity and upland conditions (Becker & Asch, 2005).

3.3.1. Iron-toxic lowland conditions

Flag Leaf Length (FLL), Flag Leaf Width (FLW), No. of Tillers (Ntill), No. of Effective Tillers (Etill), Root Length (RL), Root Width (RW), Root Volume (RV), Seeds per Panicle (SPP), Dry Root Weight (DRW).

Correlations among root, shoot, and yield-related traits indicate that some morphological characteristics varied together under the lowland test condition (Table 6). These associations may help identify traits for further evaluation, but they do not establish that a particular root trait caused improved tillering or reduced iron uptake

Table 6. Correlations among shoot, root, and yield characters in iron-toxic lowland conditions

	FLL	FLW	Ntill	Etill	RL	RW	RV	SPP	DRW
FLL	1	0.189	-0.15	-0.11	0.263	-0.148	0.222	0.226	0.093
FLW		1	-0.106	-0.058	0.309	0.168	.472**	.440*	.379*
Ntill			1	.980**	.476**	.687**	.451*	-0.043	.620**
Etill				1	.502**	.713**	.491**	-0.049	.677**
RL					1	.566**	.750**	.481**	.596**
RW						1	.758**	0.178	.640**
RV							1	0.358	.740**
SPP								1	0.092

**. Correlation is significant at the 0.01 level (2-tailed)
*. Correlation is significant at the 0.05 level (2-tailed)

Root length alone cannot demonstrate Fe^{2+} exclusion, and iron uptake, root oxidation, and related physiological responses were not directly measured in this study.

Root width was correlated with tiller number and effective tillers in the present dataset. This association identifies a relationship among measured traits; it does not demonstrate that thicker roots reduce Fe^{2+} uptake or oxidative damage. Such mechanisms would require direct physiological or chemical measurements.

Root volume was correlated with selected yield-related traits. A larger measured root volume may be a useful morphological characteristic for selection, but the present correlations do not establish that root volume increased nutrient acquisition or caused higher reproductive performance. Nutrient availability and uptake were not directly measured.

Dry root weight was associated with several root traits and effective tiller number. These relationships describe covariation among the measured traits and should not be interpreted as proof that greater root biomass caused improved nutrient acquisition or reduced yield losses under iron stress.

Flag leaf width was positively correlated with seeds per panicle and root volume in the lowland dataset. These associations suggest that the traits may be worth considering together during further screening; they do not demonstrate that wider leaves caused higher seed production or maintained photosynthetic capacity under iron toxicity.

3.2. Upland conditions

Under the upland test condition, root width was correlated with effective tiller and tiller number (Table 7). These relationships may be useful when screening breeding material, but root penetration and access to deeper soil moisture were not measured directly. Therefore, the correlations should not be interpreted as direct evidence of drought tolerance or water acquisition.

Dry root weight was associated with flag leaf width and tiller number, while flag leaf length was correlated with selected shoot and root traits. These results indicate coordination among measured morphological characteristics; however, photosynthetic efficiency, soil moisture, and plant water status were not measured and cannot be inferred directly from these correlations.

Table 7: Correlations among shoot, root, and yield characters in upland conditions

	FLL	FLW	PL	Ntill	Etill	RL	RW	RV	DRW
FLL	1	.718**	.749**	0.147	0.114	0.205	0.285	0.287	0.422*
FLW		1	.504**	0.128	0.092	0.253	0.282	0.257	.385*
PL			1	-0.087	-0.066	-0.057	-0.098	-0.104	0.119
Ntill				1	.979**	0.306	.579**	.493**	.416*
Etill					1	0.22	.498**	0.374	0.323
RL						1	.585**	.755**	0.241
RW							1	.841**	.512**
RV								1	.707**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
Flag Leaf Length (FLL), Flag Leaf Width (FLW), Panicle Length (PL), No of Tillers (Ntill), No of Effective Tillers (Etill), Root Length (RL), Root Width (RW), Root Volume (RV), Seeds per Panicle (SPP), Dry Root Weight (DRW)

3.3. Estimation of genotypic and phenotypic coefficient of variation of the F4 generation six rice crosses under iron–toxic lowland conditions.

PCV values were generally higher than GCV values in the reported calculations (Table 8), which may indicate that observed phenotypic variation includes environmental variation in addition to genotypic variation.

Flag leaf length and width showed moderate GCV values across crosses, with Cross 6 exhibiting the highest GCV for both traits. This suggests that Cross 6 carries stronger genetic potential for improvement in leaf dimensions — a characteristic relevant to photosynthetic capacity under stress (Theerawitaya et al., 2022). Tiller number and effective tillers also showed high GCV in Cross 6, reflecting substantial genetic variability for these yield-related traits.

Variation in root width, root volume, and dry root weight was observed among the crosses. These differences may help identify material for further evaluation. However, GCV alone does not establish heritability or prove that a trait is under strong genetic control

Table 8: Genotypic and phenotypic coefficients of variation of shoot, root and yield traits under iron-toxic lowland conditions

	Flag Leaf Length							**Flag Leaf Width**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	27.3	31.9	27.1	31.0	26.0	29.2	Mean	1.6	1.6	1.3	1.7	1.2	3.66
PCV	12.01	25.04	21.38	19.75	20.12	17.89	PCV	21.51	23.49	20.94	22.40	23.19	20.22
GCV	6.92	9.37	12.65	13.78	15.37	5.34	GCV	16.22	9.54	13.38	13.57	18.10	2.75
	No. of Tillers							**No. of Effective Tillers**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	7.69	6.59	5.9	6.22	6.35	5.48	Mean	7.56	6.34	5.75	6.26	6.16	5.11

PCV	22.46	39.51	42.44	32.15	37.59	32.51	PCV	22.83	39.31	41.96	30.72	36.52	27.03
GCV	8.21	19.42	21.41	17.47	37.74	13.85	GCV	8.35	17.36	14.42	1.77	33.97	10.87
	Root Length							**Root Width**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	15.86	15.52	15.37	14.30	13.90	14.97	Mean	4.36	4.17	3.69	4.11	3.94	3.66
PCV	20.32	21.71	17.18	20.91	25.51	17.86	PCV	23.89	26.00	22.96	18.89	24.00	20.22
GCV	6.93	12.49	7.09	8.29	21.56	2.43	GCV	15.6	2.02	17.27	4.93	21.18	2.75
	Root Volume							**Seeds per Panicle**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	40	34.30	33.47	33.44	22.58	27.12	Mean	147.2	165.08	156.19	186.17	160.44	155.96
PCV	35.98	61.90	54.74	61.49	63.09	31.15	PCV	41.16	43.18	59.08	42.74	27.93	36.38
GCV	20.23	34.81	46.67	60.67	36.72	10.95	GCV	34.26	19.20	51.16	26.98	15.45	23.23
	Panicle Weight							**1000 Grain Weight**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	2.70	3.49	2.96	3.56	3.22	2.64	Mean	31.17	31.11	29.02	37.57	30.60	34.22
PCV	77.69	51.40	56.98	42.74	31.81	28.74	PCV	12.87	37.54	20.96	27.07	24.61	22.20
GCV	68.32	15.03	33.09	53.69	25.18	6.02	GCV	5.66	36.07	18.68	5.50	10.66	7.12
	Dry Root Weight												
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13							
Mean	11.87	10.95	9.5	9.70	7.25	5.41							
PCV	58.92	66.25	72.28	64.79	71.91	52.59							
GCV	28.92	30.73	56.90	49.76	55.32	42.02							

3.4. Estimation of genotypic and phenotypic coefficient of variation of the F4 generation six rice crosses under the upland conditions

Table 9: Genotypic and phenotypic coefficients of variation of shoot, root and yield traits under the upland conditions

	Flag Leaf Length							**Flag Leaf Width**					
z	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	31.8	29.00	28.9	27.0	27.3	27.1	Mean	1.4	1.3	1.2	1.3	1.2	1.1
PCV	20.96	26.82	16.77	26.70	28.30	17.9	PCV	24.65	24.21	23.41	26.25	22.01	14.1
GCV	15.93	13.22	7.51	20.78	20.30	8.3	GCV	22.78	18.10	14.02	8.63	18.58	7.5
	No. of Tillers							**No. of Effective Tillers**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	7.5	6.5	7.11	6.41	7.27	8.8	Mean	6.7	6.2	6.93	6.27	7.02	8.39
PCV	34.56	39.76	42.68	40.54	40.22	35.75	PCV	36.52	39.43	44.41	38.96	40.19	35.82
GCV	16.62	25.14	31.45	29.15	18.76	25.62	GCV	18.39	24.46	32.60	14.00	25.80	22.26
	Root Length							**Root Width**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	12.19	13.07	11.64	12.30	10.95	11.2	Mean	2.85	2.88	2.69	2.88	2.61	2.91
PCV	32.92	70.46	24.67	20.69	23.46	22.35	PCV	33.77	26.68	30.51	27.39	33.40	25.31
GCV	27.58	63.48	13.18	14.97	12.31	17.60	GCV	13.50	8.66	23.65	12.27	20.20	21.44
	Root Volume							**Seeds per Panicle**					
	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13		Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13
Mean	25.5	21	17.46	20.17	18.78	19.33	Mean	107.66	112.88	106.70	108.66	105.13	88
PCV	64.15	74.80	90.40	64.94	82.77	66.66	PCV	43.81	46.79	45.36	49.33	51.70	36.06
GCV	37.00	59.31	87.79	53.05	74.41	53.58	GCV	32.50	36.60	34.59	37.86	27.84	31.02
	Dry Root Weight												

	Cross 1	Cross 2	Cross 3	Cross 4	Cross 6	Cross 13							
Mean	9.03	7.84	7.97	7.58	8.21	7.48							
PCV	65.97	78.00	78.77	66.78	77.51	68.99							
GCV	62.25	70.98	56.11	45.44	67.81	54.87							

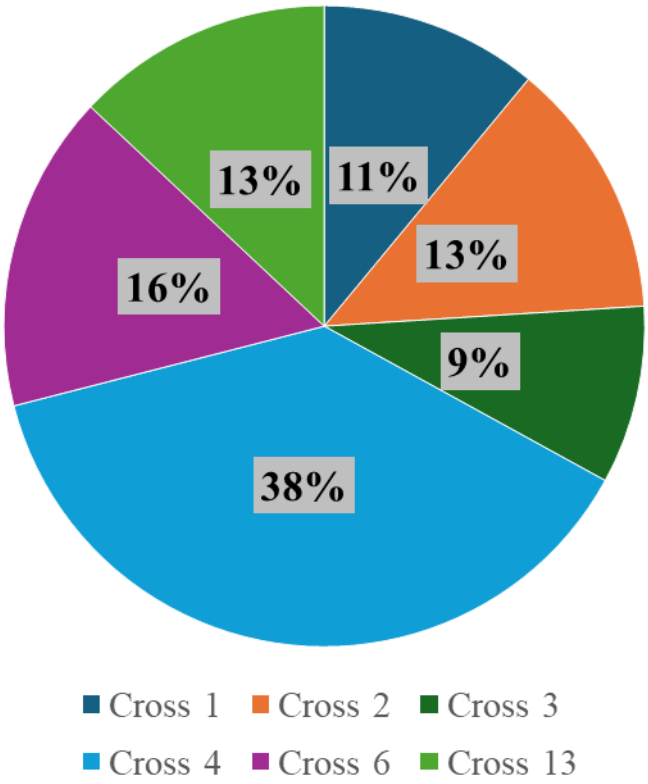

Figure 2: Distribution of selected rice lines for the F_5 generation

Under the upland condition, the reported PCV and GCV values suggest variation in several shoot and root traits (Table 9). The magnitude and ranking of these estimates should be interpreted only after the calculations have been verified. Because soil moisture and plant water status were not measured, these estimates alone cannot confirm drought tolerance. Variation in tiller number, root length, and root volume was observed among crosses under the upland condition. These traits may be considered in subsequent screening, but the present estimates do not by themselves establish a genetic basis for performance or demonstrate drought tolerance. The reported variation in dry root weight and seeds per panicle may be useful for identifying candidates for further evaluation. GCV alone is not evidence of heritability or strong genetic control.

3.5. Selected Lines for F5 Generation

Figure 2 summarizes the rice lines selected for advancement to the F_5 generation based on the selection criteria used in the breeding programme. Root and

yield-related traits were considered together when identifying candidate lines. Cross 4 contributed the largest proportion (38%) of the selected material, followed by Cross 6 (16%), Cross 2 and Cross 13 (13% each), Cross 1 (11%), and Cross 3 (9%). This distribution represents the contribution of each cross to the total selected population and should not be interpreted as a direct measure of comparative overall performance.

The selected F_5 lines represent breeding material for further evaluation. Their performance should be confirmed in subsequent trials with clearly characterized environments and adequate replication before conclusions are drawn about stress tolerance or suitability for varietal development.

4. Conclusions

The study documented variation in root, shoot, and yield-related traits among F_4-generation rice crosses, their parental entries, and standard checks evaluated under lowland and upland field conditions. In the lowland trial, Cross 4 had the greatest mean root volume among the crosses (35.0 cm^3) and the highest mean seeds per panicle (206.17), while Cross 1 had the longest mean root length (16.54 cm). In the upland trial, Cross 13 recorded the highest mean tiller number (9.00), and Cross 6 had the greatest mean dry root weight among the crosses (9.48 g). Correlations among measured traits identified associations that may guide further screening, but they do not establish causal physiological mechanisms. GCV and PCV provided descriptive estimates of variation and were not used as substitutes for heritability estimates. Cross 4, Cross 13, Cross 6, and other selected material merit further evaluation in replicated trials with characterized soil and moisture conditions. The present screening results do not independently confirm iron-toxicity tolerance, drought adaptation, or suitability for varietal release.

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Author Contributions

Conceptualization, MCM; methodology, MCM; investigation, MCM, MKM; data curation, KMGPD; formal analysis, KMGPD, MCM; writing—original draft, KMGPD, TTD; and writing—review and editing were contributed by the authors. All authors reviewed and approved the final manuscript.

Data Accessibility

The data supporting the findings of this study are available at the Labuduwa Rice Research Station, Department of Agriculture, Sri Lanka. Upon request, data could be accessed

Declaration of conflict of interest

The authors declare no conflicts of interest.

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