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## Evaluation of genotypic variation in seedling vigour traits under different seed priming treatments in rice (*Oryza sativa* L.)

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### Abstract

Seedling growth traits and biomass accumulation are key determinants of successful crop establishment in rice, particularly under water-limited rice production systems. Seed priming has been studied as an effective strategy for improving seedling growth; however, information on genotypic variation in priming response remains limited. Therefore, the present investigation aimed to evaluate the effects of different seed priming treatments on seedling growth traits and biomass accumulation in diverse rice genotypes and to identify superior rice genotypes. A pot experiment was conducted using 25 diverse rice genotypes and four seed priming treatments, including Control, Hydropriming, 2% CaHPO<sub>4</sub> (Calcium Hydrogen Phosphate) and 14% KCl (Potassium Chloride). Seedling traits including shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight were recorded at 45 days after sowing (DAS).

Analysis of variance revealed significant effects of genotype, priming treatment, and genotype × treatment interaction for all measured traits, indicating differential responses among genotypes. Among the treatments, KCl priming consistently produced the greatest improvement in seedling growth and biomass accumulation. Correlation analysis showed strong positive relationships among growth traits and biomass accumulation, suggesting coordinated seedling development. Hierarchical clustering and principal component analyses further revealed substantial phenotypic diversity and identified Kapurkali, Swara Samrudhi, Ratnasundari, Siddhagiri, and Raibhog as highly distinct and responsive genotypes to priming treatments and maintained superior seedling growth characteristics.

The findings indicate that KCl priming is an effective seed priming treatment for improving early seedling morphological traits and biomass accumulation in rice. The identified responsive genotypes may serve as valuable genetic resources for breeding programs aimed at improving seedling establishment and adaptation to water-limited rice cultivation systems.

**Keywords:** Rice, Seedling traits, Biomass accumulation, Seed priming, KCl priming, Hydropriming, Seedling vigour

### 1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide and serves as the primary staple food for more than half of the global population, particularly in Asia (Chauhan *et al.*, 2015; Sandhu *et al.*, 2021; Jang *et al.*, 2021; Zhang *et al.*, 2023; Negi *et al.*, 2024) [4, 23, 9, 25]. With continued population growth and economic development, the global demand for rice is expected to increase further, necessitating the development of high-yielding and climate-resilient production systems.

Early seedling traits are critical both physiologically and agronomically in contributing to successful crop establishment, especially in water-limited rice cultivation systems like direct-seeded rice (DSR) (Negi *et al.*, 2024; Maila *et al.*, 2025; Dey *et al.*, 2026) [18, 14, 7]. Early seedling establishment is facilitated by traits such as rapid, uniform germination, seedling emergence, mesocotyl elongation, vigorous vegetative growth, root development, and biomass accumulation. Vigorous seedlings are more efficient in acquiring water, nutrients, and light, thereby enhancing their ability to compete with weeds and tolerate environmental stresses such as heat, water, and osmotic stress (Anandan *et al.*, 2020; Bharamappanavara *et al.*, 2023) [1, 3].

Several component traits, including coleoptile length, chlorophyll content, root vigour, and early biomass production, collectively determine seedling establishment and subsequent crop performance (Dey *et al.*, 2026)<sup>[7]</sup>.

Despite its importance, early seedling traits have received limited attention as a direct selection criterion in conventional rice breeding programs due to their quantitative nature and complex genetic control (Dey *et al.*, 2026)<sup>[7]</sup>. This limitation is particularly relevant in less-water requiring systems, where rapid and uniform seedling establishment is essential for maximizing productivity and resource-use efficiency. A better understanding of the variability in seedling vigour traits among rice genotypes is therefore necessary to develop varieties better suited for DSR cultivation.

Among various approaches for improving early seedling establishment in rice crops, seed priming has emerged as an effective and economical technique. Priming treatments initiate pre-germinative metabolic activities, resulting in faster and more uniform germination and improved seedling growth. Hydropriming, in particular, has been reported to enhance root development, water uptake, and drought avoidance mechanisms, thereby improving seedling performance under moisture-limited conditions (Choi *et al.*, 2025)<sup>[5]</sup>. Potassium-based product such as KCl reported to be outstanding in improving seedling performance under crop establishment (Kushwaha *et al.*, 2018; Monajjem *et al.*, 2023; Wang *et al.*, 2022)<sup>[11, 16, 24]</sup>. Apart from that, calcium-based products like CaCl<sub>2</sub> and CaHPO<sub>4</sub> reported to enhance the seedling performance and vigour (Monajjem *et al.*, 2023; Wang *et al.*, 2022)<sup>[16, 24]</sup>. Since rice is highly susceptible to drought stress and osmotic stress during germination and early seedling growth because of its shallow root system and more water requirement, improved seedling establishment through seed priming can play a significant role in enhancing crop resilience under stress condition (Panda *et al.*, 2021; Choi *et al.*, 2025)<sup>[5, 20]</sup>. In addition to being an invaluable source of genetic variation, local landraces frequently have advantageous characteristics including longer mesocotyls, more robust and adaptable root systems, and better nutrient uptake efficiency (Puri *et al.*, 2010; Negi, 2025)<sup>[21, 19]</sup>. Therefore, studying regional landraces can reveal significant genetic variation that could be used to create better cultivars that are especially suited to DSR settings (Puri *et al.*, 2010)<sup>[21]</sup>. Because of their ecological sustainability in a variety of agroclimatic conditions and their capacity to support both ecological sustainability and climate-resilient rice production, local landraces (LLRs) present viable alternatives. Agronomic techniques like seed priming are essential for improving seedling emergence and early establishment in addition to genetic advancements (Farooq *et al.*, 2006; Mahajan *et al.*, 2011, Choudhary *et al.*, 2017; Khalid *et al.*, 2021)<sup>[8, 13, 6, 10]</sup>. However, the effectiveness of seed priming is often influenced by the genetic background of the genotype, resulting in differential responses among rice varieties. Hydropriming has been shown to improve seedling growth under limited soil moisture conditions, with a 1.2-fold increase in growth rate reported at 8-15% soil

moisture (Matsushima and Sakagami, 2013)<sup>[15]</sup>. However, the magnitude of this response varies among genotypes, suggesting that genetic factors strongly influence the effectiveness of priming treatments (Nakao *et al.*, 2020)<sup>[17]</sup>. However, there has been no discussion of how primed seed rice acquires drought tolerance during the heterotrophic period from sowing to young seedlings, especially the development of aboveground and underground organs and their drought tolerance.

Such genotype-specific variation highlights the need to evaluate diverse rice germplasm under different priming treatments to identify responsive genotypes and suitable priming strategies. Therefore, the present investigation was undertaken to assess genotypic variation in seedling growth traits under different seed priming treatments in rice seedlings. The specific objectives were to evaluate the role of different seed priming treatments on seedling vigour traits in rice and to identify the most effective priming treatment and rice genotypes for improved seedling establishment.

## 2. Materials and Methods

A diverse set of 25 rice (*Oryza sativa* L.) genotypes comprising local landraces, improved cultivars, and hybrids was used in the present study. Details of the genotypes and their sources are provided in Table 1. Four seed treatments evaluated were untreated seeds (control), hydropriming, 2% CaHPO<sub>4</sub> (calcium hydrogen phosphate) priming, and 14% KCl (potassium chloride) priming.

The experiment was conducted under pot conditions using a Factorial Completely Randomized Design (FCRD) with three replications. Seeds of each genotype were subjected to priming treatments by soaking in the treatment solutions for 24 hours. Following priming, the primed seeds were thoroughly rinsed with distilled water to remove any residual chemicals and air-dried at room temperature for 24 hours before sowing. The treated seeds were sown in pots (25 x 25 x 20.7 cm dimensions) filled with a 1:1 mixture of black and red soil. The whole experiment was maintained under uniform environmental and management conditions throughout the experimental period. 20 seeds were sown per pot, which were later thinned, and only 10 healthy seedlings were selected to evaluate seedling traits.

Seedling observations were recorded at 45 days after sowing (DAS). The evaluated shoot-related traits included SL (shoot length), SFW (shoot fresh weight), and SDW (shoot dry weight). Root-related traits included RL (root length), RFW (root fresh weight), and RDW (root dry weight).

Data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of genotype, priming treatment, and their interaction on seedling growth traits. Mean comparisons were performed at both  $P \leq 0.05$  and  $P \leq 0.01$  significance levels. R software version 4.5.1 was used for data analysis, multivariate principal component analysis, Pearson correlation analysis among seedling traits, hierarchical clustering of rice genotypes, scatter plots showing the relationship between shoot and root biomass, and violin-boxplots for seedling traits at 45 DAS.

**Table 1:** Rice genotypes used as experimental material in Experiment 3 under Objective 1

| Sr. No. | Genotypes      | Source                     |
|---------|----------------|----------------------------|
| 1       | Ratnasundari   | ARS, Radhanagari, Kolhapur |
| 2       | Raibhog        | ARS, Maval, Pune           |
| 3       | Ambemohar Gara | ARS, Maval, Pune           |
| 4       | Kalbhat        | ARS, Maval, Pune           |
| 5       | Barangmoti     | ARS, Radhanagari, Kolhapur |
| 6       | Kapurkali      | ARS, Radhanagari, Kolhapur |
| 7       | Indrayani      | ARS, Maval, Pune           |

|    |                  |                            |
|----|------------------|----------------------------|
| 8  | RTN Purple       | ARS, Radhanagari, Kolhapur |
| 9  | Siddhagiri       | ARS, Radhanagari, Kolhapur |
| 10 | Tej              | Private sector             |
| 11 | Kundalika        | ARS, Radhanagari, Kolhapur |
| 12 | Ambemohar Khadka | ARS, Maval, Pune           |
| 13 | Bela             | ARS, Radhanagari, Kolhapur |
| 14 | Kala Bela        | ARS, Radhanagari, Kolhapur |
| 15 | Pawana           | ARS, Radhanagari, Kolhapur |
| 16 | Phule Samrudhi   | ARS, Maval, Pune           |
| 17 | Kalajirga        | ARS, Radhanagari, Kolhapur |
| 18 | Bhogavati        | ARS, Radhanagari, Kolhapur |
| 19 | Kalikatti        | ARS, Radhanagari, Kolhapur |
| 20 | Prabhatjeera     | ARS, Radhanagari, Kolhapur |
| 21 | Ghansal          | ARS, Radhanagari, Kolhapur |
| 22 | Swara Sanrudhi   | Private sector             |
| 23 | CR Dhan 311      | Private sector             |
| 24 | Telangana Sona   | Private sector             |
| 25 | Jira Kalam       | ARS, Maval, Pune           |

### 3. Results

#### 3.1 Effect of Seed Priming on Early Seedling Growth Traits at 45 DAS

Seed priming treatments significantly influenced rice seedling growth and development at 45 DAS (Days after Sowing) (Table No. 2). The findings demonstrate that rice genotypes' responses to various priming treatments exhibit significant genetic heterogeneity. Seed priming improves seedling vigour traits in all genotypes in comparison to the control. Hydropriming

demonstrates favourable but relatively moderate effects in comparison to other priming agents. Among all priming treatments, KCl consistently demonstrates the most substantial positive outcomes in shoot and root growth traits, followed by CaHPO<sub>4</sub>. Increased shoot elongation, increased biomass accumulation, and improved root growth across multiple genotypes demonstrated the statistically superior performance of KCl-primed seedlings.

**Table 2:** Genotypic variation in seedling growth traits under different Priming treatments including CaHPO<sub>4</sub>, Hydropriming, and KCl at 45 DAS

| Priming agent      | Gen ID | Genotypes        | Morphological parameters @ 45 DAS |                               |                               |         |                               |                               |
|--------------------|--------|------------------|-----------------------------------|-------------------------------|-------------------------------|---------|-------------------------------|-------------------------------|
|                    |        |                  | SL (cm)                           | SFW (mg plant <sup>-1</sup> ) | SDW (mg plant <sup>-1</sup> ) | RL (cm) | RFW (mg plant <sup>-1</sup> ) | RDW (mg plant <sup>-1</sup> ) |
| Control            | G04    | Ambemohar Gara   | 21.13                             | 234.53                        | 86.93                         | 4.83    | 38.73                         | 8.57                          |
|                    | G05    | Ambemohar Khadka | 18.60                             | 236.50                        | 94.73                         | 5.60    | 54.63                         | 14.80                         |
|                    | G10    | Barangmoti       | 16.07                             | 227.93                        | 88.23                         | 4.13    | 20.27                         | 11.00                         |
|                    | G22    | Bela             | 16.30                             | 140.40                        | 54.37                         | 4.27    | 27.60                         | 16.63                         |
|                    | G29    | Bhogavati        | 17.10                             | 162.67                        | 66.57                         | 4.87    | 45.10                         | 19.37                         |
|                    | G47    | CR Dhan 311      | 12.87                             | 98.03                         | 40.30                         | 5.00    | 26.10                         | 9.50                          |
|                    | G40    | Ghansal          | 12.77                             | 69.13                         | 28.87                         | 5.80    | 32.10                         | 12.10                         |
|                    | G43    | Indrayani        | 19.37                             | 199.43                        | 78.13                         | 5.07    | 38.90                         | 19.43                         |
|                    | G66    | Jira Kalam       | 18.57                             | 154.37                        | 72.70                         | 3.63    | 16.90                         | 10.00                         |
|                    | G48    | Kala Bela        | 15.20                             | 94.07                         | 45.70                         | 4.03    | 47.33                         | 19.20                         |
|                    | G45    | Kalajirga        | 17.73                             | 129.03                        | 48.20                         | 5.87    | 23.10                         | 10.73                         |
|                    | G46    | Kalbhat          | 20.63                             | 215.57                        | 85.80                         | 3.97    | 21.83                         | 13.80                         |
|                    | G50    | Kalikatti        | 16.40                             | 132.57                        | 62.30                         | 4.37    | 15.30                         | 8.83                          |
|                    | G08    | Kapurkali        | 19.30                             | 689.37                        | 259.80                        | 7.33    | 76.37                         | 35.63                         |
|                    | G55    | Kundalika        | 20.03                             | 165.47                        | 62.67                         | 5.70    | 34.90                         | 13.10                         |
|                    | G56    | Pawana           | 15.87                             | 153.13                        | 63.70                         | 4.83    | 51.13                         | 21.40                         |
|                    | G09    | Phule samrudhi   | 14.23                             | 78.27                         | 30.97                         | 5.10    | 29.83                         | 13.50                         |
|                    | G60    | Prabhatjeera     | 15.03                             | 69.40                         | 32.33                         | 4.13    | 26.87                         | 5.73                          |
|                    | G61    | Raibhog          | 22.27                             | 259.80                        | 93.80                         | 4.10    | 13.40                         | 7.40                          |
|                    | G59    | Ratnasundari     | 23.27                             | 315.03                        | 125.90                        | 6.03    | 41.03                         | 26.57                         |
| CaHPO <sub>4</sub> | G63    | RTN Purple       | 14.80                             | 135.83                        | 62.83                         | 3.97    | 40.27                         | 25.87                         |
|                    | G67    | Siddhagiri       | 25.37                             | 306.07                        | 119.60                        | 5.70    | 90.70                         | 28.03                         |
|                    | G69    | Swara Samrudhi   | 16.53                             | 149.73                        | 75.43                         | 7.13    | 30.77                         | 18.77                         |
|                    | G70    | Tej              | 18.97                             | 249.70                        | 89.03                         | 5.83    | 38.97                         | 24.73                         |
|                    | G44    | Telangana Sona   | 12.43                             | 52.73                         | 31.13                         | 2.83    | 11.00                         | 7.93                          |
|                    | G04    | Ambemohar Gara   | 30.33                             | 627.70                        | 106.23                        | 6.53    | 64.37                         | 45.77                         |
|                    | G05    | Ambemohar Khadka | 25.27                             | 411.07                        | 143.03                        | 7.80    | 114.67                        | 38.83                         |
|                    | G10    | Barangmoti       | 24.63                             | 559.07                        | 198.87                        | 4.37    | 37.97                         | 13.83                         |
|                    | G22    | Bela             | 17.93                             | 166.50                        | 78.97                         | 5.63    | 41.50                         | 25.80                         |
|                    | G29    | Bhogavati        | 32.27                             | 641.53                        | 266.90                        | 6.87    | 51.97                         | 35.67                         |
|                    | G47    | CR Dhan 311      | 15.97                             | 116.27                        | 50.50                         | 5.53    | 31.47                         | 15.90                         |
|                    | G40    | Ghansal          | 17.77                             | 151.97                        | 50.80                         | 5.87    | 37.80                         | 15.10                         |
|                    | G43    | Indrayani        | 21.37                             | 276.77                        | 97.07                         | 5.20    | 73.63                         | 31.63                         |
|                    | G66    | Jira Kalam       | 20.47                             | 208.90                        | 90.13                         | 6.20    | 236.10                        | 25.87                         |
|                    | G48    | Kala Bela        | 23.23                             | 421.07                        | 164.53                        | 4.57    | 49.70                         | 32.43                         |

|              |                |                  |       |         |        |       |        |        |
|--------------|----------------|------------------|-------|---------|--------|-------|--------|--------|
|              | G45            | Kalajirga        | 20.10 | 326.47  | 97.23  | 8.57  | 46.87  | 17.67  |
|              | G46            | Kalbhat          | 29.30 | 904.60  | 336.90 | 6.63  | 134.17 | 84.30  |
|              | G50            | Kalikatti        | 19.17 | 183.40  | 86.23  | 4.87  | 21.97  | 13.97  |
|              | G08            | Kapurkali        | 29.23 | 998.81  | 321.18 | 11.07 | 255.60 | 75.67  |
|              | G55            | Kundalika        | 23.13 | 201.90  | 76.00  | 7.13  | 35.20  | 21.77  |
|              | G56            | Pawana           | 23.07 | 592.43  | 259.90 | 8.07  | 57.77  | 34.13  |
|              | G09            | Phule samrudhi   | 22.23 | 457.80  | 186.77 | 5.80  | 74.77  | 30.37  |
|              | G60            | Prabhatjeera     | 29.50 | 177.33  | 45.47  | 9.20  | 85.03  | 39.27  |
|              | G61            | Raibhog          | 28.53 | 583.43  | 211.23 | 6.97  | 51.90  | 33.90  |
|              | G59            | Ratnasundari     | 30.20 | 836.33  | 269.47 | 9.57  | 43.07  | 29.33  |
|              | G63            | RTN Purple       | 23.43 | 330.83  | 118.73 | 6.93  | 115.73 | 32.80  |
|              | G67            | Siddhagiri       | 34.03 | 906.50  | 281.03 | 7.67  | 152.63 | 72.07  |
|              | G69            | Swara Samrudhi   | 20.23 | 458.40  | 115.57 | 8.77  | 57.70  | 19.90  |
|              | G70            | Tej              | 20.03 | 319.77  | 144.77 | 6.67  | 46.93  | 26.97  |
| Hydropriming | G44            | Telangana Sona   | 12.67 | 102.50  | 44.87  | 4.37  | 12.53  | 9.00   |
|              | G04            | Ambemohar Gara   | 22.43 | 289.40  | 230.03 | 6.43  | 57.10  | 30.60  |
|              | G05            | Ambemohar Khadka | 21.53 | 394.77  | 171.33 | 7.27  | 125.47 | 37.87  |
|              | G10            | Barangmoti       | 20.27 | 247.90  | 93.03  | 6.47  | 88.30  | 31.87  |
|              | G22            | Bela             | 19.07 | 479.63  | 162.87 | 6.30  | 114.00 | 60.13  |
|              | G29            | Bhogavati        | 19.20 | 233.53  | 75.70  | 5.47  | 47.87  | 23.77  |
|              | G47            | CR Dhan 311      | 14.47 | 177.40  | 70.50  | 6.07  | 41.30  | 20.00  |
|              | G40            | Ghansal          | 25.77 | 251.20  | 96.43  | 6.40  | 48.90  | 18.40  |
|              | G43            | Indrayani        | 21.50 | 344.60  | 122.90 | 6.27  | 101.47 | 24.93  |
|              | G66            | Jira Kalam       | 21.73 | 185.03  | 81.07  | 5.80  | 35.93  | 24.83  |
|              | G48            | Kala Bela        | 22.23 | 598.27  | 243.40 | 4.60  | 65.03  | 43.60  |
|              | G45            | Kalajirga        | 24.10 | 149.33  | 61.90  | 6.80  | 30.13  | 13.67  |
|              | G46            | Kalbhat          | 20.87 | 392.83  | 153.53 | 5.37  | 34.70  | 21.10  |
|              | G50            | Kalikatti        | 17.27 | 136.50  | 79.07  | 5.40  | 58.77  | 40.40  |
|              | G08            | Kapurkali        | 29.00 | 1151.03 | 374.77 | 12.07 | 462.20 | 149.07 |
|              | G55            | Kundalika        | 22.93 | 268.07  | 95.87  | 5.87  | 70.33  | 44.67  |
|              | G56            | Pawana           | 19.30 | 294.17  | 124.67 | 5.17  | 72.07  | 29.03  |
|              | G09            | Phule samrudhi   | 15.57 | 150.10  | 60.23  | 5.60  | 34.43  | 19.40  |
|              | G60            | Prabhatjeera     | 16.33 | 110.30  | 57.33  | 4.43  | 28.47  | 10.63  |
|              | G61            | Raibhog          | 26.63 | 343.97  | 128.57 | 4.87  | 18.77  | 11.43  |
|              | G59            | Ratnasundari     | 24.87 | 531.50  | 207.73 | 8.37  | 44.77  | 29.90  |
|              | G63            | RTN Purple       | 25.97 | 748.63  | 259.63 | 6.53  | 159.40 | 55.80  |
|              | G67            | Siddhagiri       | 29.13 | 327.93  | 134.97 | 11.10 | 152.93 | 59.70  |
|              | G69            | Swara Samrudhi   | 21.40 | 345.97  | 135.05 | 11.97 | 93.93  | 32.93  |
|              | G70            | Tej              | 23.20 | 606.77  | 219.70 | 6.30  | 79.63  | 36.97  |
|              | G44            | Telangana Sona   | 16.20 | 105.77  | 55.53  | 5.77  | 24.80  | 17.63  |
| KCl          | G04            | Ambemohar Gara   | 32.33 | 517.63  | 190.03 | 7.20  | 71.43  | 40.97  |
|              | G05            | Ambemohar Khadka | 23.00 | 250.53  | 105.70 | 5.80  | 67.27  | 39.00  |
|              | G10            | Barangmoti       | 24.17 | 496.59  | 173.46 | 5.36  | 48.99  | 21.30  |
|              | G22            | Bela             | 17.37 | 234.33  | 102.03 | 5.93  | 45.83  | 29.97  |
|              | G29            | Bhogavati        | 20.47 | 496.97  | 108.13 | 6.53  | 50.17  | 31.30  |
|              | G47            | CR Dhan 311      | 19.10 | 214.67  | 84.07  | 6.33  | 46.67  | 26.57  |
|              | G40            | Ghansal          | 22.17 | 203.83  | 66.60  | 6.77  | 45.83  | 16.70  |
|              | G43            | Indrayani        | 19.60 | 264.03  | 97.00  | 5.50  | 63.67  | 19.50  |
|              | G66            | Jira Kalam       | 19.20 | 217.07  | 82.77  | 5.73  | 552.83 | 34.17  |
|              | G48            | Kala Bela        | 21.20 | 292.33  | 108.27 | 7.73  | 100.57 | 31.40  |
|              | G45            | Kalajirga        | 27.80 | 576.43  | 172.57 | 7.37  | 51.2   | 20.33  |
|              | G46            | Kalbhat          | 24.30 | 538.83  | 212.73 | 4.07  | 22.6   | 13.83  |
|              | G50            | Kalikatti        | 19.10 | 137.77  | 62.73  | 4.87  | 28.43  | 18.43  |
|              | G08            | Kapurkali        | 33.90 | 1345.39 | 386.11 | 14.83 | 469.03 | 161.72 |
|              | G55            | Kundalika        | 23.40 | 253.92  | 90.48  | 4.73  | 68.03  | 42.73  |
|              | G56            | Pawana           | 19.70 | 302.70  | 126.17 | 7.97  | 66.07  | 160.47 |
|              | G09            | Phule samrudhi   | 20.33 | 1098.90 | 417.07 | 9.33  | 435.27 | 136.43 |
|              | G60            | Prabhatjeera     | 20.90 | 299.23  | 114.60 | 7.07  | 37.77  | 20.20  |
|              | G61            | Raibhog          | 34.53 | 828.30  | 292.47 | 6.07  | 33.8   | 17.90  |
|              | G59            | Ratnasundari     | 32.17 | 1163.73 | 389.03 | 7.67  | 46.33  | 26.80  |
|              | G63            | RTN Purple       | 21.97 | 422.67  | 164.43 | 8.10  | 135.03 | 79.00  |
|              | G67            | Siddhagiri       | 31.07 | 801.83  | 255.70 | 9.53  | 129.6  | 82.77  |
|              | G69            | Swara Samrudhi   | 31.30 | 2780.57 | 655.97 | 17.20 | 1172.3 | 86.47  |
|              | G70            | Tej              | 23.33 | 950.43  | 344.47 | 8.57  | 132.87 | 65.83  |
|              | G44            | Telangana Sona   | 17.33 | 116.73  | 52.13  | 4.97  | 22.17  | 15.10  |
| Factor - A   | Priming agents |                  |       |         |        |       |        |        |
|              | S.E (m)±       |                  | 0.13  | 0.19    | 0.23   | 0.03  | 0.22   | 2.42   |
|              | C.D at 5%      |                  | 0.36  | 0.52    | 0.64   | 0.07  | 0.60   | 6.74   |

|                           |           |      |      |      |      |      |       |
|---------------------------|-----------|------|------|------|------|------|-------|
|                           | C.D at 1% | 0.47 | 0.69 | 0.84 | 0.09 | 0.80 | 8.89  |
| Factor - B                | Genotypes |      |      |      |      |      |       |
|                           | S.E (m)±  | 0.32 | 0.47 | 0.57 | 0.06 | 0.54 | 6.04  |
|                           | C.D at 5% | 0.89 | 1.31 | 1.59 | 0.18 | 1.51 | 16.84 |
|                           | C.D at 1% | 1.17 | 1.73 | 2.10 | 0.23 | 1.99 | 22.21 |
| Interaction effect of A×B |           |      |      |      |      |      |       |
|                           | S.E (m)±  | 0.64 | 0.94 | 1.14 | 0.13 | 1.08 | 12.08 |
|                           | C.D at 5% | 1.78 | 2.62 | 3.18 | 0.35 | 3.02 | 33.69 |
|                           | C.D at 1% | 2.34 | 3.45 | 4.20 | 0.47 | 3.99 | 44.43 |

\*DAS- Days After Sowing, SL- Shoot Length (cm), SFW- Shoot Fresh Weight (mg), SDW- Shoot Dry Weight (mg), RL- Root Length (cm), RFW- Root Fresh Weight (mg), RDW- Root Dry Weight (mg), SE(m)- Standard Error of Mean (±), C.D at 5%- Critical Difference at 5% level of significance, C.D at 1%- Critical Difference at 1% level of significance

Significant genotypic differences in shoot growth were observed across different treatments and interaction effects. With priming treatments, noticeable increases in shoot length were observed in genotypes such as Raibhog, Ambemohar Gara, Ratnasundari, Bhogavati, Siddhagiri, and Swara Samrudhi, especially with KCl and CaHPO<sub>4</sub> priming. Telangana Sona, on the other hand, exhibited a weaker response to seed priming, with comparatively lower shoot growth across treatments. Priming treatments had a significant impact at  $P \leq 0.05$  and  $P \leq 0.01$  significance levels on the seedling biomass accumulation at 45 DAS. Swara Samrudhi, Kapurkali, Ratnasundari, and Phule Samrudhi showed the greatest statistically significant increases in both shoot fresh weight and shoot dry weight with KCl priming compared to the control. Additionally, seed priming increased root development. Under KCl priming, the largest root biomass and length were observed, especially in genotypes such as Kapurkali and Swara Samrudhi.

Although its effects were not as strong as those shown with chemical priming treatments, hydropriming also enhanced root growth in several genotypes. The importance of genetic background in determining priming effectiveness is highlighted by the strong genotype  $\times$  treatment interaction. While Telangana Sona and CR Dhan 311 showed slight improvements, genotypes such as Kapurkali, Swara Samrudhi, Ratnasundari, Siddhagiri, Phule Samrudhi, and Raibhog demonstrated considerable positive responses to priming treatments. These results show significant genetic diversity in the enhancement of seedling vigour after seed priming. All priming treatments showed a positive correlation between root dry weight (RDW) and shoot dry weight (SDW) (Figure 1). KCl-primed seedlings showed the greatest root and shoot biomass among all priming treatments. Other priming treatments, including hydropriming and CaHPO<sub>4</sub>, also showed favourable relationships between RDW and SDW.

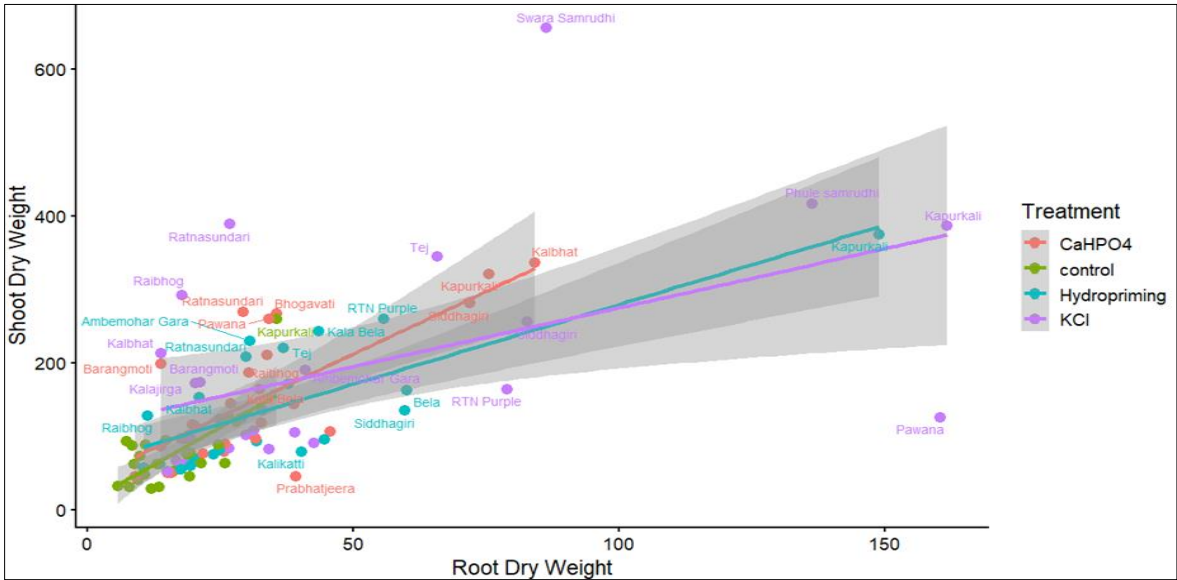


**Fig 1:** The relationship between root dry weight (RDW) and shoot dry weight (SDW) of 25 rice genotypes subjected to seed priming treatments at 45 DAS, as demonstrated by a scatter plot. The coloured points in the figure represent the mean value of a genotype under a particular priming treatment. Coloured regression lines show treatment-specific linear trends, while shaded areas represent the 95% confidence intervals. Positive slopes indicate a robust correlation between the accumulation of root and shoot biomass across priming treatments.

3.2 Correlation Analysis among Seedling Traits

Correlation analysis demonstrates a coordinated pattern of shoot and root development in various priming treatments, showing substantial positive relationships among all evaluated seedling traits at 45 DAS (Figure 2). SFW and SDW showed the highest correlation ( $r = 0.96$ ), indicating that higher dry matter accumulation was strongly linked to increases in fresh biomass. The substantial positive correlations between shoot fresh weight and root fresh weight ( $r = 0.77$ ), root length ( $r = 0.74$ ), and shoot

length ( $r = 0.67$ ) demonstrated the deeper connection between biomass production and seedling growth. Similarly, there was a positive correlation between root length and root fresh weight ( $r = 0.70$ ), shoot fresh weight ( $r = 0.74$ ), and shoot dry weight ( $r = 0.69$ ), suggesting that seedlings with larger root systems tend to accumulate more biomass above ground. Additionally, there were moderate correlations between root dry weight and shoot dry weight ( $r = 0.56$ ), root length ( $r = 0.58$ ), and root fresh weight ( $r = 0.51$ ).

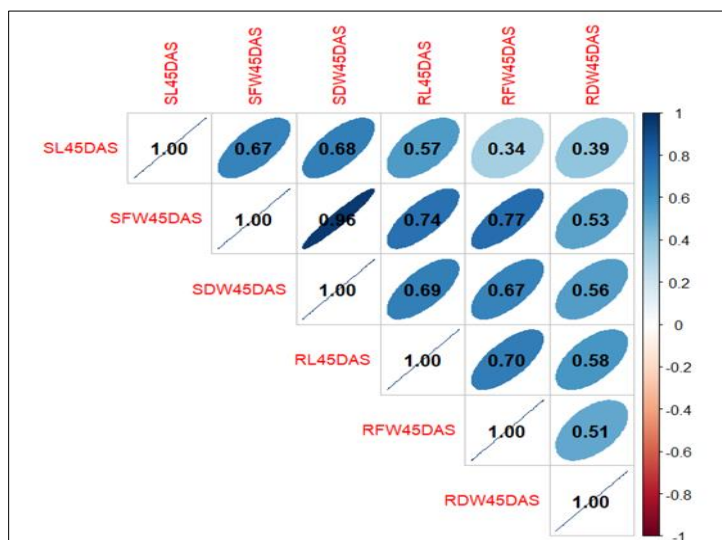


**Fig 2:** Pearson correlation matrix demonstrating the relationships among the observed seedling traits across 25 rice genotypes under four different seed priming treatments. The correlation strength and direction are shown by the ellipse shape and colour intensity, while correlation coefficients ( $r$ ) are shown in the upper triangle. Stronger positive correlations are shown by darker and narrower ellipses. SL- Shoot length, SFW-shoot fresh weight, SDW-shoot dry weight, RL-root length, RFW- root fresh weight, RDW- root dry weight, and 45 DAS- at 45 days after sowing.

### 3.3 Hierarchical Clustering of Rice Genotypes

The 25 rice genotypes were categorised into three main clusters (Figure 3) using hierarchical cluster analysis based on six seedling traits recorded at 45 DAS.

Only two genotypes, Kapurkali and Swara Samrudhi, made up Cluster I. These genotypes were separated from the other genotypes at the greatest linkage distance. These genotypes' unusual grouping suggests a distinctive seedling traits profile marked by increased biomass accumulation and seedling vigour. Thirteen genotypes, including Telangana Sona, CR Dhan 311,

Kalikatti, Jira Kalam, Kalajirga, Ghansal, Prabhatjeera, Bhogavati, Barangmoti, Bela, Kala Bela, Indrayani, and Kundalika, were included in Cluster II.

Ten genotypes, including Ambemohar Khadka, RTN Purple, Pawana, Phule Samrudhi, Ambemohar Gara, Raibhog, Kalbhat, Tej, Ratnasundari, and Siddhagiri, were found in Cluster III. Although the genotypes in this cluster were different from those in Cluster II, they showed similar biomass accumulation patterns and seedling vigour traits.

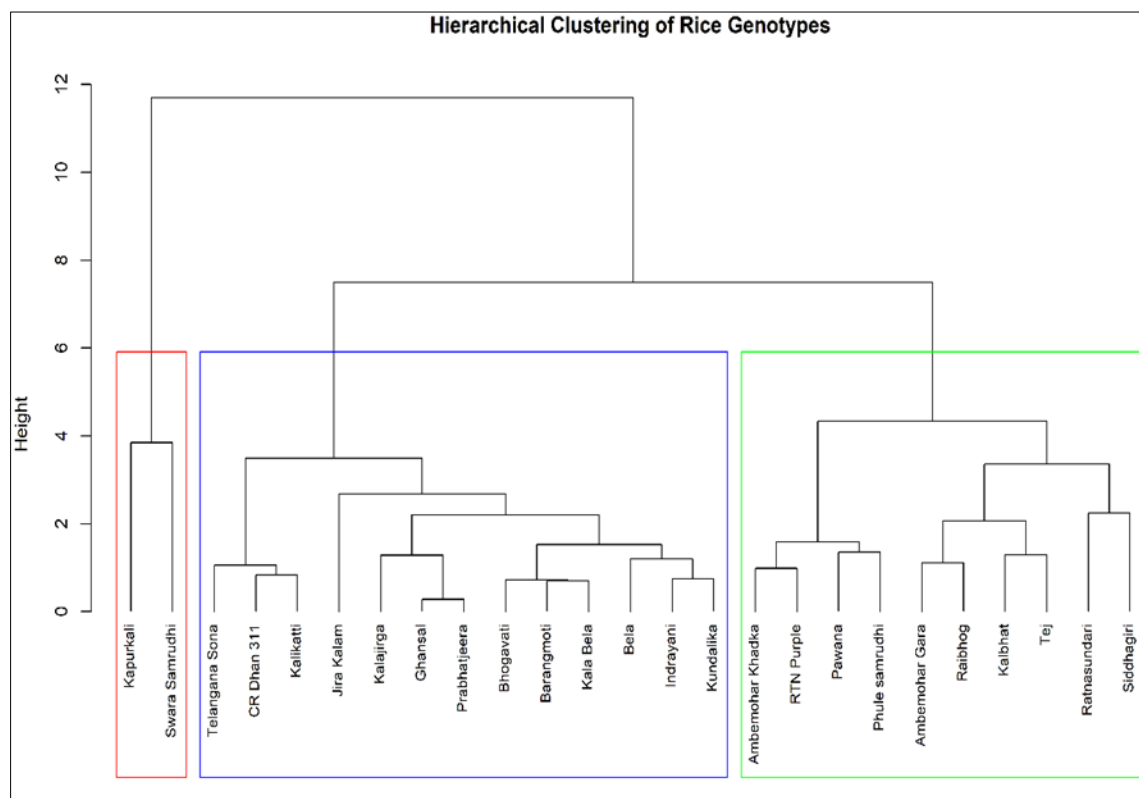


**Fig 3:** Hierarchical clustering of rice genotypes based on seedling traits measured at 45 DAS. Standardised data of observed seedling traits were clustered using Ward's hierarchical agglomerative approach. The degree of morphological divergence among genotypes was reflected in the identification of three primary clusters. The dendrogram's height shows the degree of genotypic variation in seedling traits among different priming treatments.

### 3.4 Principal Component Analysis of seedling morphological and biomass Traits

The multivariate correlations among the 25 rice genotypes based on six seedling traits recorded at 45 DAS were evaluated using principal component analysis (PCA) (Figure 4). 87.1% of the variation was explained by the first two principal components, PC1 and PC2, which contributed 73.1% and 14.0%, respectively. The high percentage of explained variation suggests that the first two components adequately represented the differential responses among the assessed genotypes. Shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight were all measured characteristics that positively associated with PC1. Thus, PC2 exhibited variance related to the relative contribution of shoot and root growth features, while PC1 mainly indicated overall seedling traits and biomass

accumulation.

The PCA biplot revealed clear differentiation among genotypes based on their seedling growth performance. Kapurkali and Swara Samrudhi were distinctly separated from the remaining genotypes, indicating their unique morphological traits and biomass. Similarly, Ratnasundari, Siddhagiri, Raibhog, Ambemohar Gara, and Kalbhat were closely associated with shoot-related traits, particularly shoot length and shoot dry weight, suggesting enhanced above-ground growth potential. Genotypes including Phule Samrudhi, Pawana, and RTN Purple were closely linked to root-associated characteristics, such as root fresh weight and root dry weight. Telangana Sona, CR Dhan 311, Jira Kalam, Kalikatti, and Bela, on the other hand, were located far from the main vigour-related trait vectors, indicating relatively poor seedling growth performance.

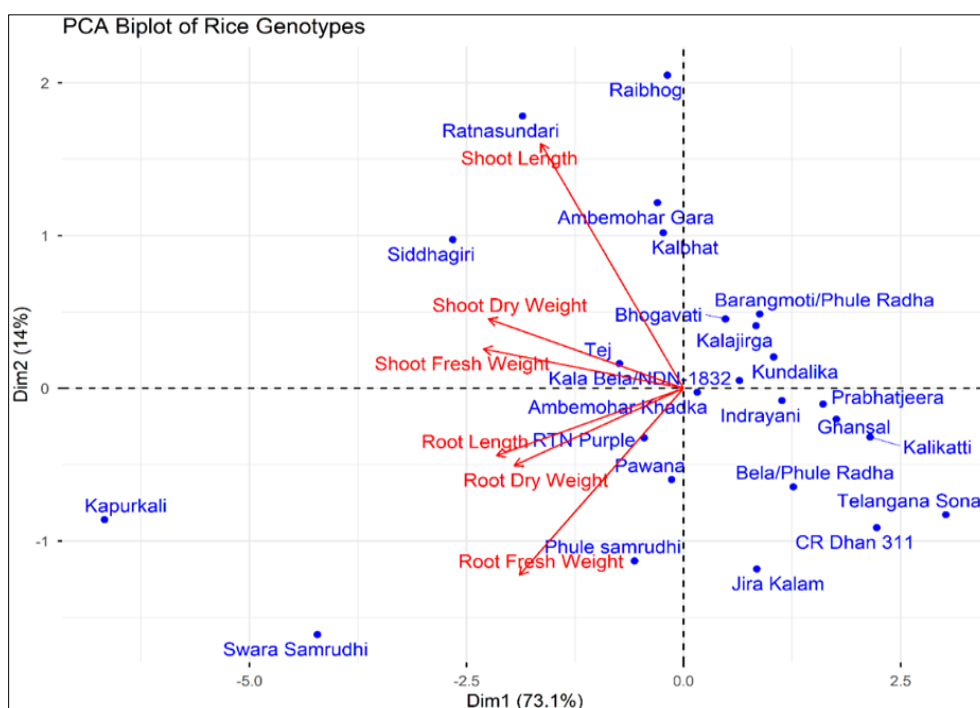


**Fig 4:** 25 rice genotypes were plotted using principal component analysis (PCA) based on six morphological characteristics assessed at 45 DAS. PC1 and PC2 accounted for 87.1% of the cumulative variability, explaining 73.1% and 14.0% of the overall variation, respectively. Blue points show genotype scores, and red vectors show trait loadings. The length and direction of vectors show how each attribute contributes to genotype difference.

### 3.5 Violin boxplots showing Seedling Growth Traits distribution at 45 DAS

At 45 DAS, the violin-box plots revealed substantial variation in seedling growth traits and biomass accumulation among rice genotypes and priming treatments (Figure 5). The genotype Swara Samrudhi, represented the highest observed value and the widest distribution of RL at 45 DAS, under KCl priming. Hydropriming also showed improved root length with genotype Kapurkali showing higher performance under this priming treatment. The control treatment, on the other hand, showed a tighter range and relatively lower root length values. Both RDW and RFW responded favourably to KCl priming. Under KCl, both RFW and RDW showed the largest outlying values, with genotype Kapurkali recording the highest RDW and genotype Swara Samrudhi displaying very high RFW. Compared to root-related traits and biomass accumulation, SL showed little variation; however, KCl priming once more recorded the greatest values, with genotype Raibhog exhibiting greater shoot elongation. Similarly, under KCl priming, SFW and SDW were often higher. With the highest accumulation of shoot biomass,

genotype Swara Samrudhi emerged as a prominent outlier, indicating increased vigor and dry matter production. Additionally, for biomass-related traits, genotype Kapurkali consistently showed up among the best-performing outliers in both hydropriming and control treatments. Overall, KCl priming produced larger maximum values and wider trait ranges, suggesting a positive association between KCl priming and seedling growth and biomass accumulation at 45 DAS. The identified outlier genotypes, particularly Swara Samrudhi, Kapurkali, and Raibhog, may represent valuable genetic resources for improving seedling vigor under priming conditions.

### Discussion

Significant genotypic diversity in seedling vigour traits was found in this study, which also showed how seed priming can improve early rice seedling growth. Genotypic variation has a significant impact on the response to seed priming, as evidenced by significant treatment, genotype, and genotype  $\times$  treatment interaction effects reported for all evaluated parameters. For better crop establishment, this variation offers potential to find

responsive genotypes and create focused seed enhancement approaches. KCl priming consistently resulted in the most gains in biomass accumulation, shoot and root growth, and overall seedling vigour among the assessed treatments. Previous studies have suggested that increased osmotic control, membrane stability, nutrient mobilization, and activation of metabolic pathways during germination and early growth may all contribute to the increased performance of primed seedlings (Anwar *et al.*, 2021; Rhaman *et al.*, 2025; Zhang *et al.*, 2025)<sup>[2, 26, 22]</sup>. Potassium is essential for cell growth, water relations, carbohydrate translocation, and enzyme activation—all of which support strong seedling establishment and higher biomass output (Anwar *et al.*, 2021)<sup>[2]</sup>. The advantageous effects observed under KCl priming

may be associated with improved osmotic adjustment, membrane integrity, and metabolic process activation during germination (Anwar *et al.*, 2021; Rhaman *et al.*, 2025; MacDonald and Mohan, 2025)<sup>[2, 22, 12]</sup>, which may be results in enhanced seedling growth traits and increased biomass production. Similarly, CaHPO<sub>4</sub> priming might have increased phosphorus availability in the early stages of growth, which would have encouraged dry matter buildup, cell division, and root proliferation. Although its effects were often less noticeable than those of chemical priming, hydropriming probably enhanced seedling performance through regulated seed hydration and activation of pre-germinative metabolic activity.

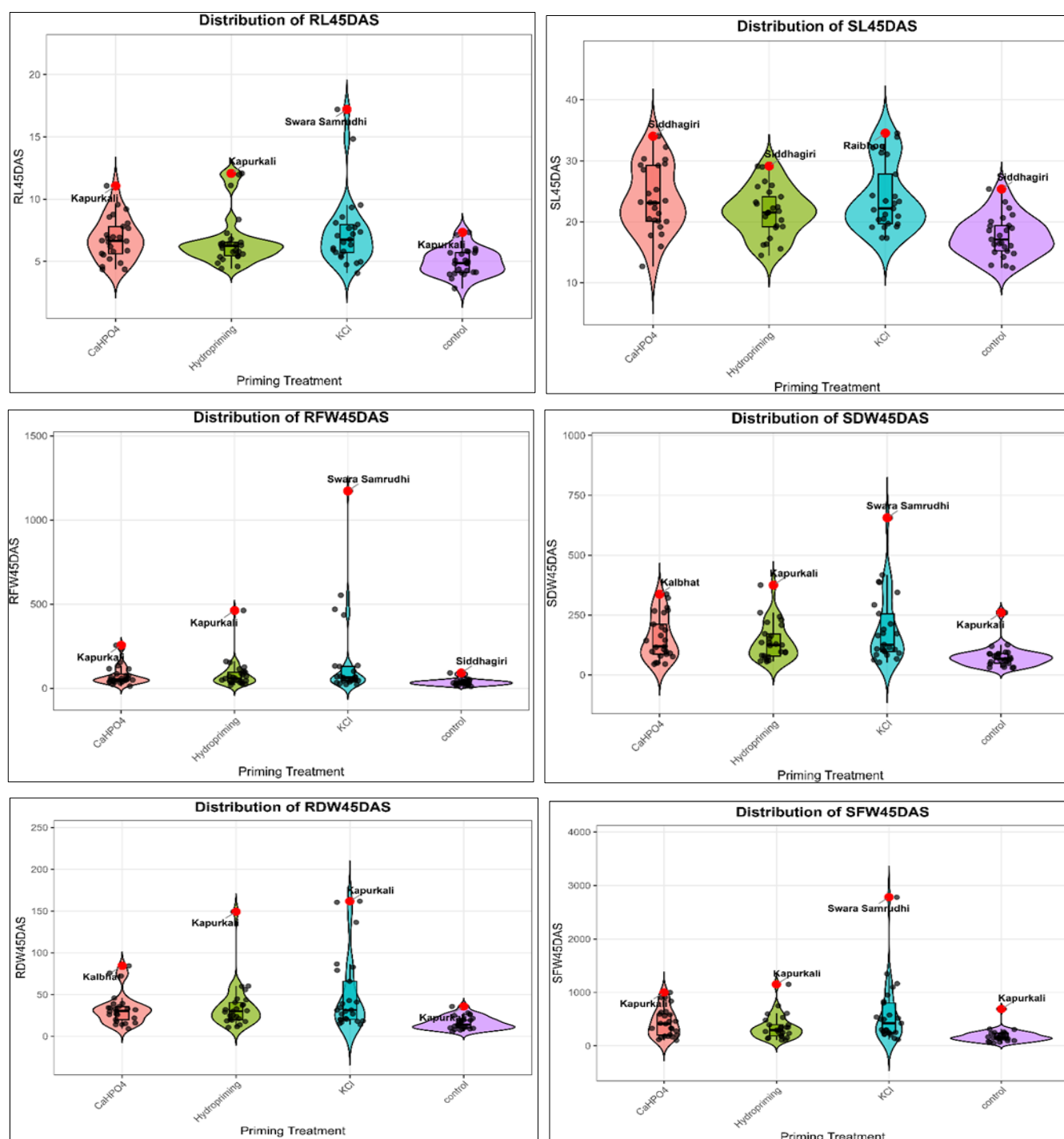


**Fig 5:** The distribution of six seedling growth traits among 25 rice genotypes under four seed priming treatments (CaHPO<sub>4</sub>, Hydropriming, KCl, and Control) at 45 DAS is displayed in violin-box plots. Individual points correspond to genotype means, boxplots show the median and interquartile range, and violin plots show the distribution of genotypes. Red points and labels indicate the best-performing genotype for each attribute within each treatment. Shoot length (SL45DAS), shoot fresh weight (SFW45DAS), shoot dry weight (SDW45DAS), root length (RL45DAS), root fresh weight (RFW45DAS), and root dry weight (RDW45DAS) are among the characteristics.

Strong positive relationships between shoot and root characteristics associate coordinated plant development with seedling vigour traits. Significant variability among the assessed genotypes was further revealed by multivariate analyses. The enhanced vigour-related traits and robust responsiveness to priming treatments of Kapurkali and Swara Samrudhi were consistently identified as phenotypically genotypes by both principal component analysis and hierarchical clustering. The reliability of the observed genotype categorization is strengthened by the measurements of traits and evaluation of genotype relationships by using clustering, PCA, and correlation studies, which also shows how effective morphological traits and biomass accumulation are for identifying variations in seedling vigour among rice genotypes.

The broader trait distributions shown under KCl priming further imply that this priming treatment reveal genotypic variability expression, making it easier to identify top performers. Swara Samrudhi, Kapurkali, Ratnasundari, Siddhagiri, and Raibhog are examples of genotypes that consistently showed improved growth and biomass accumulation, suggesting that they could be useful genetic resources for breeding programs targeted at enhancing early seedling establishment and adaptation to water-limited rice systems.

### Conclusion

The present study revealed considerable genotypic variation in seedling growth and biomass traits among 25 rice genotypes subjected to different priming treatments. Seed priming enhanced both shoot and root development at 45 DAS, with KCl priming producing the most consistent improvements across growth and biomass-related traits. Significant positive correlations among shoot and root parameters indicated coordinated seedling development and underscored the contribution of root growth to early plant establishment. Correlation analysis, hierarchical clustering, and principal component analysis collectively demonstrated substantial phenotypic diversity among the evaluated genotypes and facilitated the identification of superior performers. Swara Samrudhi, Kapurkali, Ratnasundari, Siddhagiri, and Raibhog showed strong responses to priming treatments and maintained favourable growth characteristics. The distinct clustering of Kapurkali and Swara Samrudhi further highlighted their potential utility as donor genotypes in breeding programmes aimed at improving early seedling performance. Overall, KCl priming proved to be the most effective treatment for enhancing seedling growth under the conditions of this study. Further evaluation under field and direct-seeded rice conditions is necessary to confirm the stability of these responses and their potential contribution to crop establishment and yield.

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