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PHENOTYPIC CHARACTERIZATION AND TRAIT CORRELATIONS OF EARLY SEEDLING VIGOUR AND ITS COMPONENT TRAITS IN A SUBSET OF RICE (*Oryza sativa* L.) 3K PANEL

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ABSTRACT

Early seedling vigour is a key determinant of successful crop establishment under dry direct-seeded rice (DDSR) systems, where rapid and uniform seedling emergence enhances competitiveness against weeds and improves adaptation to limited soil moisture. Despite its agronomic significance, comprehensive phenotypic assessment of seedling vigour-related traits across diverse rice germplasm remains limited. The present study evaluated early seedling vigour in 203 rice accessions representing the Indian subset of the 3,000 Rice Genomes Project, together with five check varieties. Phenotyping was conducted under controlled laboratory conditions using the paper towel method, and observations were recorded for germination percentage, mesocotyl length, coleoptile length, shoot length, root length, seedling length, total fresh weight, total dry weight and the derived indices Seedling Vigour Index-I (SVI-I) and Seedling Vigour Index-II (SVI-II). Analysis of variance revealed highly significant ($P < 0.01$) genotypic differences for all measured traits, indicating substantial genetic variability within the evaluated panel. Mesocotyl length exhibited the greatest phenotypic variation, ranging from 0.22 to 5.53 cm, while the seedling vigour indices also displayed wide variability. In contrast, coleoptile length showed comparatively limited variation. Correlation analysis demonstrated significant positive associations between mesocotyl length and coleoptile length ($r = 0.39$), with both traits also positively correlated with shoot length and overall seedling length. SVI-I and SVI-II exhibited a strong positive correlation ($r = 0.90$) and were significantly associated with dry matter accumulation, highlighting their effectiveness as indicators of early seedling performance. The identification of genotypes combining superior mesocotyl elongation with high seedling vigour indices provides valuable genetic resources for breeding rice varieties adapted to DDSR cultivation. Overall, the study demonstrates that laboratory-based phenotypic screening offers an efficient first-stage approach for identifying promising germplasm to improve seedling establishment and adaptation in water-limited rice production systems.

Keywords : Seedling vigour, mesocotyl elongation, coleoptile length, 3,000 Rice Genomes Project, direct-seeded rice, germplasm characterization, *Oryza sativa*.

Introduction

Rice (*Oryza sativa* L.) remains the staple food for more than half of the global population, with cultivated area exceeding 165 million hectares and annual production surpassing 500 million tonnes (FAO, 2023). In Asia, where over 90% of the world's rice is

produced and consumed, the crop occupies a central position in food security frameworks and rural livelihoods. However, conventional puddled transplanted rice production faces mounting challenges from diminishing water resources, rising labour costs, and the cascading effects of climate change on

agricultural systems (Kumar and Ladha, 2011). These pressures have propelled dry direct-seeded rice (DDSR) as a viable alternative production system, wherein pre-germinated or dry seeds are sown directly into non-puddled soil, thereby eliminating the water-intensive nursery establishment and transplanting operations (Farooq *et al.*, 2011; Chauhan, 2012).

The successful adoption of DDSR hinges critically on the capacity of sown seeds to emerge rapidly and establish uniformly under variable soil conditions. Early seedling vigour, defined as the collective capacity of a seedling to emerge swiftly, accumulate biomass, and establish a functional root system, determines the probability of survival during the vulnerable germination-to-establishment transition (Finch-Savage *et al.*, 2010). Vigorous seedlings possess several competitive advantages: they suppress weed growth through rapid canopy closure, exhibit enhanced tolerance to intermittent drought through deeper root penetration, and display improved nutrient acquisition efficiency during the critical early growth phase (Okami *et al.*, 2011; Singh *et al.*, 2017). Conversely, poor seedling vigour leads to patchy stands, prolonged vulnerability to biotic and abiotic stresses, and ultimately, yield penalties that can reach 15-30% under unfavourable conditions (Zhao *et al.*, 2006).

Two anatomical traits occupy pivotal positions in the seedling vigour architecture of rice: mesocotyl elongation and coleoptile elongation. The mesocotyl, an internodal tissue located between the coleoptilar node and the base of the seminal root system, serves as the primary engine for elevating the shoot apex from deeper soil layers toward the surface (Watanabe *et al.*, 2001; Lee *et al.*, 2017). In direct-seeding systems with sowing depths of 3-5 cm, which are common under mechanized drilling or surface broadcasting followed by light tillage, mesocotyl elongation can contribute 30-70% of the total emergence rate (Zhan *et al.*, 2020). Genotypes possessing inherently longer mesocotyls demonstrate superior emergence rates under deep sowing and transient submergence conditions, a trait particularly valuable in rainfed lowland and aerobic rice ecosystems (Alibu *et al.*, 2012; Yang *et al.*, 2020). The physiological basis of mesocotyl elongation involves gibberellin-mediated cell division and expansion in the sub-apical meristem, processes that are regulated by light quality through phytochrome signalling and negatively modulated by strigolactones (Hu *et al.*, 2010; Gao *et al.*, 2012).

The coleoptile, a protective sheath enveloping the plumule and first leaves, plays an equally indispensable role in seedling emergence. Its

elongation capacity determines whether the emerging shoot can penetrate the soil surface, particularly under conditions of increased sowing depth or surface residue cover (Turner *et al.*, 1982; Alibu *et al.*, 2011). In oxygen-limited environments characteristic of waterlogged soils, coleoptile elongation is often the only viable pathway for seedling emergence, as the coleoptile can extend through anaerobic soil layers whereas leaf expansion remains suppressed (Huang *et al.*, 2003). The functional complementarity between mesocotyl and coleoptile elongation has been recognized as a key determinant of emergence success under deep sowing, with both traits required to achieve complete seedling establishment (Dilday *et al.*, 1990; Zhan *et al.*, 2020).

To integrate multiple seedling vigour components into selection criteria, plant breeders have employed composite indices. Seedling vigour index-I (SVI-I), computed as the product of germination percentage and seedling length, captures the overall capacity for emergence and early growth (Addanki *et al.*, 2019). Seedling vigour index-II (SVI-II), derived from germination percentage and total dry weight, reflects the efficiency of biomass accumulation during establishment. Both indices have demonstrated utility in identifying genotypes with superior field emergence and early vigour in rice (Addanki *et al.*, 2019) and related cereal crops. The integration of these indices with component trait measurements enables breeders to discriminate between genotypes that excel in specific aspects of seedling development versus those with balanced performance across multiple traits.

Genetic dissection of seedling vigour components has accelerated with the advent of genome-wide association studies (GWAS) leveraging high-density genotypic data. The 3,000 Rice Genomes Project (3K-RGP) has generated re-sequencing data for diverse accessions representing the major rice subspecies and geographic origins, providing an unprecedented resource for linking phenotypic variation with underlying genetic polymorphisms (Wang *et al.*, 2018). Recent GWAS analyses have identified quantitative trait loci (QTL) for mesocotyl length on chromosomes 1, 3, 4, 6, 7, 8, 10, and 12, with some loci co-localizing with previously reported gibberellin biosynthesis and signalling genes (Wu *et al.*, 2015; Lu *et al.*, 2016; Wang *et al.*, 2021). Similarly, coleoptile length QTL have been mapped to chromosomes 1, 3, 5, 6, and 11, with promising candidate genes including expansins and xyloglucan endotransglycosylases implicated in cell wall loosening (Dwivedi, 2016). However, the majority of association studies have relied on relatively small panels or bi-parental populations, and

comprehensive phenotypic characterization of large diversity panels with explicit relevance to DDSR breeding remains scarce.

The Indian subcontinent harbors exceptional rice genetic diversity, with the 3K-RGP Indian subset encompassing landraces, traditional varieties, and improved lines adapted to diverse agro-ecological zones. Phenotypic evaluation of this subset for seedling vigour components offers dual benefits: identification of superior donor genotypes for breeding programmes targeting DDSR adaptation, and establishment of robust phenotype-genotype datasets for downstream association mapping. Against this backdrop, the present study was designed with the following objectives: (i) to evaluate early seedling vigour-related traits in 203 rice accessions representing the Indian subset of the 3K-RGP; (ii) to assess the nature and magnitude of genetic variability and inter-trait correlations; and (iii) to identify superior genotypes with enhanced mesocotyl elongation and seedling vigour indices for potential deployment in DDSR breeding programmes.

Materials and Methods

Experimental Material

The present investigation comprised 203 rice (*Oryza sativa* L.) accessions sourced from the Indian subset of the 3,000 Rice Genomes Project (3K-RGP). The germplasm was procured from ICAR-National Institute of Seed Science and Technology (NISST), Regional Station, Bengaluru, Karnataka, India. The panel represented broad genetic diversity encompassing indica and aus subspecies, landraces, traditional cultivars, and improved breeding lines adapted to diverse agro-ecological zones of the Indian subcontinent. In addition to the 3K-RGP accessions, five check varieties with contrasting seedling vigour characteristics were included: Brown Gora, an aus landrace recognized for superior seedling vigour and early maturity; DRR Dhan 44, a drought-tolerant variety developed at ICAR-IIRR; Sahbhagi Dhan (IR 74371-70-1-1), a drought-tolerant mega-variety widely cultivated in rainfed ecosystems; Sabita, a variety reported to possess high seedling vigour; and IR 64, an elite high-yielding variety characterized by comparatively low early seedling vigour.

Seed Preparation and Germination Assay

Uniformly sized, well-filled grains were manually selected and residual seed dormancy, commonly encountered in freshly harvested rice germplasm, was mitigated by exposing seeds to a controlled temperature of 50° C for five days in a hot air oven (Memmert, Germany). Following dormancy breaking,

seeds were surface-sterilized in 0.6% (v/v) sodium hypochlorite solution for 15 minutes with intermittent agitation to ensure uniform contact, and subsequently rinsed three times with sterile distilled water to remove residual hypochlorite.

Paper Towel Method

The paper towel method, standardized for high-throughput phenotyping of rice seedling vigour components (Addanki *et al.*, 2019), was employed with minor modifications. Brown paper towels (30 x 20 cm) were autoclaved at 121° C for 20 minutes and moistened uniformly with sterile distilled water. Fifty surface-sterilized seeds of each accession were placed at equidistant intervals on the moistened paper towels. The experimental layout followed a completely randomized design (CRD) with three replications. The paper towels with seeds were covered with a transparent polythene sheet to prevent mechanical damage and minimize moisture loss. The setup was folded from both ends toward the center and secured with rubber bands to maintain consistent seed-to-paper contact. Each replicate setup was appropriately labelled with the accession identity and arranged in a slanting position (approximately 30 degrees from horizontal) in a plastic tray containing sterile distilled water at the base. The trays were maintained in a growth room under controlled conditions (temperature 28 +/- 2° C, relative humidity 70 +/- 5%, 12-hour photoperiod with fluorescent illumination at 300 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

Trait Measurements

Data were recorded on five randomly selected seedlings from each replication at 14 days after incubation in growth chamber for all traits. Germination percentage (GP) was calculated as the ratio of normally germinated seedlings to the total number of seeds, expressed as a percentage. Mesocotyl length (ML) was measured as the distance from the base of the seminal root to the coleoptilar node using a centimetre scale with 0.1 cm precision. Coleoptile length (CL) was recorded from the coleoptilar node to the tip of the coleoptile. Shoot length (SL) was measured from the coleoptilar node to the tip of the longest leaf, while root length (RL) represented the distance from the seed base to the tip of the longest seminal root. Total seedling length (SDL) was computed as the sum of shoot and root lengths. Seedling fresh weight (SFW) and seedling dry weight (SDW) were recorded using an electronic analytical balance (Shimadzu AUW220D, Japan) after oven-drying at for 72 hours for dry weight determination. Two composite indices were calculated following Addanki *et al.* (2019): seedling vigour index-I (SVI-I)

= germination percentage (%) x seedling length (cm);
and seedling vigour index-II (SVI-II) = germination
percentage (%) x total dry weight (g).

Statistical Analysis

Analysis of variance (ANOVA) was performed for all ten traits using a completely randomized design model in R software version 4.5.1 (R Core Team, 2024). The significance of genotypic mean sum of squares was tested against the error mean sum of squares using F-tests at $P < 0.05$ and $P < 0.01$ probability levels. Coefficient of variation (CV) was computed as the ratio of standard deviation to the grand mean, expressed as a percentage. Descriptive statistics including mean, minimum, maximum, range, and standard deviation were computed for each trait. Pearson correlation coefficients among all trait pairs were estimated and their significance tested using t-tests. The distribution of mesocotyl and coleoptile lengths was visualized using violin plots to assess the shape and dispersion of phenotypic variation. All graphical outputs were generated using the ggplot2 package in R (Wickham, 2016).

Results

Analysis of Variance

Analysis of variance revealed highly significant differences ($P < 0.01$) among the 208 genotypes (203 accessions + 5 checks) for all ten seedling vigour traits evaluated (Table 1). The F values ranged from 695.64 for coleoptile length to 23,841.02 for shoot length, indicating that genetic differences among accessions explained a substantial proportion of the total phenotypic variation. The error mean squares were notably small relative to genotypic mean squares for all traits, reflecting high precision in the experimental measurements. The coefficient of variation (CV) was lowest for shoot length (0.27%), root length (0.22%), and seedling length (0.23%), suggesting that these

traits were measured with high reproducibility. Moderate CV values were observed for germination percentage (3.71%), mesocotyl length (1.37%), coleoptile length (1.51%), and total fresh weight (2.14%), while SVI-I (3.69%) and SVI-II (3.81%) exhibited comparatively higher coefficients of variation, attributable to the compounding of variation from their component traits. The highly significant genotypic mean squares for all traits indicate the presence of substantial additive genetic variation within this panel, providing a sound basis for selection and downstream genetic mapping.

For mesocotyl length, the highest values were recorded in genotypes N4, N149, N137, N150, N8, N115, N26, N9, N31, and N144, with values ranging from approximately 4.7 to 5.5 cm. Similarly, the highest coleoptile lengths were observed in N31, N21, N44, N4, N30, N60, N75, N34, N276, and N33, ranging from about 3.5 to 4.4 cm. Notably, N4 and N31 were common among the top performers for both mesocotyl and coleoptile length, indicating their consistently superior elongation ability (Fig. 1).

For Seedling Vigour Index-I, the leading genotypes were N56, N54, N25, N26, N39, N46, N22, N15, N28, and N16, with values ranging from approximately 3,950 to 4,225. In contrast, the highest Seedling Vigour Index-II values were recorded in N43, N60, N58, N22, N28, N27, N39, N54, N197, and N56, with values ranging from about 17.5 to 22.5. A considerable overlap was observed between the two vigour indices, as N56, N54, N22, N28, and N39 were common to both groups. The occurrence of common genotypes across ML and CL as well as SVI-I and SVI-II suggests consistent superiority for related seedling vigour traits within these accessions, although the majority of genotypes exhibited trait-specific performance (Fig. 1).

Table 1: Analysis of Variance for Early Seedling Vigour Traits in subset of 3K Panel of Rice using Paper Towel Method.

Sl. No.	Traits	Mean sum of squares		F value	CV (%)
		Genotypes	Error		
	Degrees of freedom	207	416		
1.	Germination percentage (%)	2270.96 **	1.99	1142.81	3.71
2.	Mesocotyl length (cm)	8.36 **	0.00	5265.38	1.37
3.	Coleoptile length (cm)	1.22 **	0.00	695.64	1.51
4.	Shoot length (cm)	50.98 **	0.00	23841.02	0.27
5.	Root length (cm)	29.98 **	0.00	16580.06	0.22
6.	Seedling length (cm)	105.22 **	0.01	15058.27	0.23
7.	Total fresh weight (mg)	0.46 **	0.00	1150.08	2.14
8.	Total dry weight (mg)	0.01 **	0.00	3979.79	1.09
9.	Seedling vigour index I	3856609.32 **	2710.35	1422.92	3.69
10.	Seedling vigour index II	145.83 **	0.07	2056.91	3.81

** = Significant at 99% interval ($P \leq 0.01$)

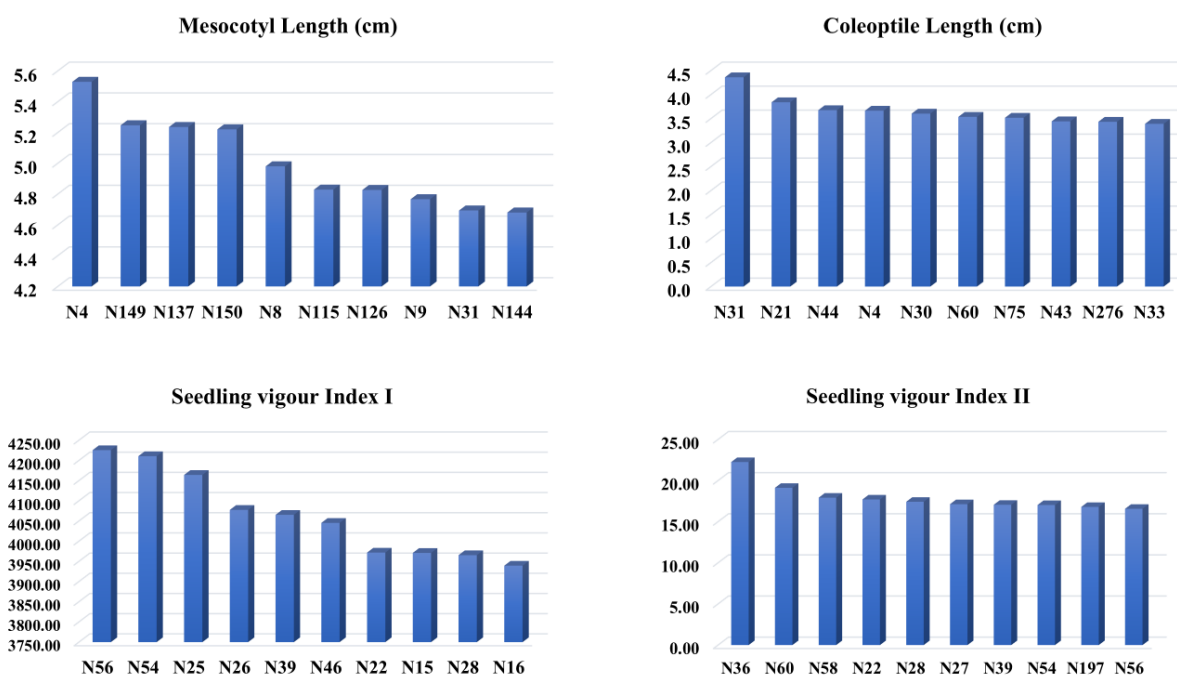


Fig. 1 : Top-performing genotypes for mesocotyl length (ML), coleoptile length (CL), Seedling Vigour Index-I (SVI-I), and Seedling Vigour Index-II (SVI-II).

Descriptive Statistics

Considerable phenotypic diversity was observed across all evaluated traits (Table 2). Germination percentage ranged from 9.33% to 99.33% with a mean of 67.32% and standard deviation of 28.41%, indicating substantial variation in germination capacity among accessions. This wide range reflects the inclusion of diverse germplasm types, from landraces with variable dormancy characteristics to improved varieties with high germinability. Mesocotyl length displayed the widest absolute range among morphological traits, varying from 0.22 cm to 5.53 cm (mean = 2.30 cm, SD = 1.24 cm). This 25-fold difference between the shortest and longest mesocotyls underscores the considerable genetic variation available for selection of deep-sowing adaptation. The continuous distribution of mesocotyl length without distinct clustering suggests polygenic inheritance consistent with previous quantitative genetic analyses (Wu *et al.*, 2015; Lu *et al.*, 2016).

Coleoptile length ranged from 1.54 cm to 4.36 cm (mean = 2.57 cm, SD = 0.46 cm), exhibiting a narrower distribution compared to mesocotyl length. The coefficient of variation for coleoptile length (17.9%) was substantially lower than that for mesocotyl length (53.9%), indicating that coleoptile elongation is relatively more conserved across diverse

germplasm. Shoot length varied from 7.56 cm to 25.70 cm (mean = 16.35 cm), while root length ranged from 10.80 cm to 26.21 cm (mean = 20.06 cm). The generally longer roots compared to shoots resulted in a mean root-to-shoot length ratio greater than unity, a pattern characteristic of seedlings evaluated under optimal moisture conditions where root system development is not constrained by soil impedance. Total seedling length ranged from 21.68 cm to 47.64 cm, reflecting the combined variation in shoot and root elongation.

Biomass-related traits also showed considerable variation. Total fresh weight ranged from 0.05 mg to 1.53 mg (mean = 0.86 mg), while total dry weight varied from 0.02 mg to 0.25 mg (mean = 0.14 mg). The fresh-to-dry weight ratio provides an indirect indicator of tissue hydration status, which can reflect metabolic activity and osmotic adjustment capacity during early seedling growth. The composite indices SVI-I and SVI-II exhibited wide ranges: 312.94 to 4,225.56 for SVI-I (mean = 1,796.32) and 0.56 to 22.25 for SVI-II (mean = 6.92). The broad ranges of these composite indices, which integrate germination efficiency with growth parameters, suggest that substantial gains in overall seedling vigour can be achieved through targeted selection.

Table 2: Descriptive Statistics for Early Seedling Vigour Traits in subset of 3K Panel of Rice using Paper Towel Method.

Sl. No.	Traits	Mean	Max	Min	Range	SD
1.	Germination percentage (%)	67.32	99.33	9.33	90.00	28.41
2.	Mesocotyl length (cm)	2.30	5.53	0.22	5.31	1.24
3.	Coleoptile length (cm)	2.57	4.36	1.54	2.82	0.46
4.	Shoot length (cm)	16.35	25.70	7.56	18.14	3.18
5.	Root length (cm)	20.06	26.21	10.80	15.41	2.71
6.	Seedling length (cm)	36.41	47.64	21.68	25.96	4.69
7.	Total fresh weight (mg)	0.86	1.53	0.05	1.47	0.23
8.	Total dry weight (mg)	0.14	0.25	0.02	0.23	0.04
9.	Seedling vigour index I	1796.32	4225.56	312.94	3912.62	1051.15
10.	Seedling vigour index II	6.92	22.25	0.56	21.69	4.81

Distribution of Mesocotyl and Coleoptile Length

The violin plot comparing the distributions of mesocotyl and coleoptile lengths revealed distinct patterns of phenotypic variation (Fig. 2). Mesocotyl length displayed a broad, right-skewed distribution with a substantially larger interquartile range and multiple extreme values at both ends of the distribution. The presence of several accessions with mesocotyl lengths exceeding 4.5 cm, alongside a considerable proportion of genotypes with mesocotyls shorter than 1.0 cm, indicates the presence of transgressive segregation in this diversity panel. In contrast, coleoptile length exhibited a more symmetric, approximately normal distribution with most genotypes clustered around the median value of approximately 2.5 cm. The smaller interquartile range and fewer

extreme observations for coleoptile length confirm its comparatively lower phenotypic variability relative to mesocotyl length.

Although the median values of the two traits were broadly similar (approximately 2.3 cm for mesocotyl and 2.5 cm for coleoptile), the markedly different dispersion patterns have important implications for breeding. The high variability of mesocotyl length suggests strong potential for selecting genotypes with superior deep-sowing adaptation, while the more constrained variation of coleoptile length may reflect stronger functional constraints on this trait. The continuous, approximately normal distribution of both traits supports their suitability for quantitative genetic analysis and marker-assisted selection approaches.

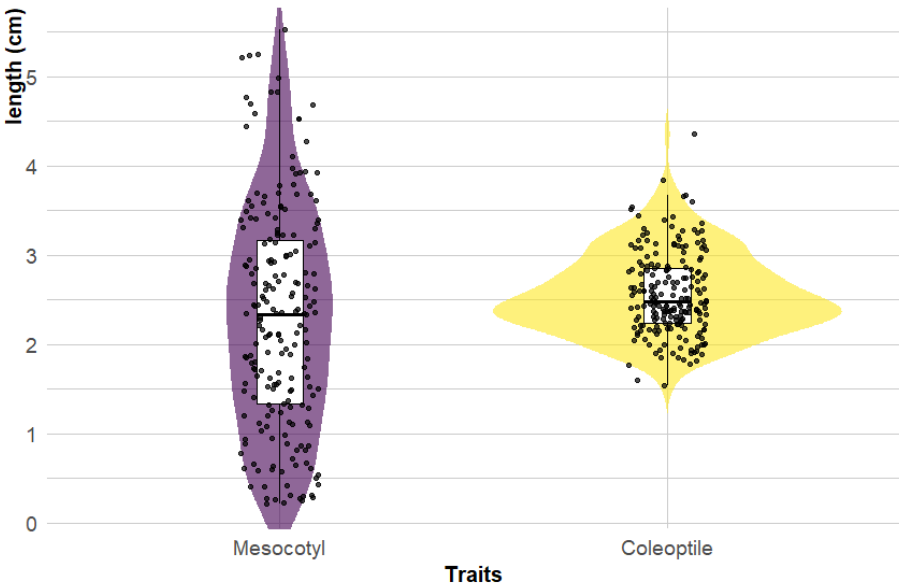


Fig. 2 : Distribution of mesocotyl length (ML) and coleoptile length (CL) among 203 rice accessions from the Indian subset of the 3K rice panel

Correlation Analysis

Pearson correlation analysis among the ten seedling vigour traits revealed several significant associations that provide insights into the underlying trait architecture and inform selection strategies (Fig. 3). Germination percentage exhibited strong positive correlations with both composite indices: SVI-I ($r = 0.82$, $P < 0.001$) and SVI-II ($r = 0.72$, $P < 0.001$). This pattern is mathematically expected given that germination percentage is a multiplicative component of both indices, but the strength of these associations also confirms that germination efficiency is a major determinant of overall seedling vigour. Interestingly, germination percentage showed weak negative correlation with mesocotyl length ($r = -0.22$, $P < 0.01$), suggesting that accessions with rapid germination may not necessarily possess long mesocotyls, and that these traits may respond differentially to selection pressures.

Mesocotyl length demonstrated significant positive correlations with coleoptile length ($r = 0.39$, $P < 0.001$), shoot length ($r = 0.49$, $P < 0.001$), and seedling length ($r = 0.29$, $P < 0.01$). The positive correlation between mesocotyl and coleoptile lengths ($r = 0.39$) is particularly noteworthy from a breeding perspective, as it suggests that selection for either trait would confer partial improvement in the other, facilitating simultaneous enhancement of both emergence pathways. This association aligns with previous reports by Dilday *et al.* (1990) and Alibu *et al.* (2012), who documented coordinated elongation of these tissues in rice seedlings. The absence of significant correlation between mesocotyl length and the two seedling vigour indices ($r = -0.13$ and $r = -0.15$ for SVI-I and SVI-II, respectively) reflects the dominance of germination percentage in the index calculations, which masks the contribution of mesocotyl elongation in genotypes with suboptimal germination.

Coleoptile length showed highly significant positive correlations with shoot length ($r = 0.55$, $P < 0.001$) and seedling length ($r = 0.40$, $P < 0.001$), but was not significantly associated with root length ($r = 0.045$, ns). This differential association pattern reflects the anatomical continuity between the coleoptile and shoot tissues, both of which derive from the plumule, versus the independent developmental origin of the root system from the radicle. Coleoptile length also correlated positively with SVI-I ($r = 0.25$, $P < 0.001$)

and SVI-II ($r = 0.20$, $P < 0.01$), indicating that longer coleoptiles contribute to enhanced overall seedling vigour as captured by these composite indices.

Shoot length and root length were moderately correlated ($r = 0.25$, $P < 0.001$), suggesting partial independence in the genetic control of above-ground and below-ground growth during the seedling stage. Both traits exhibited strong positive correlations with total seedling length ($r = 0.82$ and $r = 0.76$, respectively, $P < 0.001$), as expected given their additive contribution to this composite measure. Root length also correlated significantly with fresh weight ($r = 0.34$, $P < 0.001$), dry weight ($r = 0.20$, $P < 0.01$), and both vigour indices ($r = 0.24$ and $r = 0.16$, $P < 0.001$ and $P < 0.05$, respectively), underscoring the importance of root system development for early biomass accumulation and vigour expression.

The two composite indices, SVI-I and SVI-II, were very strongly inter-correlated ($r = 0.90$, $P < 0.001$), confirming their concordance as overall measures of seedling vigour. SVI-I showed significant positive associations with shoot length ($r = 0.41$), seedling length ($r = 0.41$), and root length ($r = 0.24$), while SVI-II correlated strongly with dry weight ($r = 0.39$, $P < 0.001$). Rao *et al.* (2020) independently demonstrated that seedling height and fresh weight together accurately predict dry biomass accumulation in rice seedlings, reinforcing that SVI-II effectively captures the biomass efficiency component of seedling vigour. Akshitha *et al.* (2020) similarly reported germination percentage as the dominant driver of vigour index values, with shoot length and dry weight as secondary contributors, consistent with the correlation structure observed in the present study. The strongest correlation for SVI-II after the SVI-I association was with dry weight ($r = 0.66$, $P < 0.001$), reinforcing that SVI-II effectively captures the biomass accumulation component of seedling vigour. Dry weight also emerged as a hub trait, correlating significantly with fresh weight ($r = 0.37$), SVI-I ($r = 0.66$), and SVI-II ($r = 0.39$), all at $P < 0.001$. These correlation patterns collectively suggest that a multi-trait selection index incorporating mesocotyl length, coleoptile length, and dry matter accumulation would be more effective than single-trait selection for improving overall seedling vigour under DDSR conditions.

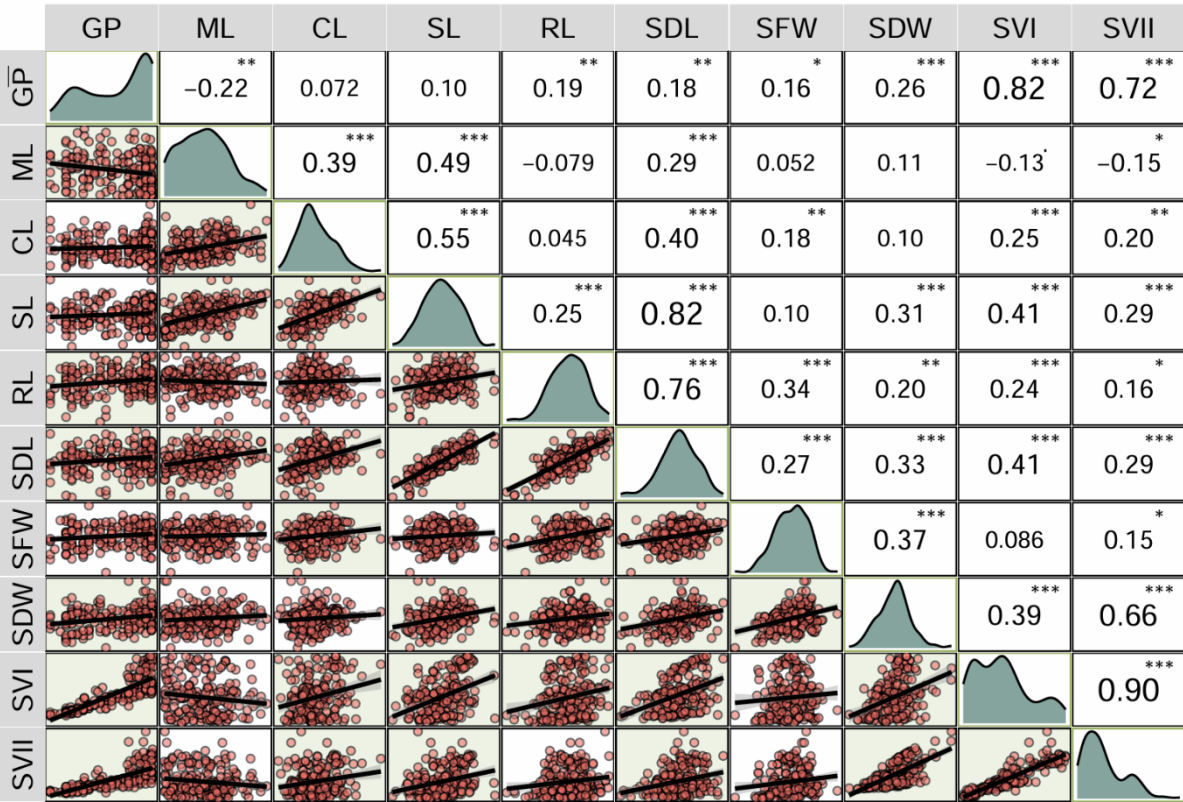


Fig. 3 : Correlation matrix depicting Pearson correlation coefficients among ten early seedling vigour traits in 203 rice accessions from the Indian subset of the 3,000 Rice Genomes Project. GP: germination percentage; ML: mesocotyl length; CL: coleoptile length; SL: shoot length; RL: root length; SDL: seedling length; SFW: seedling fresh weight; SDW: seedling dry weight; SVI-I: seedling vigour index-I; SVI-II: seedling vigour index-II. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Identification of Superior Genotypes

Based on the combined performance across multiple seedling vigour traits, several accessions distinguished themselves as consistently superior performers. Accessions with mesocotyl length exceeding 4.5 cm, representing the top 5% of the distribution, were identified as promising donors for deep-sowing adaptation. These accessions, predominantly of aus and indica subspecies origin, exhibited mesocotyl lengths substantially greater than all check varieties including Brown Gora, which is recognized for its seedling vigour. The superior mesocotyl elongation in these accessions, when combined with their above-average coleoptile lengths, suggests enhanced capacity for seedling emergence from sowing depths of 4-5 cm, which are typical of mechanized DDSR systems.

For the composite indices, accessions ranking in the top decile for both SVI-I and SVI-II were identified as elite genotypes with balanced performance across germination efficiency and growth parameters. These accessions surpassed the best check variety (Brown Gora) for both indices, demonstrating their potential breeding value. The correlation between SVI-I and

SVI-II ($r = 0.90$) ensured substantial overlap between the top-performing groups identified by each index, yet some accessions showed differential ranking, reflecting variation in the relative contribution of seedling length versus dry weight to overall vigour. Accessions combining high SVI-II with above-average mesocotyl length are particularly valuable as they integrate efficient germination, robust biomass accumulation, and enhanced deep-sowing emergence capacity.

Notably, several accessions of landrace origin exhibited exceptional mesocotyl elongation despite moderate germination percentages, resulting in intermediate SVI rankings. These genotypes represent valuable genetic resources for dissecting the specific genetic architecture of mesocotyl elongation independent of germination efficiency, and could serve as specialized donors in crossing programmes aimed at combining superior mesocotyl length with high germinability. The diverse genetic backgrounds of the identified superior accessions, spanning multiple geographic origins within the Indian subcontinent, reduce the risk of genetic vulnerability associated with narrow breeding pools and offer opportunities for

pyramiding complementary alleles for enhanced seedling vigour.

Discussion

The present study provides one of the most comprehensive phenotypic characterizations of early seedling vigour traits in a large, geographically representative subset of the 3,000 Rice Genomes Project. The evaluation of 203 Indian rice accessions using a standardized, high-throughput paper towel method revealed substantial genetic variation for all ten seedling vigour components, identified promising trait associations relevant to selection strategies, and pinpointed superior genotypes with potential value for DDSR breeding programmes. The findings are discussed in the context of existing literature and their implications for rice improvement.

Genetic Variability and Breeding Potential

The highly significant genotypic mean squares observed for all traits ($P < 0.01$) confirm the presence of substantial additive genetic variation within the Indian 3K-RGP subset. This result is consistent with previous studies reporting significant genetic variability for seedling vigour components in smaller rice panels. For instance, Yang *et al.* (2020) documented significant differences in mesocotyl length among 290 rice accessions evaluated under multiple sowing depths, while Lu *et al.* (2016) reported broad variation for mesocotyl elongation in 469 indica accessions subjected to GWAS. Similarly, Subramanian *et al.* (2025) evaluated 510 Indian rice germplasm lines and reported highly significant genetic variance for all seedling vigour traits, and Anandan *et al.* (2016) established that ESV observations recorded at 14 DAS reliably estimate seedling vigour in large rice panels. The magnitude of variation observed in our study, particularly the 25-fold range in mesocotyl length (0.22–5.53 cm), exceeds the variability reported in most previous studies and underscores the value of mining large diversity panels for extreme phenotypes (Mahender *et al.*, 2015).

The coefficient of variation was remarkably low for structural length traits (shoot length: 0.27%; root length: 0.22%; seedling length: 0.23%), reflecting both high measurement precision under controlled laboratory conditions and the relatively narrow environmental variance component for these traits in the absence of soil impedance and moisture stress. By contrast, the higher CV values for SVI-I (3.69%) and SVI-II (3.81%) derive from the compounding of variation from their multiplicative components, a statistical property that amplifies the observable range of composite indices relative to their components. This

compounding effect, while inflating the apparent variability, also enhances the selection differential achievable for overall vigour improvement.

Mesocotyl and Coleoptile Elongation: Comparative Variation

The contrasting distribution patterns of mesocotyl and coleoptile lengths carry significant biological and breeding implications. The broad, right-skewed distribution of mesocotyl length with multiple extreme values suggests that this trait has evolved under diversifying selection pressures across different agro-ecological niches. In deep-sowing systems practiced in rainfed upland and aerobic rice environments, strong directional selection would favour accessions with elongated mesocotyls capable of pushing the shoot apex to the soil surface from greater depths (Ohno *et al.*, 2018; Zhan *et al.*, 2020). Conversely, in shallow-sowing or transplanting systems, mesocotyl elongation may be selectively neutral or even disadvantageous if it is associated with metabolic costs that divert resources from other growth processes. The presence of accessions with extremely short mesocotyls (< 0.5 cm) in our panel, predominantly from lowland ecotypes, supports this niche-differentiation hypothesis.

The comparatively narrower variation in coleoptile length (range: 1.54–4.36 cm) suggests stronger functional constraint on this trait, possibly reflecting its essential role in protecting the emerging plumule across all sowing conditions. Coleoptile elongation represents a more conserved adaptive response that operates across the full range of sowing depths, whereas mesocotyl elongation becomes critical primarily under deeper sowing (Turner *et al.*, 1982; Zhan *et al.*, 2020). This interpretation aligns with the findings of Lee *et al.* (2017), who demonstrated that coleoptile length remains the primary determinant of emergence at shallow sowing depths (1–2 cm), while mesocotyl elongation increasingly dominates at depths exceeding 3 cm. Akshitha *et al.* (2020) similarly reported a substantially lower coefficient of variation for coleoptile length compared to mesocotyl length across diverse rice germplasm, corroborating the comparatively constrained phenotypic range of coleoptile elongation observed in the present study. The complementary but differentially variable nature of these two traits suggests that breeding for DDSR adaptation should prioritize mesocotyl length as the primary selection target, with coleoptile length serving as a secondary trait to ensure emergence under shallow sowing or crusted soil conditions.

Trait Associations and Indirect Selection

The correlation matrix revealed several trait associations with practical significance for breeding programme design. The moderate positive correlation between mesocotyl and coleoptile lengths ($r = 0.39$) suggests partial sharing of genetic determinants for elongation growth in these two tissues. This association has been reported consistently across multiple studies (Dilday *et al.*, 1990; Alibu *et al.*, 2012; Yang *et al.*, 2020; Barik *et al.*, 2022) and may reflect pleiotropic effects of gibberellin signalling pathways or cell wall loosening enzymes that operate in both mesocotyl and coleoptile tissues. From a selection standpoint, this positive correlation is advantageous because it permits concurrent improvement of both traits through selection for either one, reducing the number of independent selection criteria required in early generation screening.

The strong correlation between SVI-I and SVI-II ($r = 0.90$) indicates that either index can serve as an effective proxy for overall seedling vigour in phenotypic screening programmes. However, the differential associations of these indices with component traits offer nuanced selection opportunities. SVI-I correlated more strongly with shoot length and seedling length, which capture the spatial expansion of the seedling, whereas SVI-II showed its strongest component correlation with dry weight ($r = 0.66$), reflecting metabolic efficiency and resource accumulation. Depending on the target production environment, breeders may prioritize one index over the other: SVI-I may be more relevant for environments where rapid canopy closure is critical for weed suppression, while SVI-II may better predict performance under resource-limited conditions where metabolic efficiency determines survival.

The negative correlation between germination percentage and mesocotyl length ($r = -0.22$), though modest, warrants consideration. This antagonistic association suggests that some accessions may have evolved rapid germination as an alternative strategy to mesocotyl elongation for ensuring seedling emergence. In shallow-sowing systems typical of broadcast DDSR, rapid germination and early leaf expansion may confer equivalent establishment advantage to mesocotyl elongation, and the observed negative correlation may reflect adaptation to different sowing depths within the panel. This finding reinforces the need for multi-trait selection indices that balance germination efficiency with elongation capacity rather than relying on single-trait selection (Mahender *et al.*, 2015).

Implications for Direct-Seeded Rice Breeding

The identification of accessions with mesocotyl lengths exceeding 4.5 cm, surpassing all check varieties including the vigorous landscape Brown Gora, represents a significant outcome of this study. These accessions provide novel genetic resources for introgressing superior mesocotyl elongation into elite breeding lines without the yield penalties often associated with traditional landrace donors. Field evidence from Ohno *et al.* (2018) confirms that mesocotyl lengths exceeding 4 cm translate directly into improved seedling establishment rates under DDSR conditions, validating the breeding value of the superior accessions identified in the present study. The availability of re-sequencing data for the entire 3K-RGP panel (Wang *et al.*, 2018) enables immediate initiation of association mapping to identify the underlying genetic variants, accelerating the transition from phenotypic selection to marker-assisted breeding.

The paper towel method employed in this study offers a robust, cost-effective, and high-throughput platform for first-tier screening of large germplasm collections. The method's correlation with field emergence under DDSR has been validated in previous studies (Addanki *et al.*, 2019; Barik *et al.*, 2022; Anandan *et al.*, 2016), and the controlled conditions eliminate the confounding effects of soil heterogeneity, moisture gradients, and biotic stresses that complicate field-based phenotyping. However, we acknowledge that laboratory-based measurements may not fully capture genotype $\times$ environment interactions that modulate seedling vigour expression under field conditions. Therefore, the top-performing accessions identified in this study should be validated through multi-location field trials under actual DDSR conditions before deployment in breeding programmes.

The integration of our phenotypic dataset with the publicly available genomic data from the 3K-RGP opens avenues for GWAS and genomic selection applications. Previous GWAS studies for mesocotyl length have identified significant SNPs near gibberellin biosynthesis genes (Wu *et al.*, 2015), auxin transporters (Wang *et al.*, 2021), and cell wall-related genes (Lu *et al.*, 2016; Subramanian *et al.*, 2025). The comprehensive QTL catalogue spanning chromosomes 1, 3, 5, 6, 9, 11, and 12 for multiple ESV traits (Mahender *et al.*, 2015) provides a comparative genomic framework within which novel loci detectable from the present panel may be interpreted. The expanded sample size and enhanced phenotypic resolution of our dataset may enable detection of additional loci with smaller effect sizes that collectively explain a larger proportion of the genetic

variance. Furthermore, the haplotype diversity captured in the Indian subset may reveal novel functional variants not detected in previous studies dominated by East Asian germplasm.

Methodological Considerations

Several methodological aspects of this study merit discussion. The use of 50 seeds per replication, while providing robust estimates of germination percentage, may have introduced micro-environmental variation within replicates due to positional effects within the paper towel setup. However, the completely randomized design and the inclusion of three replications should have effectively controlled for such variation. The measurement of mesocotyl length at 14 DAS captures the maximum elongation phase under dark/skotomorphogenic conditions, which approximates the growth condition experienced by mesocotyl tissue buried under soil. The strong correlation between laboratory-measured mesocotyl length and field emergence from depth, reported by Lee *et al.* (2017) and Yang *et al.* (2020), supports the ecological validity of our phenotyping approach. Redona and Mackill (2009) further demonstrated that QTLs for shoot length and dry weight measured under greenhouse conditions co-localize with field early vigour QTLs, providing additional evidence that controlled laboratory phenotyping reliably predicts field performance. Padhi *et al.* (2023) validated this further by confirming that accessions identified as superior using the paper roll method under laboratory conditions also outperformed the high-vigour check Sabita under actual DSR field conditions across two consecutive seasons.

The choice of 14 DAS as the measurement time point represents a balance between capturing maximum elongation growth and avoiding the confounding effects of secondary leaf expansion and root branching that become prominent after this stage. The five-day dormancy-breaking treatment at 50 degrees C was effective in ensuring uniform germination across accessions, but may have differentially affected genotypes with varying dormancy intensities. Future studies could benefit from including dormancy-related traits as covariates in the analysis to account for their potential influence on germination dynamics.

Conclusion

The phenotypic evaluation of 203 rice accessions from the Indian subset of the 3,000 Rice Genomes Project revealed substantial genetic variation for all ten early seedling vigour traits, with highly significant genotypic differences for germination percentage,

mesocotyl length, coleoptile length, shoot and root lengths, seedling length, biomass parameters, and two composite seedling vigour indices. Mesocotyl length exhibited the widest phenotypic range (0.22-5.53 cm), highlighting considerable scope for selecting genotypes with superior deep-sowing adaptation. The significant positive correlation between mesocotyl and coleoptile lengths ($r = 0.39$) supports concurrent improvement of both emergence-related traits through integrated selection strategies.

Several accessions with mesocotyl lengths exceeding 4.5 cm, surpassing the best check variety Brown Gora, were identified as novel genetic resources for DDSR breeding. The strong inter-correlation between SVI-I and SVI-II ($r = 0.90$) confirms their utility as complementary overall measures of seedling vigour, while their differential associations with component traits offer nuanced selection opportunities tailored to specific target environments. The laboratory-based paper towel method proved effective as a first-tier screening tool for large germplasm collections, and the resulting phenotypic dataset provides a valuable resource for downstream genome-wide association studies aimed at dissecting the genetic architecture of seedling vigour in rice. Future research should focus on validating the superior accessions under multi-location field conditions and integrating the phenotypic data with genomic information to accelerate marker-assisted breeding for improved DDSR adaptation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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