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ORIGINAL RESEARCH

Assessment of Drought Tolerance in Rice Landraces via Seedling-Based Indices

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ABSTRACT: Landraces serve as a vital reservoir of genetic diversity, offering allelic variation crucial for breeding resilient cultivars. However, with the increasing frequency and intensity of drought due to climate change, identifying drought-resilient rice varieties is crucial to ensure sustainable rice production. Although, Nepal hosts a vast diversity of rice landraces, yet their potential for drought stress tolerance remains underexplored. This study evaluated 25 rice landraces for drought tolerance at the seedling stage using a completely randomized design (CRD) in 250 ml disposable cups under three moisture regimes: 60% field capacity (FC), 100% FC, and saturated conditions. Ten quantitative traits were analyzed, and significant variation was observed among landraces and across moisture conditions. Correlation analysis suggested that, under 60% FC, root length showed a significant positive correlation with shoot length, root: shoot ratio, and fresh root weight, whereas a negative correlation was noted between root: shoot ratio and root number. Germination percentage remained unaffected by moisture conditions. Principal component analysis revealed a positive connection of root length and root-to-shoot ratio towards 60% FC, while shoot length, fresh weight, dry weight, and root number were associated with saturated conditions. Among the landraces, Manamurey demonstrated superior performance across studied traits. These findings highlight the potential of specific landraces for drought resilience and emphasize the need for further evaluations at vegetative and reproductive stages to confirm their utility in breeding programs.

KEYWORDS: Drought stress, field capacity, landraces, rice, seedling stage.

1. Introduction

Rice, a major staple food of Nepal, surpasses all other cereal crops in terms of acreage and yield and ranks third globally after wheat and maize. The total area of land under rice cultivation is estimated to be 1,477,378 hactare (ha) with annual production of 5,130,625 metric (mt) ton and productivity of 3.47 mt/ha (MOALD, 2021). Rice contributes about 21% of AGDP in Nepal which is equivalent to almost 10% of the national GDP (Aryal et al., 2022). Generally, Nepal is considered one of the centers of diversity of rice landraces (Ghimire et al., 2018). Landraces comprise a major component of Nepal's rice production system, accounting for about 70% of the country's total rice area (Amgai and Joshi, 2004). Variations in altitude, geography, physical and climatic circumstances have enriched the country with immense genetic diversity in the form of landraces or traditional cultivars of rice. Landraces are maintained and managed by the farmers in their fields for diverse purposes, including Indigenous rites and beliefs, and their immense adaptability to changing conditions over time and across different environments (Bajracharya et al., 2010). Landraces possess specific traits to adapt for better local adaptation in their environment, various socio-economic, and cultural values (Rayamajhi and Thakuri, 2023). They are adapted to marginal to high fertile soil, drought to deep water, different planting seasons, different climatic conditions, pest and disease infestation (Joshi, 2017).

Climatic change has affected agricultural productivity which is dependent on water resources. Unpredictable climate change has created water cycle disruptions leading to drought stress (Kompas et al., 2024). Decreased precipitation and shifting rainfall patterns are primary drivers of the emergence of drought stress on a global scale. (Fahad et al., 2017). Drought stress leads to the annual loss of 12 million hectares of agricultural land with the loss of 20 million tons of grain (Tripathi et al., 2024). Drought has led to a significant loss in yield, influencing physiological, molecular, and biochemical processes such as photosynthesis, nitrogen metabolism, and assimilation (Qiao et al., 2024). In addition, it affects the elongation and expansion growth impairing the germination of rice seedlings of modern varieties, reducing the tiller number, plant height, and biomass production (Jarin et al., 2024). From an irrigation perspective, 52% of the total agricultural land remains rainfed, with only 48% irrigated, of which 39% receives year-round irrigation (Irrigation Master Plan, 2019). The lack of proper maintenance and associated regulatory mechanisms creates an inadequate supply of irrigation in the irrigated areas of the country (Aryal et al., 2022).

Rice landraces have been fading out due to the absolute use of exotic advanced modern rice varieties. Despite having the ability to thrive in harsh conditions, the genetic resources of landraces have been declining at an alarming rate (Kharel et al., 2022). Rice landraces, despite having low yield are believed to be adopted in the local environment (Rijal, 2010), resistant to both biotic and abiotic stresses, and are considered reservoirs of genetic potential, whereas modern rice varieties don't hold such qualities (Tiwari et al., 2018). Therefore, the preservation and utilization of these landraces can help develop improved varieties with enhanced drought tolerance.

In light of the escalating challenges driven by climate change and water scarcity, it is imperative to identify drought-resilient landraces to secure sustainable rice production. Therefore, the objective of this study is to evaluate local landraces under drought conditions by analyzing and comparing root and shoot traits in the seedling stage and to identify promising landraces that can be utilized in breeding programs for enhanced drought resilience.

2. Material and methods

2.1. Experimental site and design

The experiment was carried out in the greenhouse of the Institute of Agriculture and

Animal Science, Lamjung Campus, situated in Sundarbazar, Lamjung, Nepal. Specially, the research site is located in the mid-hill region at 610 meters above sea level, with geographic coordinates of 28.12° N latitude and 84.41° E longitude. The research was undertaken in July and August 2024, during a period marked by the region's characteristic warm and humid climate.

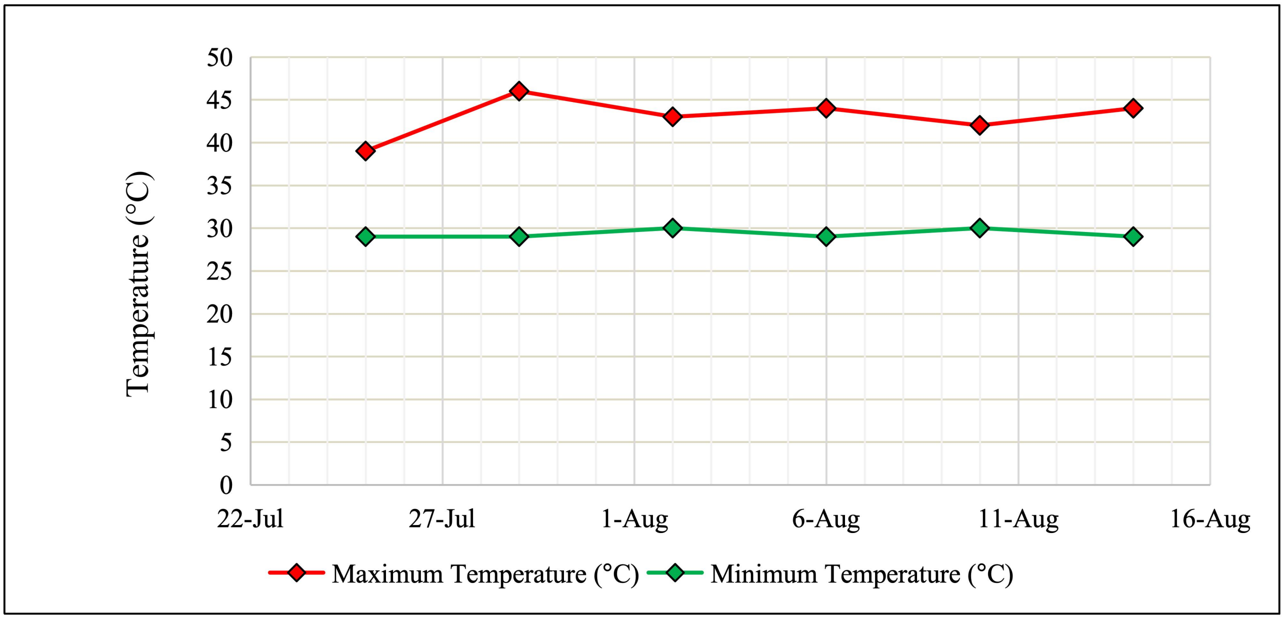

Figure 1. Environment conditions during the research period.

Table 1. List of rice landraces tested in the experiment.

Treatment	Landraces	Treatment	Landraces
T1	Kattikey	T14	Jarneli
T2	Rambilash	T15	Jungey
T3	Jhini	T16	Kamal
T4	Darmali	T17	Dalley Masino
T5	Basmati	T18	Gaurey
T6	Aangha	T19	Himali
T7	Mansara	T20	Jungey Kanchi
T8	Kalo Jhinuwa	T21	Manamurey
T9	Kalokattey	T22	Nouley Dalley
T10	Gurdo	T23	Pahele
T11	Pathijharey	T24	Biramphul
T12	Krishnabeli	T25	Seto Dalley
T13	Aapjhuttey		

The experiment tested three moisture conditions: 100% field capacity (D1), 60% field capacity (D2), and saturated condition (S). A total of 25 distinct rice landraces were tested, sourced from the Purkot and Ghanpokhara Community Seed Banks of Tanahun and Lamjung, respectively. These landraces encompass a broad spectrum of genetic traits and exhibit distinctive attributes that enable them to thrive under harsh environmental conditions. The experiment was conducted using a two-factor factorial design under a completely randomized design (CRD). Factor A comprised 25 distinct rice landraces as outlined in Table 1, while Factor B encompassed three specific moisture regimes: 100% field capacity (FC), 60% FC, and saturated condition.

The experimental setup comprised 225 disposable cups, each with a capacity of 250 ml, representing 25 landraces × 3 moisture conditions × 3 replications. Each cup accommodated two seedlings, resulting in a total of 450 seedlings for the entire study. The planting medium was prepared by combining a 1:2:1 ratio of sand, sandy loam soil, and farmyard manure (FYM). Each cup was filled with 200 grams of this mixture. Similarly, six seeds were sown per cup, and after five days of seedling establishment, the seedlings were thinned to maintain two per cup.

Water management was carefully implemented to maintain the desired moisture conditions. The field capacity (FC) was determined by saturating 200 grams of planting media and allowing it to drain for 48 hours. After this period, the weight of the planting media was recorded as 233.92 g. The oven-dried weight of the media, obtained by drying it at 72°C for 48 hours, was 159.97 g. The weight of the soil water at field capacity was calculated as 73.95 g, which is the difference between the saturated weight and the oven-dried weight. Subsequently, the weight of soil water at 60% of field capacity was determined to be 44.37 g. These calculations ensured precise control over the moisture levels in the planting media.

2.2 Data collection

Germination percentage was calculated by determining the proportion of seeds germinating successfully under controlled conditions. The following formula was used to calculate the germination percentage:

Germination Percentage (GP) = $\frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds Sown}} \times 100$ (Mamun et al., 2018).

Root length (RL) was measured as the distance from the base of the plant (where it connects to the plant) to the tip of the longest root, recorded in centimeters using a measuring scale. Similarly, shoot length (SL) was determined as the distance from the base of the plant (where it connects to the root) to the tip of the longest flag leaf, also measured in centimeters using a measuring scale. The root-to-shoot ratio (RL/SL) was calculated by dividing the root length by the shoot length. Fresh root weight (FRW) and fresh shoot weight (FSW) were determined by weighing freshly washed roots and shoots, respectively, on a precision balance, with their weights recorded in grams. Total plant weight (TPW) was measured by weighing the entire plant, including roots and shoots, on a precision balance. Fresh plant samples were oven-dried at 70±5 for 48 hours to determine the dry weight (Badr et al., 2020). Root dry weight (RDW) and shoot dry weight (SDW) were obtained by oven-drying fresh root and shoot samples at 70±5°C for approximately 48 hours and measuring their weights in grams. Finally, root number (RN) was determined by manually counting the number of washed roots. These standardized techniques ensured consistent and accurate data collection for the study.

2.3. Statistical analysis

The observed data were entered in MS Excel (2021), and the interaction effect was visualized by SigmaPlot. Statistical analyses, including Analysis of Variance, mean separation, and F-tests, were conducted at a 5% significance level. Boxplots, correlation analysis, and principal component analysis were executed using R (version 4.4.1).

3. Results

3.1. Comparison of rice landraces for traits

Table 2 illustrates the results of the analysis of variance for 10 quantitative traits, examining the effects of landraces, conditions, and their interaction at 21 days.

3.2 Mean performance comparison of the study parameters

3.2.1. Germination percentage

A significant difference in germination was observed among the 25 rice landraces. Among the tested landraces, Darmali, Jhini, and Kattikey exhibited the highest germination rates, with a percentage of 98.148%. In contrast, Biramphul showed the lowest germination rate at 81.481%, followed closely by Seto Dalley at 81.185%.

3.2.2. Root length

A significant difference in root length was observed among the 25 rice landraces. As shown in Table 3, Manamurey had the highest mean root length of 17.25 cm, statistically similar to Biramphul, Rambilash, Pahele, Kamal, Jungey Kanchi, and Seto Dalley. The lowest root length was recorded for Pathijharey with a mean value of 13.156 cm.

Table 2. Analysis of variance of studied traits.

S.N.	Traits	Mean sum of square			
		Landraces (DF=24)	Condition (DF=2)	Landraces*Condition (DF=48)	Error (DF=150)
1	Germination percentage	151.44^{**}	34.57^{ns}	120.73^{*}	81.84
2	Root length	12.16^{***}	203.47^{***}	4.68^{ns}	3.98
3	Shoot length	167.7^{***}	1299.4^{***}	31.3^{***}	12.8
4	Root: shoot ratio	0.0480^{***}	1.2540^{***}	0.0142^{**}	0.0078
5	Fresh root weight	0.00463^{***}	0.05342^{***}	0.00213^{***}	0.00042
6	Fresh shoot weight	0.01873^{***}	0.22429^{***}	0.00397^{***}	0.00097
7	Total plant weight	0.0378^{***}	0.4966^{***}	0.0088^{***}	0.0019
8	Root dry weight	0.0000640^{***}	0.0003217^{***}	0.0000242^{***}	0.0000111
9	Shoot dry weight	0.000358^{***}	0.003807^{***}	0.000163^{***}	0.000040
10	Root number	10.5^{***}	665.6^{***}	3.4^{ns}	2.2

Note: *, **, and *** denote significance at 5%, 1%, and 0.1%, respectively, whereas ns denotes non-significant.

Table 3. Mean separation table for quantitative traits in twenty-five rice landraces.

Factor A: Landraces										
Landraces	GP	RL	SL	RL/SL	FRW	FSW	TPW	RDW	SDW	RN
Aangha	94.444^{c}	13.678^{fgh}	34.450^{bcd}	0.406^{kl}	0.140^{cdef}	0.246^{abcd}	0.386^{bcde}	0.017^{cdef}	0.047^{cdefg}	9.333^{f}
Aapjhuttey	96.296^{b}	15.133^{bcdefg}	30.733^{efghi}	0.503^{defghi}	0.094^{kl}	0.177^{efghi}	0.271^{jkl}	0.012^{hi}	0.039^{j}	8.278^{l}
Basmati	88.889^{f}	13.506^{gh}	25.144^{lmno}	0.580^{bc}	0.121^{ghi}	0.151^{ghi}	0.272^{jkl}	0.018^{bcd}	0.043^{fghij}	8.944^{i}
Biramphul	81.481^{h}	16.928^{ab}	30.056^{fghij}	0.576^{bc}	0.106^{hijkl}	0.203^{defgh}	0.309^{ghijk}	0.015^{defg}	0.041^{ghij}	8.389^{k}
Dalley Masino	96.296^{b}	15.017^{cdefg}	23.239^{o}	0.685^{a}	0.101^{jkl}	0.128^{i}	0.229^{l}	0.010^{i}	0.032^{k}	7.000^{r}
Darmali	98.148^{a}	15.344^{bcdefg}	35.628^{abc}	0.453^{hijkl}	0.143^{cde}	0.260^{abcd}	0.403^{bcd}	0.021^{a}	0.054^{ab}	9.056^{h}
Gaurey	94.444^{c}	13.800^{efgh}	31.639^{defg}	0.444^{hijkl}	0.142^{cdef}	0.208^{cdefg}	0.350^{defg}	0.016^{cdefg}	0.039^{ij}	9.833^{d}
Gurdo	94.444^{c}	13.556^{fgh}	31.061^{efgh}	0.458^{hijkl}	0.091^{l}	0.209^{cdefg}	0.306^{ghijk}	0.015^{defg}	0.042^{ghij}	8.222^{l}
Himali	94.444^{c}	15.144^{bcdefg}	29.639^{ghijk}	0.547^{cde}	0.131^{defg}	0.235^{bcde}	0.366^{cdef}	0.016^{cdefg}	0.042^{ghij}	9.222^{g}
Jarneli	96.296^{b}	14.117^{defgh}	37.694^{ab}	0.393^{l}	0.145^{bcd}	0.280^{ab}	0.425^{b}	0.018^{bcd}	0.054^{ab}	10.000^{c}
Jhini	98.148^{a}	14.372^{defgh}	24.728^{mno}	0.591^{bc}	0.117^{ghij}	0.212^{cdefg}	0.330^{fghi}	0.014^{efgh}	0.041^{ghij}	7.000^{r}
Jungey	94.444^{c}	13.861^{efgh}	26.944^{jklmn}	0.551^{cb}	0.126^{efg}	0.208^{cdefg}	0.334^{efgh}	0.013^{ghi}	0.042^{ghij}	7.556^{o}
Jungey Kanchi	90.741^{e}	15.400^{abcdef}	27.694^{ijklm}	0.587^{bc}	0.100^{jkl}	0.159^{fghi}	0.259^{kl}	0.015^{defgh}	0.039^{j}	7.444^{p}
Kalo Jhinuwa	96.296^{b}	14.522^{defgh}	26.594^{klmn}	0.557^{bcd}	0.113^{ghijk}	0.146^{hi}	0.258^{kl}	0.013^{ghi}	0.045^{defgh}	7.944^{m}
Kalokattey	94.444^{c}	14.239^{defgh}	27.789^{hijklm}	0.529^{cdef}	0.100^{jkl}	0.176^{efghi}	0.276^{ijkl}	0.015^{defg}	0.045^{efghi}	7.556^{o}
Kamal	94.444^{c}	16.906^{ab}	28.211^{hijkl}	0.621^{ab}	0.116^{ghij}	0.160^{fghi}	0.275^{ijkl}	0.014^{fgh}	0.040^{hij}	7.778^{n}
Kattikey	98.148^{a}	14.361^{defgh}	33.383^{cdef}	0.438^{ijkl}	0.105^{ijkl}	0.218^{bcdef}	0.323^{fghij}	0.017^{bcde}	0.050^{bcde}	9.111^{h}
Krishnabeli	88.889^{f}	14.550^{defgh}	27.889^{hijklm}	0.527^{cdefg}	0.125^{efgh}	0.166^{fghi}	0.291^{hijk}	0.013^{ghi}	0.039^{j}	8.722^{j}
Manamurey	88.889^{f}	17.250^{a}	38.694^{a}	0.462^{ghijk}	0.179^{a}	0.303^{a}	0.483^{a}	0.020^{ab}	0.057^{a}	9.944^{c}
Mansara	94.444^{c}	14.283^{defgh}	35.200^{bc}	0.419^{jkl}	0.163^{ab}	0.266^{abc}	0.430^{ab}	0.015^{defg}	0.051^{bc}	10.611^{a}
Nouley Dalley	92.593^{d}	13.689^{fgh}	27.856^{hijklm}	0.525^{cdefg}	0.123^{fghi}	0.213^{cdefg}	0.336^{efgh}	0.013^{ghi}	0.039^{hij}	9.833^{d}
Pahele	96.296^{b}	15.633^{abcde}	34.511^{bcd}	0.471^{fghijk}	0.121^{ghi}	0.233^{bcde}	0.354^{cdefg}	0.019^{abc}	0.053^{ab}	10.333^{b}
Pathijharey	90.741^{e}	13.156^{h}	23.889^{no}	0.580^{bc}	0.118^{ghij}	0.182^{efghi}	0.300^{ghijk}	0.016^{defg}	0.039^{j}	7.333^{q}
Rambilash	92.593^{d}	16.572^{abc}	32.950^{cdefg}	0.506^{defgh}	0.149^{bcd}	0.258^{abcd}	0.407^{bc}	0.016^{cdefg}	0.049^{bcdef}	9.556^{e}
Seto Dalley	85.185g	15.728^{abcd}	33.828^{cde}	0.48^{efghij}	0.156^{bc}	0.248^{abcd}	0.405^{bcd}	0.021^{a}	0.051^{bcd}	9.333^{f}
F-test	**	***	***	***	***	***	***	***	***	***
Mean	93.259	14.829	30.377	0.515	0.125	0.210	0.335	0.0157	0.0444	8.733
LSD	0.0417	1.858	3.332	0.0665	0.019	0.0624	0.0551	0.003	0.00589	0.0838
CV (%)	9.679	13.453	11.777	13.852	16.397	31.905	17.64	21.124	14.240	1.030
SEM	0.820	0.232	0.863	0.014	0.004	0.009	0.012	0.0005	0.0012	0.216
Factor B: Moisture condition										
Conditions	GP	RL	SL	RL/SL	FRW	FSW	TPW	RDW	SDW	RN
D1	93.111^{a}	15.086^{b}	31.224^{b}	0.504^{b}	0.123^{b}	0.205^{b}	0.328^{b}	0.016^{b}	0.046^{b}	8.033^{b}
D2	94.000^{a}	16.33^{a}	25.857^{c}	0.649^{a}	0.099^{c}	0.157^{c}	0.257^{c}	0.013^{c}	0.036^{c}	6.166^{c}
S	92.666^{a}	13.069^{c}	34.052^{a}	0.391^{c}	0.152^{a}	0.267^{a}	0.419^{a}	0.017^{a}	0.049^{a}	12.00^{a}
F test	NS	***	***	***	***	***	***	***	***	***
Mean	93.259	14.829	30.377	0.515	0.124	0.210	0.335	0.157	0.04441	8.733
LSD	2.912	0.643	1.154	0.023	0.006	0.021	0.019	0.001	0.00204	0.0290
CV (%)	9.679	13.452	11.777	13.852	16.397	31.905	17.647	21.124	14.240	1.030
SEM	0.391	0.950	2.403	0.074	0.015	0.031	0.046	0.001	0.004	1.719
Interaction (A*B)	*	NS	***	**	***	***	***	***	***	NS

Note: LSD: Least Significant Difference; CV: Coefficient of Variation; SEM: Standard Error of Mean *, **, and *** denote significance at 5%, 1%, and 0.1%, respectively, whereas NS denotes non-significant. $^{a\text{-}r}$ means with the same set of letters are not significantly different. GP: Germination percentage; RL: Root length; SL: Shoot length; RL/SL: Root shoot ratio; FRW: Fresh root weight; FSW: Fresh shoot weight; TPW: Total plant weight; RDW: Root dry weight; SDW: Shoot dry weight; RN: Root number.

In contrast, Notable variability in root length was observed across the three moisture conditions: S (13.069), D1 (15.086), and D2 (16.333) However, the interaction between landraces and moisture conditions was found to be statistically non-significant. The greater root length observed under drought stress aligns with the findings of Kaysar et al. (2023).

3.2.3. Shoot length

Landraces displayed notable variations in shoot length. As presented in Table 3, Manamurey showed the highest shoot length with a mean value of 38.694 cm, which was statistically similar to Jarneli and Darmali. Conversely, Dalley Masino recorded the shortest shoot length, with a mean value of 23.239 cm. Similarly, there was a statistically significant interaction between landraces and moisture conditions. Under saturated conditions, Manamurey exhibited the longest shoot length of 43.28 cm, followed by Mansara (41.92), Jarneli (40.7), Seto Dalley (40.37), and Himali (39.25). At 100% field capacity (FC), Jarneli achieved the greatest shoot length with a mean value of 42.65 cm, statistically comparable to Darmali (42.5), Manamurey (41.35), and Pahele (40.45). Under drought conditions at 60% FC, Rambilash demonstrated the highest shoot length with a mean value of 32 cm, which was statistically similar to Manamurey (31.45), Mansara (30.48), and Aangha (30.42). These findings align with Patel et al. (2021), who reported reduced shoot lengths under drought stress.

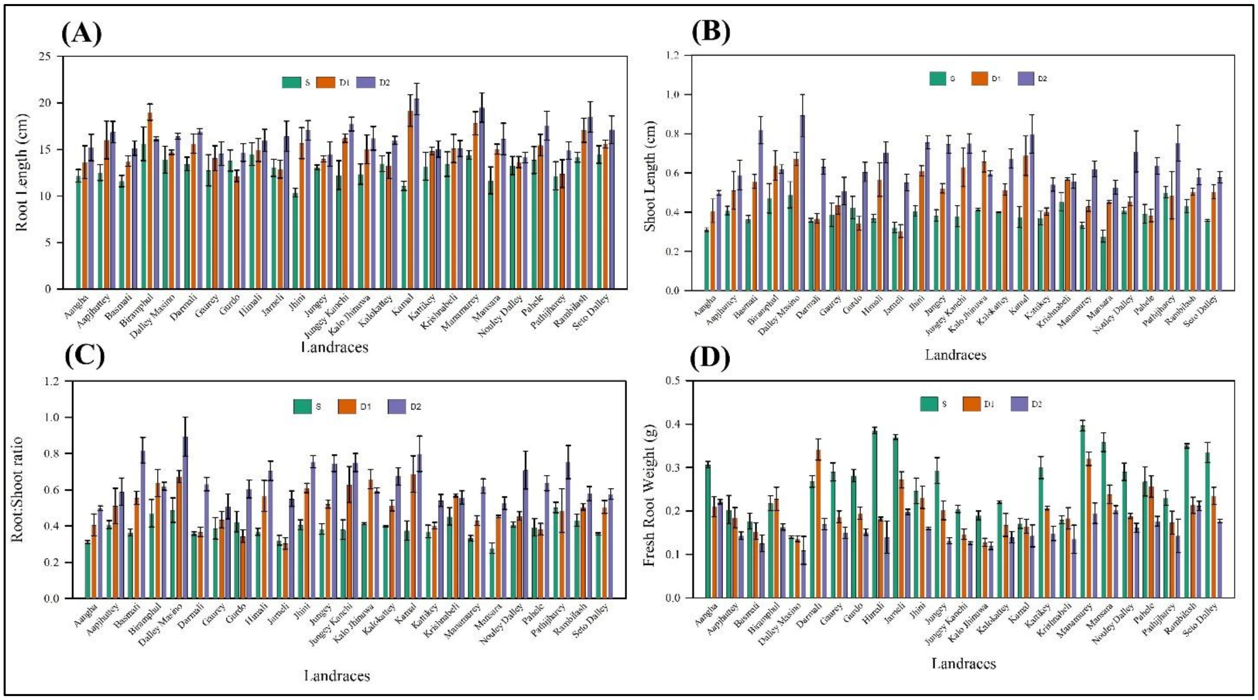

Figure 2. (A) Mean performance comparison of 25 rice landraces in different moisture conditions regarding root length. Interaction is non-significant. (B) Interaction effect between landraces and moisture condition regarding shoot length. (C)Interaction effect between landraces and moisture conditions regarding root: shoot ratio. (D) Interaction effect between landraces and moisture conditions regarding fresh root weight.

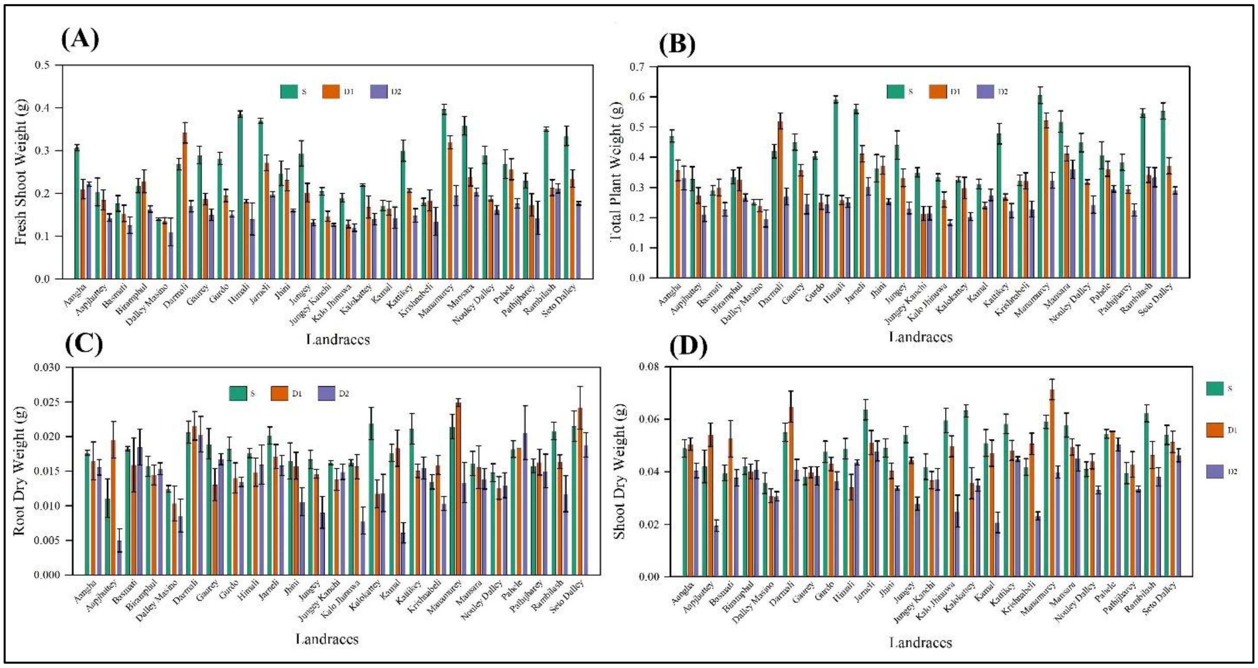

Figure 3. Interaction effect between landraces and moisture conditions in terms of (A) fresh shoot weight, (B) total plant weight, (C) root dry weight, and (D) shoot dry weight.

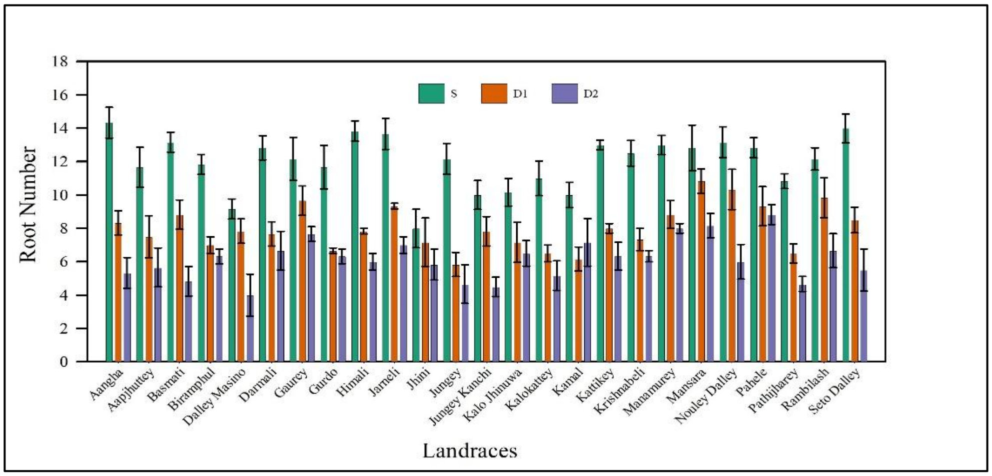

Figure 4. Mean performance comparison of rice landraces in different moisture conditions regarding root number. Interaction is non-significant.

These findings suggest that drought stress promotes the development of longer roots in rice landraces, thereby increasing the root-to-shoot ratio. This observation aligns with the results reported by Hou et al. (2022)

3.2.5. Fresh root weight

Substantial variations in fresh root weight were observed among the different rice landraces, as shown in Table 3. Similarly, the interaction between landraces and moisture conditions was also statistically significant. Seto Dalley exhibited the highest fresh root weight in saturated conditions, with a mean value of 0.218 g. This result was statistically comparable to Jarneli (0.19), Himali (0.206), Rambilash (0.196), and Manamurey (0.208).

At 100% field capacity (FC), Manamurey was found to incur the greatest fresh root weight, with a mean value of 0.201 g, statistically similar to Darmali (0.178) and Mansara (0.173). Under drought conditions (60% FC), Mansara demonstrated the highest fresh root weight, with a mean value of 0.156 g, statistically consistent with Kamal (0.131) and Manamurey (0.128). As presented in Table 3, Manamurey recorded the highest overall fresh root weight, with a mean value of 0.179 g, statistically comparable to Mansara (0.163). These results align with the findings of Dien et al. (2017), who reported that plants grown under saturated conditions exhibit greater fresh root weight compared to those subjected to drought stress

3.2.6. Fresh shoot weight

Considerable variations were observed for fresh shoot weight among tested landraces ranging from 0.303g (Manamurey) to 0.128g (Dalley Masino), as shown in Table 3. Similarly, the interaction between landraces and moisture conditions was statistically significant. Under saturated conditions, Manamurey exhibited the highest fresh shoot weight, with a mean value of 0.396 g, statistically at par with Himali (0.385) and Jarneli (0.37).

At 100% field capacity (FC), Darmali recorded the highest fresh shoot weight with a mean value of 0.341 g, which was statistically similar to Manamurey (0.32). Under drought conditions (60% FC), Aangha demonstrated the greatest fresh shoot weight with a mean value of 0.221 g, statistically similar to Rambilash (0.211) and Mansara (0.203). These findings align with Saha et al. (2019), who reported a reduction in fresh shoot weight under drought conditions.

3.2.7. Total plant weight

The interaction between landraces and moisture conditions was statistically significant. Manamurey achieved the highest total plant weight under saturated conditions and 100% FC with mean values of 0.605g and 0.521g respectively.

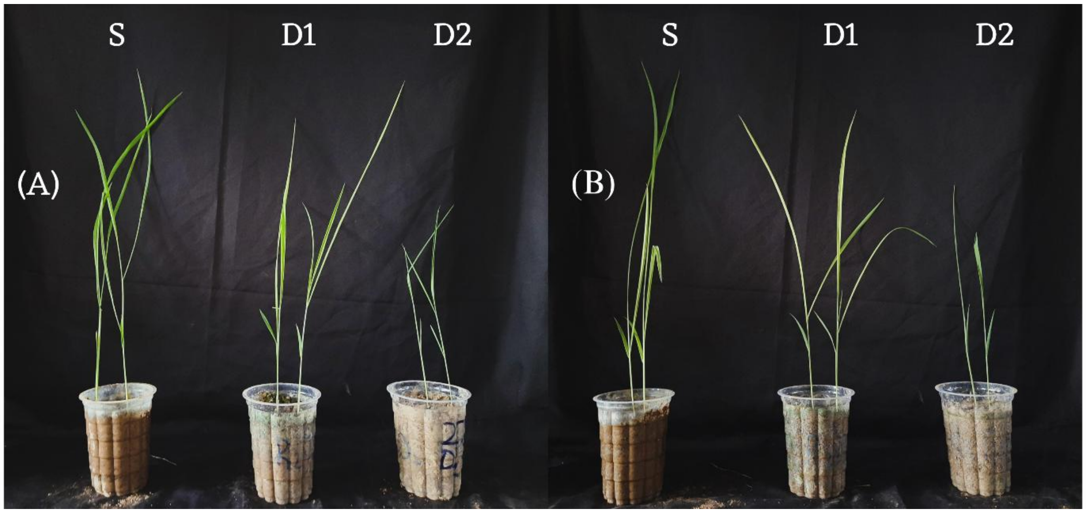

Figure 5. Photographs illustrating the response of landraces in different moisture conditions at 21 days. (A) Nouley Dalley (B) Basmati. S, D1, and D2 comprised Saturated, 100% FC, and 60% FC respectively.

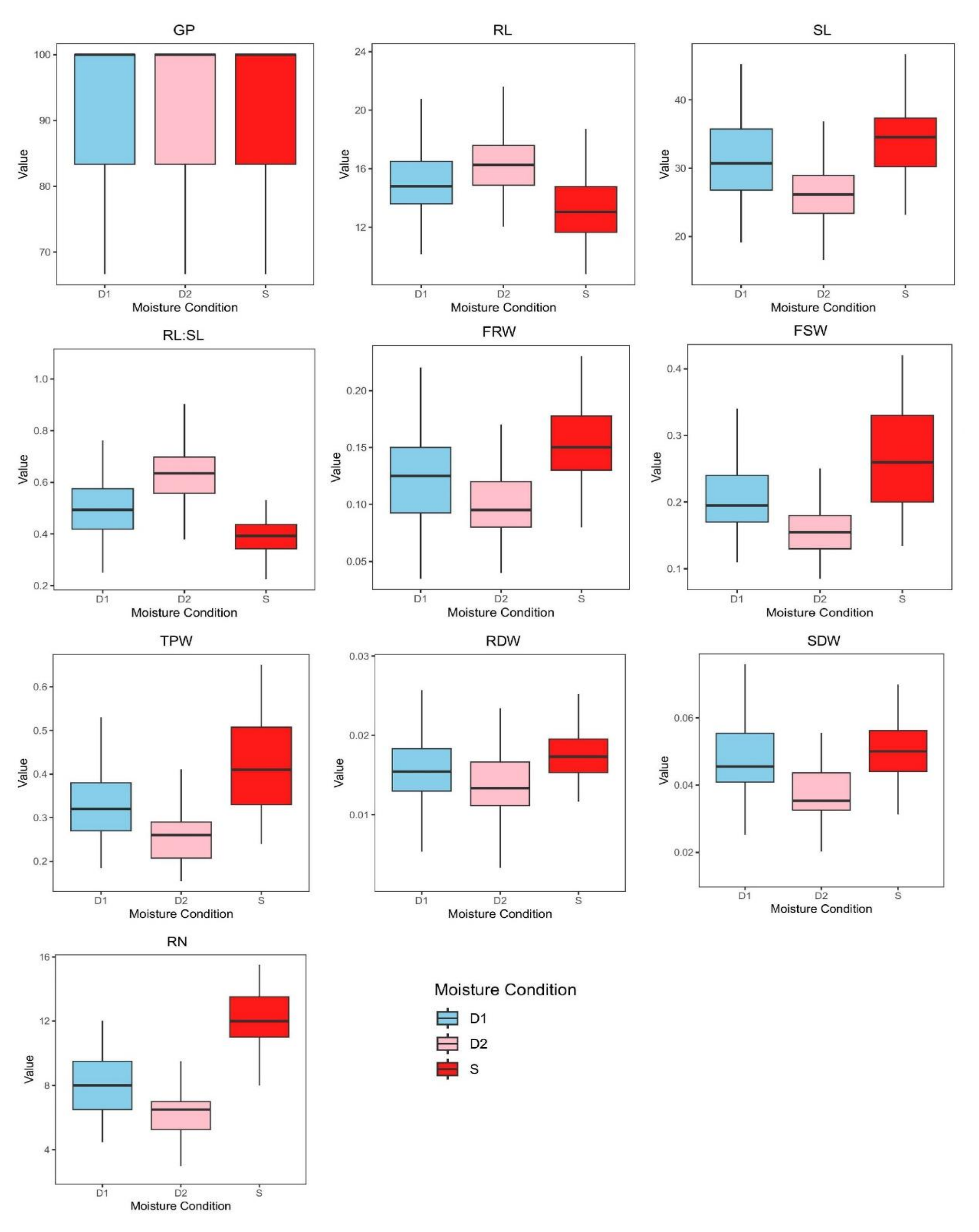

Figure 6. Box and whisker charts showing variation among landraces for the tested quantitative traits. The blue box, pink box, and red box explain the boxplot value under 100% FC, 60% FC, and saturated condition, respectively. The horizontal line inside each boxplot represents the median value. GP: Germination percentage; RL: Root length; SL: Shoot length; RL/SL: Root shoot ratio; FRW: Fresh root weight; FSW: Fresh shoot weight; TPW: Total plant weight; RDW: Root dry weight; SDW: Shoot dry weight; RN: Root number.

Under drought conditions (60% FC), Mansara exhibited the highest plant weight, with a mean value of 0.36 g, statistically consistent with Rambilash (0.333), Aangha (0.331), and Manamurey (0.323). Regarding main effects, significant differences were observed in total plant weight among the landraces, with Manamurey recording the highest mean value at 0.483 g. Similarly, Notable variability in total plant weight was also evident across the three moisture conditions, with mean values of 0.419 g under saturated conditions, 0.328 g at 100% FC, and 0.257 g under 60% FC.

3.2.8. Root dry weight

Significant differences in root dry weight were observed among tested landraces, with landraces demonstrating a considerable interaction with moisture conditions, as depicted in Table 2. Under saturated conditions, Kalokattey recorded the highest root dry weight, with a mean value of 0.0218 g, which was statistically at par with Seto Dalley (0.0215), Manamurey (0.0214), and Kattikey (0.0211). Similarly, Manamurey exhibited the highest root dry weight, under 100% FC with a mean value of 0.0249 g. Similarly, Pahele demonstrated the greatest root dry weight, under drought conditions (60% FC) with a mean value of 0.0205 g. As shown in Table 3, Seto Dalley and Darmali exhibited the highest overall root dry weight, with a mean value of 0.021 g, statistically similar to Manamurey (0.02) and Pahele (0.019). Conversely, Significant variability was also observed across the moisture conditions, with mean root dry weights of 0.017 g under saturated conditions, 0.016 g at 100% FC, and 0.013 g under 60% FC. The reduction in root dry weight with increasing water stress aligns with the findings of Patel et al. (2021)

3.2.9. Shoot dry weight

Noticeable variations in shoot dry weight were observed among the different landraces, as detailed in Table 2. Manamurey exhibited the highest shoot dry weight, with a mean value of 0.057 g, whereas Dalley Masino displayed a comparatively lower weight of 0.032 g. Similarly, the interaction between landraces and moisture conditions was also statistically significant. As illustrated in Figure 3D, under saturated conditions, Jarneli exhibited the highest shoot dry weight of 0.0636 g, which was statistically comparable to Kalokattey (0.0632) and Rambilash (0.0622). At 100% field capacity (FC), Manamurey recorded the greatest shoot dry weight, with a mean value of 0.0713 g. In addition, Pahele displayed the highest shoot dry weight, under drought conditions (60% FC) with a mean value of 0.0503 g. These findings align with those of Saha et al. (2019) and Patel et al. (2021), who reported significant reductions in shoot dry matter under stress conditions.

3.2.10 Root number

Significant differences in root number were observed among the 25 rice landraces, with landraces showing a non-significant interaction with moisture conditions.Among the 25 landraces, Mansara demonstrated the greatest overall root number, with a mean value of 10.611, which was significantly higher than the other landraces. In contrast, Dalley Masino and Jhini exhibited the lowest root numbers, both with a mean value of 7.00. Significant variability in root number was also evident across the three moisture conditions, with mean values of 12.00 under saturated conditions, 8.033 at 100% FC, and 6.166 under 60% FC. These findings are consistent with Kaysar et al. (2023), who reported a higher root count in rice landraces under saturated conditions.

3.3. Correlation between the traits

Correlation (*r*-value) was determined using a correlation matrix to identify the inter-relationship of studied traits. The significant r values among different traits varied from 32, 29, and 25 under Saturated, 100% FC, and 60% FC respectively.

Root length showed a significant positive correlation with root: shoot ratio (0.49, 0.54) under saturated and 100% FC while, a positive correlation was observed with shoot length (0.32), root dry weight (0.29), and root number (0.33) under saturated condition and fresh shoot weight (0.25) and root dry weight (0.32) in 100% FC. Under 60% FC, it showed a positive correlation with shoot length (0.36), root: shoot ratio (0.31), fresh root weight (0.36), and total plant weight (0.26). However, no significant correlation was observed for root length and root number in 100 and 60% FC.

Notably, shoot length scored a highly significant positive correlation with all the traits in a saturated condition, except fresh root weight in 100% FC and root dry weight in 60% FC. Likewise, the root: shoot ratio was negatively correlated with shoot length under all conditions (-0.65, -0.77, -0.75). There was a significant correlation observed between root: shoot ratio with fresh shoot weight (-0.27, -0.47, -0.45) including shoot dry weight (-0.40) under saturated condition, total plant weight (-0.42) under 100% FC and root number (-0.38) in case of 60% FC.

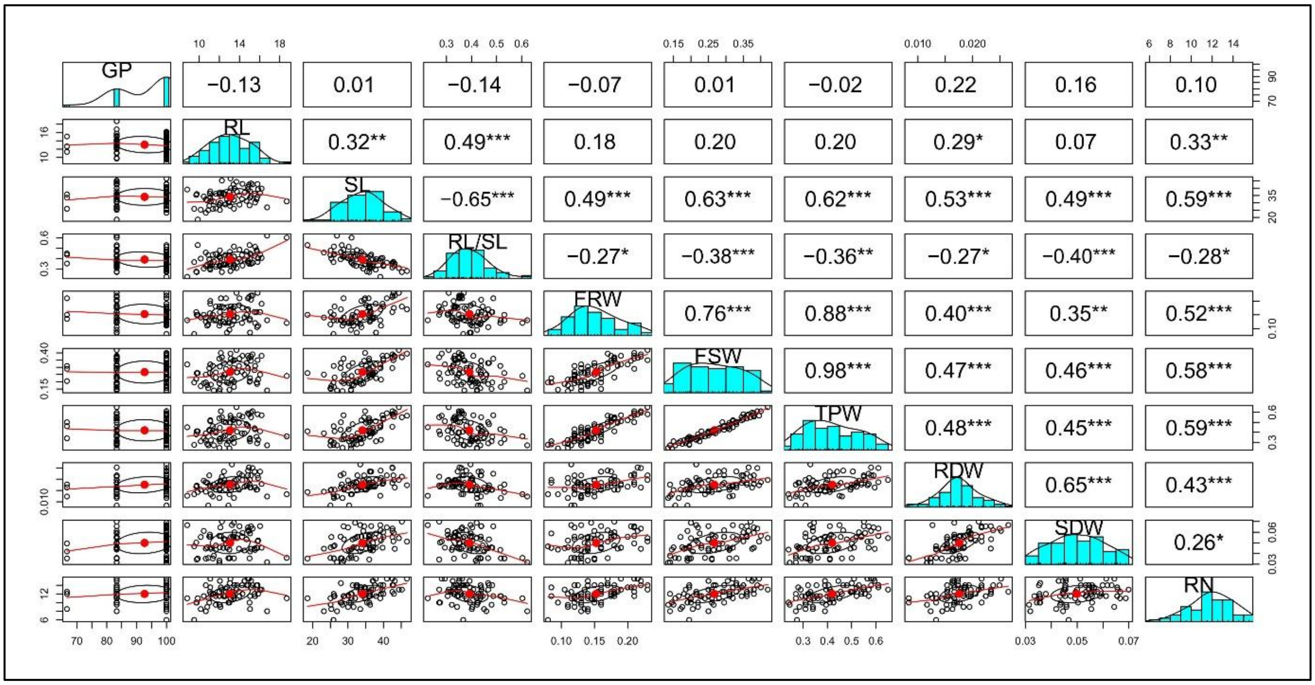

Figure 7. Correlation between studied traits under saturated condition. *, **, and *** denote significance at 5%, 1%, and 0.1%, respectively. GP: germination percentage; RL: root length; SL: shoot length; RL/SL: root shoot ratio; FRW: fresh root weight; FSW: fresh shoot weight; TPW: total plant weight; RDW: root dry weight; SDW: shoot dry weight; RN: root number.

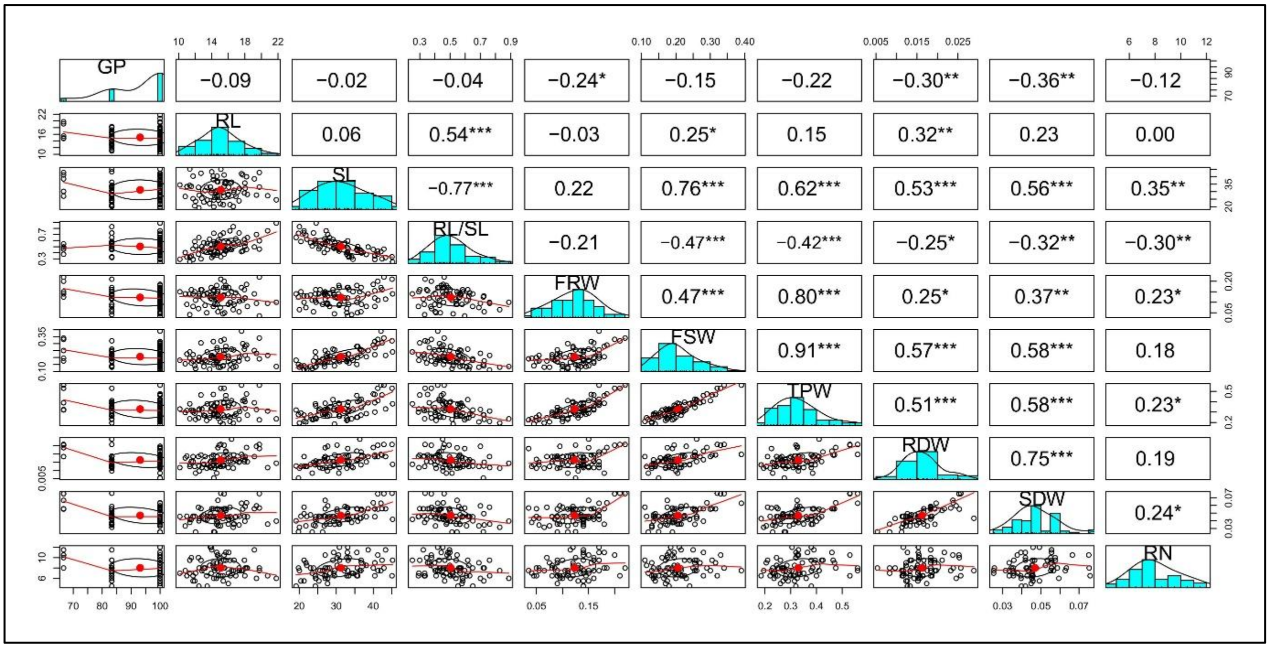

Figure 8. Correlation between studied traits under 100% FC. *, **, and *** denote significance at 5%, 1%, and 0.1%, respectively. GP: germination percentage; RL: root length; SL: shoot length; RL/SL: root shoot ratio; FRW: fresh root weight; FSW: fresh shoot weight; TPW: total plant weight; RDW: root dry weight; SDW: shoot dry weight; RN: root number.

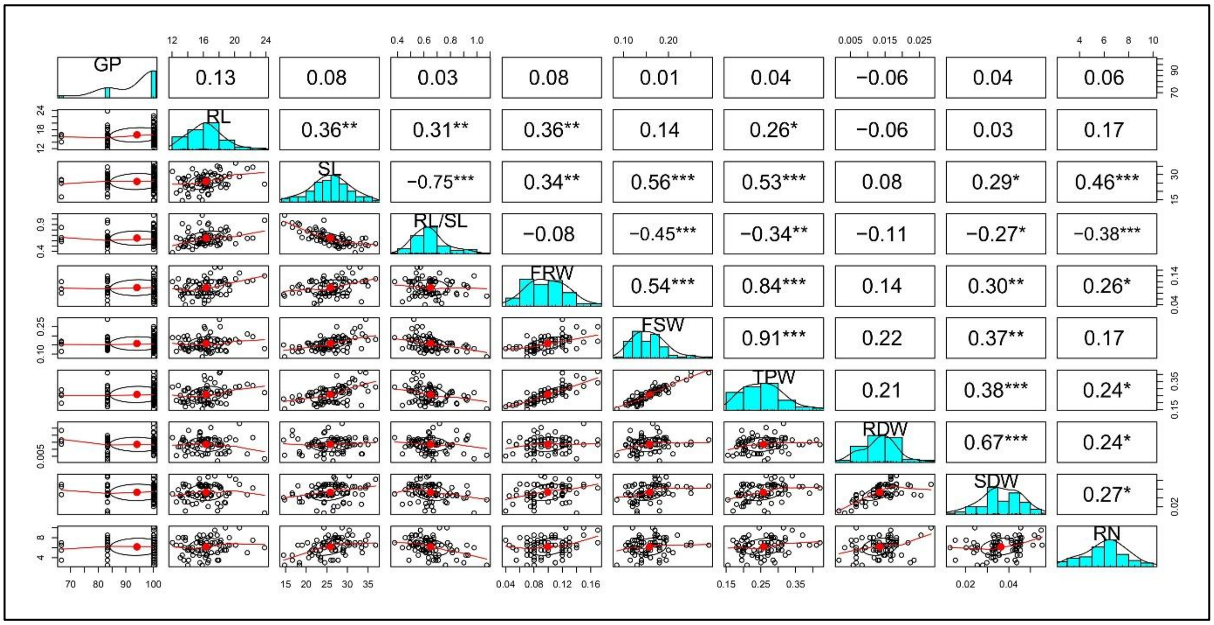

Figure 9. Correlation between studied traits under 60% FC. *, **, and *** denote significance at 5%, 1%, and 0.1%, respectively. GP: germination percentage; RL: root length; SL: shoot length; RL/SL: root shoot ratio; FRW: fresh root weight; FSW: fresh shoot weight; TPW: total plant weight; RDW: root dry weight; SDW: shoot dry weight; RN: root number.

However, a negative correlation was observed with fresh root weight (-0.27), total plant weight (-0.36), root dry weight (-0.27, -0.25), and root number (-0.28, -0.30) under saturated condition and 100% FC while, total plant weight (-0.34) and shoot dry weight (-0.27, -0.32) under 60% FC and 100% FC respectively. Fresh root weight was significantly correlated with all the traits except root length in saturated condition and root length, shoot length, and root: shoot ratio in 100% FC along with root dry weight and root: shoot ratio under 60% FC condition. Fresh shoot weight showed significant positive correlation with total plant weight (0.98, 0.91, 0.91), root dry weight (0.47, 0.57), shoot dry weight (0.46, 0.58, 0.37), and root number (0.58) under saturated, 100% FC and 60% FC respectively. There was a positive correlation of total plant weight with root dry weight (0.48, 0.51), shoot dry weight (0.45, 0.58, 0.38), and root number (0.59, 0.23, 0.24). Root dry weight showed a highly significant correlation with shoot dry weight (0.65, 0.75, 0.67) under all conditions while a positive correlation was observed with root number (0.43, 0.24) under saturated and 60% FC conditions. Shoot dry weight positively correlated with root number (0.26, 0.24, 0.27) at all moisture conditions.

3.4. Principal component analysis

The 25 rice landraces were studied through principal component analysis (PCA) biplot, where the landraces and traits are mapped based on their relationships across the first two principal components (PC1, PC2). Under the 60% FC, both axes of components (PC1 and PC2) explain a significant portion of the variance in the dataset with an eigenvalue greater than one. PC1 accounted for 46.7% while, PC2 accounted for 17.4%, totaling 64.1% of the variability (Supplementary Table 1). Furthermore, traits like RL, SL, FRW, FSW, TPW, and RN are highly associated with PC1 whereas, RDW and SDW are highly associated with PC2 (Supplementary Table 4). Thus, interpreting Figure 10 (C), Jungey Kanchi, Basmati, and Kamal are landraces located far from the origin, representing extreme or distinct characteristics compared to other landraces. Likewise, traits like FRW, TPW, FSW, RN, and SL are closely aligned (an acute angle), indicating that these traits are strongly and positively correlated.

Similarly, under 100% FC and Saturated conditions, both components explain a total of 67.7% and 70.9% of the variability in the dataset with an eigenvalue greater than one (Supplementary Table 1). Under 100% FC, traits RL, SL, FRW, FSW, TPW, RDW, and SDW are highly associated with PC1 whereas, GP and RN are highly associated with PC2 (Supplementary Table 3). Likewise, under saturated conditions, all traits except GP and SDW are associated with PC2 (Supplementary Table 2). Thus, interpreting Figure 10 (A, B) traits like RDW, SDW, FRW, FSW, TPW, RN, and SL are aligned (an acute angle) closely suggesting a significant positive correlation. This implies that landraces with higher shoot and root weight also tend to have higher root numbers and shoot length. Under all conditions, RL/SL have arrows pointing opposite to FSW, TPW, and SL, indicating a negative correlation (an obtuse angle) which means that landraces with higher shoot biomass (FSW, TPW, SL) tend to have lower root-to-shoot ratios. Interestingly, RL and RN have almost perpendicular arrows, indicating little to no correlation between these two traits (Figure 10 (B)). Similarly, the PCA-Biplot shown in Figure 10 (D), indicates the

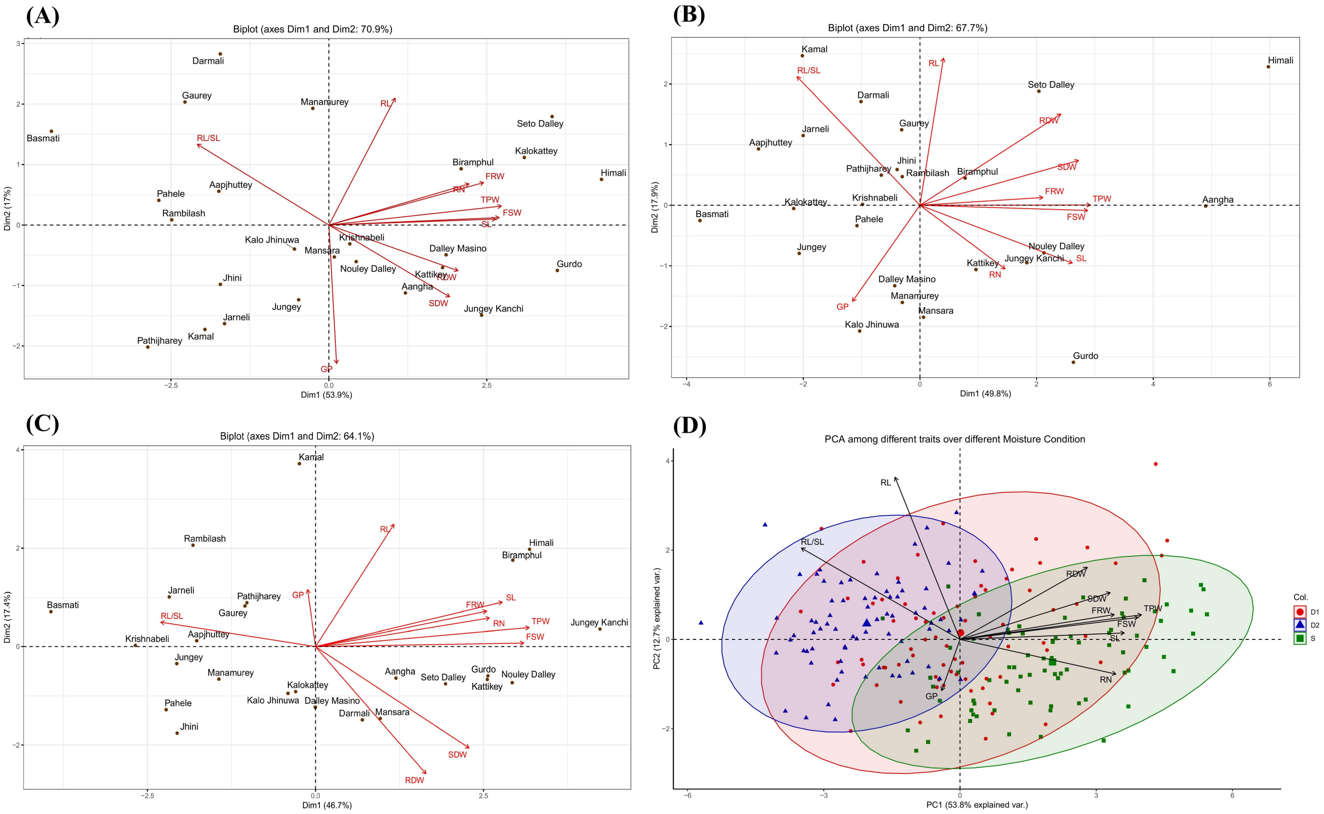

Figure 10. (A) PCA Biplot analysis for saturated condition, (B) PCA Biplot analysis for 100% FC (C) PCA Biplot analysis for 60% FC, and (D) PCA among studied traits over 60% FC, 100% FC, and saturated condition.

contribution of traits to overall variation in the dataset. PC1 explains more than half (53.8%) of the variance in the data while PC2 explains a smaller portion of the variance (12.7%) in the dataset. Together, PC1 and PC2 account for 66.5% of the total variation. Unlike the above PCA Biplots, traits like RDW, SDW, FRW, FSW, TPW, SL, and RN suggest a strong correlation between them (an acute angle). Likewise, RL/SL and RN were directed towards opposite directions, indicating a negative correlation (an obtuse angle). Traits with an arrow directed toward the environment indicate a strong association with that tested environment. Therefore, RL and RL/SL are directed towards the D2 condition (60% FC), suggesting these traits positively correlate with 60% FC. Traits like: RDW, SDW, FRW, TPW, FSW, SL, and RN are directed towards D1 (100% FC) and saturated condition suggesting these traits have a positive association with this moisture condition. However, GP falls in all moisture conditions which indicates that GP doesn't have discrimination over any moisture conditions

4. Discussion

The performance of rice landraces for drought-tolerant traits is best assessed by analyzing the variability among the tested landraces based on the evaluated traits. Numerous studies have highlighted agro-morphological diversification in rice landraces, particularly regarding vegetative traits (Mishra et al. 2018; Ndikuryayo et al. 2023). In nearly every trait under study, the

ANOVA results revealed significant differences among the genotypes under S, D1, and D2 conditions, providing strong evidence of genetic variability within the studied landraces.

Rice genotypes exhibit varied responses to drought stress, with some showing enhanced root growth as an adaptive mechanism for drought avoidance. Under drought stress, roots tend to grow toward areas with higher water content, resulting in deeper, thinner root systems that increase the total absorption surface area, thereby favoring the uptake of water and nutrients (Kou et al. 2022). However, extreme drought conditions can inhibit secondary root growth and cause primary roots to thicken with a significant reduction in branching, which ultimately leads to a decline in overall root numbers (Hassan et al. 2023). This deeper root system development is associated with increased abscisic acid (ABA) concentration in roots (Panda et al. 2021).

The signaling of ABA during water stress induces auxin biosynthesis, modifying root morphology and architecture to enhance water uptake (Kalra et al. 2024). Consequently, drought stress may promote longer roots in rice landraces, increasing root: shoot ratio (Hou et al. 2022). Studies have shown that rice genotypes exhibit varying levels of DRO1 expression, which plays a critical role in auxin signaling required for root development and gravitropic responses under drought stress (Zubaer et al. 2007; Uga et al. 2015). The observed increase in root length under drought conditions can be attributed to enhanced expression and functional variation of the DRO1 gene, integral to drought adaptation mechanisms (Uga et al. 2013).

The root: shoot ratio is a vital indicator of drought tolerance, reflecting a plant's ability to allocate resources preferentially to its root system to maximize water uptake (Xu et al. 2015). A higher root: shoot ratio signifies a strategic shift toward root development at the expense of shoot growth, enabling the plant to exploit available moisture under drought stress (Takahashi et al. 2020). This adaptive response conserves resources by slowing above-ground growth while allowing roots to explore deeper soil layers for water and nutrients (Kou et al. 2022). As illustrated in Figure 2(C), the highest root: shoot ratio was observed in the landrace Dalley Masino at 60% field capacity, suggesting its superior drought adaptation capacity. Genotypes exhibiting favorable root: shoot ratios tend to maintain physiological stability and achieve better yield performance under water-limited conditions (Hassan et al. 2023). Although plants may initially prioritize root biomass development to access moisture, as the stress persists, resource allocation may shift toward maintaining existing shoots rather than further root expansion (Sainju et al. 2017). This adaptive strategy reflects a trade-off, wherein shoot biomass increases at the expense of root growth, highlighting the delicate balance that plants maintain between root and shoot development in response to environmental stresses (Numajiri et al. 2024).

Under saturated conditions, the highest shoot biomass, including fresh shoot weight, total plant weight, and shoot length, can be attributed to the enhanced ability of plants to absorb more nutrients, which are more readily available in moist conditions, allowing for optimal shoot growth (Ros et al. 2003). As shown in Figure 3(A), the findings indicate that under saturated conditions, the rice landrace Manamurey exhibited the highest shoot fresh weight compared to other landraces. This outcome may be associated with the superior capacity of this landrace to absorb water and nutrients, along with increased stomatal conductance, which facilitates enhanced photosynthesis (Kamarudin et al. 2018). Conversely, under drought conditions, chemical and hydraulic signals from drying roots regulate stomatal closure, leading to reduced CO2 assimilation and net photosynthetic rates, which in turn contribute to a decline in shoot biomass (Hasanuzzaman et al. 2013). These findings align with Zubaer et al. (2007), who observed that shoot dry matter in Aman rice genotypes decreased with increasing water stress.

In the present study, a negative correlation was observed between shoot biomass parameters (e.g., fresh shoot weight, total plant weight, and shoot length) and the root: shoot ratio in the evaluated landraces. This phenomenon may be attributed to altered carbohydrate partitioning in rice seedlings, favoring either root or shoot growth depending on the prevailing conditions (Bui et al. 2019). Drought stress has been reported to increase the proportion of soluble sugars and starch in roots while reducing their levels in stems, driven by heightened activity of root invertase and leaf sucrose-phosphate synthase (Xu et al. 2015). This resource reallocation supports root development, enhancing water uptake efficiency and contributing to a higher root: shoot ratio. Such resource trade-offs underscore the intricate physiological mechanisms that plants employ to adapt to stress and ensure survival, providing critical insights for developing drought-resilient rice genotypes.

The results indicate that certain cultivars exhibit elongated roots without a proportional increase in root number, resulting in a negative correlation between these traits. This elongation occurred at the expense of root number, as resources are diverted toward extending existing roots rather than producing new ones, which can be seen as an adaptive mechanism for certain genotypes under drought conditions (Yang et al. 2022). Deeper roots enable plants to access moisture in lower soil layers, optimizing water absorption and enhancing drought resilience (Huang et al. 2019; Shafi et al. 2023). Moreover, several QTLs have been reported as common between root and shoot traits in two rice subgroups, indica, and japonica, suggesting a correlation between root and shoot weight, which indicates a genetic basis for their interdependence (Zhao et al. 2019). The presence of pleiotropic QTLs suggests that certain genetic factors regulate the growth of both root and shoot systems simultaneously, promoting coordinated biomass accumulation.

Traits such as longer root length (Kim et al. 2020; Zhao et al. 2019), minimal reduction in shoot length (Das et al. 2024; Islam et al. 2022), and a higher root: shoot ratio (Hussain et al. 2022) are critical indicators of drought tolerance. Based on these parameters, Manamurey emerges as a promising candidate for thriving in water-scarce conditions at the seedling stage. However, as this study primarily focuses on the seedling stage, it is essential to extend the analysis to vegetative and reproductive traits for a more comprehensive understanding of drought tolerance. The selection of drought-tolerant genotypes must also consider other factors, including biochemical responses, anatomical adaptations, environmental influences, extensive field trials, and overall plant performance. While Manamurey exhibits considerable potential, further evaluations under field conditions are necessary. Future research should prioritize assessing its ability to withstand reproductive-stage drought stress, which is crucial for its integration into breeding programs aimed at enhancing drought resistance.

5. Conclusion

Drought stress significantly influences the emergence and growth of rice roots and shoots, making it crucial to identify specific traits that could improve drought resilience. This study highlights the significant variability in root and shoot traits among rice landraces under varying moisture conditions, emphasizing the importance of evaluating germplasm for drought tolerance at the seedling stage. Root length and root: shoot ratio emerged as key indicators of drought resilience, particularly under 60% field capacity, while shoot length, fresh weight, dry weight, and root number performed better under saturated conditions. Correlation analysis revealed trade-offs in resource allocation between root and shoot, owing to the negative correlation between the root: shoot ratio and root number under drought stress. These findings underscore the potential of root traits as critical traits that can be utilized as selection criteria for drought tolerance in the seedling stage and highlight the need for further evaluations at later growth stages to validate their role in enhancing drought resilience.

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