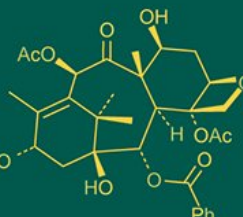
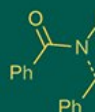
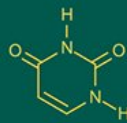


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Assessment of genetic variability, heritability and genetic advance for seedling traits associated with natural cold screening in rice (*Oryza Sativa* L.) hybrids

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Abstract

This study was conducted to estimate the extent of genetic variability, broad-sense heritability and genetic advance for important seedling-stage traits associated with cold tolerance in rice (*Oryza sativa* L.). The experimental material comprised 33 rice genotypes, including four cytoplasmic male sterile (CMS) lines, five restorers (testers), twenty F₁ hybrids and four standard checks, which were evaluated under natural low-temperature conditions during the *Rabi* 2025 season at the Regional Sugarcane and Rice Research Station (RS&RRS), Rudrur, Nizamabad. The trial was laid out in a Randomized Block Design (RBD) with two replications. Data were recorded for seven seedling traits, namely Germination%, Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Shoot Length - 8-10 DAS, Root length - 8-10 DAS, Chlorophyll Content (SPAD) and Survival Rate (%). Analysis of variance revealed highly significant differences among the evaluated genotypes for all the studied traits, indicating the existence of considerable genetic variation for improving seedling-stage cold tolerance. The small differences observed between the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) suggested that environmental influence on trait expression was relatively limited. Among the evaluated traits, Leaf Yellowing Score (1-9) recorded the highest GCV (49.89%), PCV (50.73%), broad-sense heritability (96.72%) and genetic advance as a percentage of the mean (50.08%), indicating its potential as a reliable selection criterion for identifying cold-tolerant rice genotypes. Broad-sense heritability ranged from 81.90% to 96.72%, while genetic advance as a percentage of the mean varied from 19.96% to 50.08%, demonstrating favourable prospects for genetic improvement through selection. Based on the estimated genetic parameters, Germination %, Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Root length (cm) - 8-10 DAS and Survival Rate (%) were identified as the most promising traits for the selection of superior cold-tolerant genotypes, whereas Shoot Length (cm) - 8-10 DAS and Chlorophyll Content (SPAD) also contributed to the evaluation of seedling performance under low-temperature stress. Furthermore, line × tester analysis revealed the involvement of both additive and non-additive gene effects, with dominance effects playing an important role in the inheritance of these traits. Overall, the findings provide valuable information for identifying promising parental materials and support future breeding efforts aimed at developing high-yielding rice cultivars with enhanced seedling-stage cold tolerance.

Keywords: Cold tolerance, Seedling traits, Genetic variability, Heritability, Genetic advance, Seedling Chlorophyll Content (SPAD)

Introduction

Rice (*Oryza sativa* L.) is a major staple cereal that supplies the principal source of dietary energy for more than half of the world's population, making it indispensable for global food and nutritional security. Ensuring stable rice production under adverse environmental conditions is essential to meet the increasing food demand of the growing global population. Rice accounts for nearly one-fifth of the global dietary energy supply while also contributing substantially to rural employment, household income and food security, especially in Asian countries (Khush, 2005) [8]. According to FAO (2025) (4), global rice production is projected to reach 551.5 million tonnes, emphasizing the increasing importance of rice in meeting the food requirements of a growing population. In India, rice is cultivated over approximately 51.27 million hectares, producing a record 150.18 million tonnes during 2024-25, as reported by (India stat, 2025) (7) and the Department of Agriculture and Farmers Welfare, Government of India (2025) [6]. These production figures highlight the continuing importance

of rice in ensuring food availability and nutritional security at both national and global levels.

Although considerable progress has been achieved in rice improvement and cultivation practices, maintaining consistent productivity under environmental stresses continues to be a major concern. Low-temperature stress is one of the most limiting abiotic factors affecting rice production, particularly in temperate and high-altitude subtropical ecosystems. Exposure to chilling temperatures during germination and early seedling growth severely impairs crop establishment, ultimately leading to reduced plant survival and lower grain yield (Sthapit and Witcombe, 1998) ^[19]. Chilling temperatures below 15°C disrupt several physiological processes, including seed germination, seedling growth, chlorophyll accumulation, membrane integrity and seedling survival (Shimono *et al.* 2007) ^[17]. Therefore, evaluation at the seedling stage provides an effective approach for identifying rice genotypes with improved tolerance to low-temperature stress.

A range of morphological and physiological characteristics have been widely employed to assess cold tolerance during the early stages of rice growth. Germination% and Seedling Vigour Index (SVI) are widely employed to assess early seedling establishment and tolerance to chilling conditions in rice (Monzon *et al.* 2023) ^[13]. Likewise, Shoot Length (cm) at 8-10 DAS and Root Length (cm) at 8-10 DAS are commonly measured to evaluate seedling growth, since exposure to low temperatures markedly restricts both shoot and root development (Cruz and Milach, 2004) ^[2]. Chlorophyll Content (SPAD) provides an estimate of photosynthetic efficiency and physiological health under stress, whereas the Leaf Yellowing Score (1-9) reflects the severity of cold-induced chlorosis and tissue damage (Andaya and Mackill, 2003) ^[1]. Survival Rate (%) is regarded as one of the most dependable indicators of the ability of seedlings to tolerate and recover from chilling stress (Kim and Tai, 2011) ^[9]. Together, these parameters offer valuable insights into the growth performance and physiological adaptation of rice seedlings exposed to chilling stress.

Seedling-stage cold tolerance in rice is a complex quantitative trait governed by the combined action of numerous genes and quantitative trait loci (QTLs). Advances in molecular genetics have considerably improved the understanding of the genetic basis of cold tolerance in rice. The major gene qLTG3-1, associated with improved low-temperature germinability, was identified by Fujino *et al.* (2008) ^[5]. Similarly, Ma *et al.* (2015) ^[12] reported the COLD1 gene, which enhances chilling tolerance by regulating calcium-mediated signalling pathways. In addition, several QTLs governing seedling-stage cold tolerance have been identified, further demonstrating the polygenic nature of cold tolerance in rice, as reported by Andaya and Mackill (2003) ^[1].

The availability of sufficient genetic variability is essential for the effective improvement of economically important traits through plant breeding. Estimation of genetic parameters including GCV, PCV, heritability and genetic advance helps breeders identify traits that can be effectively improved through selection. Sufficient genetic variation among rice genotypes is essential for effective selection and the enhancement of economically important traits, as emphasized by Padmaja *et al.* (2008) ^[14]. Estimation of the

mean and range further facilitates the understanding of the average performance and magnitude of variation present within the breeding material. High heritability values suggest that genetic effects contribute substantially to the expression of seedling-stage cold tolerance traits, thereby improving the efficiency of selection, as demonstrated by Fujino *et al.* (2008) ^[5] who identified the qLTG3-1 locus associated with low-temperature germinability in rice. Nevertheless, heritability alone cannot accurately predict the expected genetic gain achieved through selection. Consequently, the combined evaluation of heritability and genetic advance offers a more dependable estimate of the expected response to selection in breeding programmes. Traits characterized by high heritability along with high genetic advance are usually influenced predominantly by additive genetic effects, making them suitable targets for direct phenotypic selection. The successful identification and utilization of major loci such as qLTG3-1 for improving low-temperature germinability further highlight the importance of exploitable genetic variation in developing cold-tolerant rice cultivars (Fujino *et al.* 2008) ^[5]. Consequently, traits possessing high heritability along with substantial genetic advance represent valuable targets for breeding programmes aimed at enhancing cold tolerance in rice.

Exploitation of heterosis through hybrid rice breeding has emerged as an effective approach for enhancing grain productivity. Compared with conventional inbred cultivars, hybrid rice varieties generally produce 15-30% higher grain yield, as reported by Virmani (1994) ^[20]. Accordingly, the identification of genetically diverse parents and superior hybrids with desirable seedling characteristics is essential for developing rice cultivars capable of performing well under low-temperature conditions. In this context, the present study was undertaken to estimate the mean, range, genetic variability, heritability and genetic advance for important seedling-stage traits associated with cold tolerance in rice and to identify promising parents and hybrids that can be effectively utilized in future rice improvement programmes under low-temperature conditions.

2. Material and Methods

2.1 Experimental site and plant genetic material

The field experiment was carried out during the *Rabi* 2025 season at the Regional Sugarcane and Rice Research Station (RS&RRS), Rudrur, Nizamabad, Telangana, under naturally occurring low-temperature conditions. The location experiences tropical climatic conditions with comparatively lower temperatures during the early crop growth stage, providing a suitable environment for assessing seedling-stage cold tolerance in rice.

The experimental material comprised 33 rice genotypes developed using a line x tester mating design. The genetic material included 20 F₁ hybrids, four CMS lines (A lines) and five restorers/ testers (R). In addition, four standard checks, namely US 312 (hybrid check), KNM 118 (varietal check), Tellahamsa (cold-tolerant check) and JGL 1798 (cold-susceptible check) were included for comparison. Overall, the experimental set consisted of nine parental lines (four CMS lines and five restorers), twenty F₁ hybrids and four standard checks, making a total of 33 rice genotypes as presented in Table 1.

Table 1: Experimental material comprising parental lines, testers, F₁ hybrids and standard checks used in the study

Lines (4)	Source of collection
1.RMS6A	RARS, Polasa, Jagtial
2.JMS13A	RARS, Polasa, Jagtial
3.CMS52A	RARS, Polasa, Jagtial
4.CMS59A	RARS, Polasa, Jagtial
Testers (5)	
1.R1	RARS, Polasa, Jagtial
2. R131	RARS, Polasa, Jagtial
3. R132	RARS, Polasa, Jagtial
4. R156	RARS, Polasa, Jagtial
5. R220	RARS, Polasa, Jagtial
Hybrids (20)	
1.RMS6AXR1	RARS, Polasa, Jagtial
2.RMS6AXR131	RARS, Polasa, Jagtial
3.RMS6AXR132	RARS, Polasa, Jagtial
4.RMS6AXR156	RARS, Polasa, Jagtial
5. RMS6AXR220	RARS, Polasa, Jagtial
6. JMS13AXR1	RARS, Polasa, Jagtial
7. JMS13AXR131	RARS, Polasa, Jagtial
8. JMS13AXR132	RARS, Polasa, Jagtial
9. JMS13AXR156	RARS, Polasa, Jagtial
10. JMS13AXR220	RARS, Polasa, Jagtial
11. CMS52AXR1	RARS, Polasa, Jagtial
12. CMS52AXR131	RARS, Polasa, Jagtial
13. CMS52AXR132	RARS, Polasa, Jagtial
14. CMS52AXR156	RARS, Polasa, Jagtial
15. CMS52AXR220	RARS, Polasa, Jagtial
16. CMS59AXR1	RARS, Polasa, Jagtial
17. CMS59AXR131	RARS, Polasa, Jagtial
18. CMS59AXR132	RARS, Polasa, Jagtial
19. CMS59AXR156	RARS, Polasa, Jagtial
20. CMS59AXR220	RARS, Polasa, Jagtial
Checks (4)	
1. US312 (H)	RARS, Polasa, Jagtial
2. KNM118 (V)	RARS, Polasa, Jagtial
3. TELLAHAMSA (CT)	RARS, Polasa, Jagtial
4. JGL1798 (CS)	RARS, Polasa, Jagtial

2.2 Experimental design and crop management

The experimental material was evaluated in a Randomized Block Design (RBD) with two replications. Each genotype was planted in 3 metres of row length, maintaining a spacing of 20 cm between rows and 15 cm between plants within each plot. Standard agronomic practices and recommended crop management measures were implemented uniformly throughout the experimental period to ensure optimum crop establishment and normal plant growth

2.3 Assessment of seedling traits, data recording and statistical analysis

The recorded observations were statistically analysed to determine the mean, minimum and maximum values (range), genetic variability, broad-sense heritability and genetic advance for important seedling-stage traits related to cold conditions in rice. Data were collected for Germination %, Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Shoot Length (cm) - 8-10 DAS, Root Length (cm) - 8-10 DAS, Chlorophyll Content (SPAD) and Survival Rate (%). The experimental data were analysed using analysis of variance (ANOVA) appropriate for a Randomized Block Design (RBD) following the procedure described by Panse and Sukhatme (1985) [15]. The significance of differences among genotypes was evaluated using the F-test at the 5% and 1% probability levels.

The extent of genetic variability was estimated by calculating the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). Broad-sense heritability (h^2), genetic advance (GA) and genetic advance as per cent of mean (GAM) were also computed using standard biometrical methods to assess the inheritance pattern of the traits and to predict the effectiveness of selection for improving seedling-stage cold tolerance in rice.

3. Results and discussion

3.1 Analysis of variance

The analysis of variance revealed highly significant differences among the parents for all the seedling traits, indicating the presence of substantial genetic variability (as shown in Table 2). Significant differences among lines and testers were observed for Germination (%), Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Shoot Length (cm) at 8-10 DAS and Chlorophyll Content (SPAD). The comparison between parents and hybrids showed significant differences for Germination (%), Shoot Length (cm) at 8-10 DAS, Root Length (cm) at 8-10 DAS, Chlorophyll Content (SPAD) and Survival Rate (%), indicating the expression of heterosis for these traits. Significant line x tester interactions for most of the seedling traits suggest the important contribution of non-additive gene effects in the inheritance of seedling-stage cold tolerance. Furthermore, the relatively low error variances indicate good experimental precision

and reliability of the recorded data. These findings suggest that the identification of superior parental combinations is essential for the development of high-performing cold-tolerant rice hybrids.

The mean performance and range (minimum and maximum values) of all the seedling traits are presented in Table 3,

while their graphical representation is provided in Fig. 1 and Fig. 2. The observed variation

among the genotypes further confirms the availability of sufficient genetic variability, providing good scope for effective selection and the identification of superior parental combinations for developing cold-tolerant rice hybrids.

Table 2: Analysis of variance (ANOVA) for seedling-stage cold tolerance traits under natural field conditions in rice

Source of Variation	DF	Germination %	Seedling Vigour Index (SVI)	Leaf Yellowing Score (1-9)	Shoot length (cm) -8-10 DAS	Root length (cm)-8-10 DAS	Chlorophyll content (SPAD)	Survival Rate (%)
Replicates	1	510.069***	54577.870***	0.276	0.008	0.068*	0.173	441.379***
Treatments	28	147.397***	19138.870***	6.094***	1.541***	2.579***	20.183***	151.883***
Parents	8	128.972***	21153.010***	8.000***	1.540***	0.188***	31.677***	130.556***
Parents (Lines)	3	269.125***	38330.160***	10.667***	1.409***	0.306***	56.551***	269.125***
Parents (Testers)	4	48.150***	5399.376*	6.400***	1.272***	0.134***	14.691***	51.900***
Parents (Lines vs Testers)	1	31.803*	32636.080***	6.400***	3.007***	0.052	24.996***	29.469
Parents vs Crosses	1	199.726***	6341.546*	0.221	1.651***	5.460***	6.118***	117.805***
Crosses	19	152.400***	18964.350***	5.600***	1.536***	3.434***	16.084***	162.657***
Lines	3	110.467	24013.660	10.933	0.161	2.797	23.115	144.425
Testers	4	261.650	24530.310	6.850	0.810	5.261	20.506	202.588
Lines × Testers	12	126.467***	15846.700***	3.850***	2.122***	2.984***	12.852***	153.904***
Error	28	7.569	1443.590	0.133	0.029	0.015	0.227	7.558
Total	57	85.072	11068.190	3.064	0.772	1.276	10.029	86.065

Note: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. SPAD = Soil Plant Analysis Development (chlorophyll meter reading).

3.2 Estimates of mean, range (maximum and minimum), genetic variability, heritability and genetic advance for seedling traits under cold conditions in rice

3.2.1 Germination (%)

Germination percentage exhibited a wide range from 50.00 to 94.00%, with a mean value of 66.67%, indicating substantial genetic variability among the evaluated rice genotypes under cold stress conditions. The genotypic coefficient of variation (GCV, 17.98%) and phenotypic coefficient of variation (PCV, 18.54%) were moderate and closely comparable, suggesting that environmental influence on the expression of this trait was minimal. The trait also recorded high broad-sense heritability (94.09%) along with high genetic advance as percent of mean (35.94%), indicating the predominance of additive gene action. Therefore, germination percentage can be considered a dependable selection criterion for improving seedling-stage cold tolerance in rice through direct phenotypic selection. Similar findings were reported by Singh *et al.* (2021) ^[1], who recognized germination percentage as an important indicator of cold tolerance during the early seedling stage.

3.2.2 Shoot length (cm) - 8-10 das

Shoot length under cold stress ranged from 5.50 to 8.79 cm, with an average value of 7.25 cm, indicating the presence of moderate variation among the evaluated rice genotypes. The estimates of genotypic coefficient of variation (GCV, 12.85%) and phenotypic coefficient of variation (PCV, 13.72%) were moderate and closely associated, suggesting that environmental factors had only a minor effect on the expression of this trait. A high broad-sense heritability (87.80%) coupled with moderate genetic advance as percent of mean (24.82%) indicated the involvement of both additive and non-additive gene effects in the inheritance of shoot length. These results suggest that although phenotypic

selection can improve this trait; the magnitude of genetic progress may be moderate compared with traits exhibiting higher genetic advance. Similar observations were reported by Zhang *et al.* (2023) ^[21].

3.2.3 Root length (cm) - 8-10 das

Root length under cold stress varied from 2.50 to 8.33 cm, with a mean of 3.64 cm, indicating considerable variation among the evaluated rice genotypes. The genotypic coefficient of variation (GCV, 18.64%) and phenotypic coefficient of variation (PCV, 20.15%) were moderate, reflecting the presence of sufficient genetic variability with only a limited environmental influence on trait expression. The high broad-sense heritability (85.60%) together with high genetic advance as percent of mean (35.47%) suggested that additive gene effects predominantly govern root length. Therefore, this trait can serve as an effective selection criterion for enhancing seedling-stage cold tolerance in rice. Similar findings were reported by Li *et al.* (2024) ^[10].

3.2.4 Chlorophyll content (SPAD)

Chlorophyll content ranged from 15.77 to 29.21 SPAD units, with an average value of 21.12 SPAD units, demonstrating moderate variation among the rice genotypes under cold stress. The GCV (10.72%) and PCV (11.85%) values were relatively low and closely associated, indicating that environmental effects on chlorophyll retention were comparatively small. The trait exhibited high broad-sense heritability (81.90%) along with moderate genetic advance as percent of mean (19.96%), suggesting that although chlorophyll content is largely under genetic control, the expected response to selection would be moderate. Comparable observations were reported by Andaya and Mackill (2003) ^[1].

3.2.5 Survival rate (%)

Survival rate following cold stress ranged from 49.50 to 92.50%, with a mean value of 66.00%, indicating substantial differences in the ability of genotypes to recover after exposure to low temperatures. Moderate estimates of GCV (16.42%) and PCV (17.58%) indicated appreciable genetic variability, while the high broad-sense heritability (87.30%) and high genetic advance as percent of mean (31.24%) suggested the predominance of additive gene action. Since survival rate directly reflects the capacity of seedlings to withstand chilling stress, it is considered a dependable trait for selecting cold-tolerant rice genotypes. Similar results were documented by Zhang *et al.* (2023) ^[21].

3.2.6 Seedling vigour index (SVI)

Seedling Vigour Index (SVI) showed a broad range of variation from 483.14 to 1185.65 with a mean value of 731.19 reflecting substantial genetic diversity among the studied genotypes. The relatively high GCV (22.46%) and PCV (23.40%) values, together with the narrow difference between them, indicated that environmental influence on this trait was minimal. The combination of high broad-sense heritability

(92.11%) and high genetic advance as percent of mean (44.40%) revealed that additive genetic effects play a major role in the inheritance of seedling vigour. Hence, SVI is an effective trait for direct phenotypic selection aimed at improving seedling-stage cold tolerance. Similar findings were reported by Sadhana *et al.* (2022) ^[16].

3.2.7 Leaf yellowing score (1-9)

Among all the seedling traits evaluated, leaf yellowing score (1-9) displayed the greatest variability, with values ranging from 1.00 to 7.00 and a mean of 3.73. The high GCV (49.89%) and PCV (50.73%) values reflected abundant genetic variation for this trait, while the narrow difference between these estimates suggested only a limited influence of environmental factors. The exceptionally high broad-sense heritability (96.72%) together with a genetic advance amounting to 50.08% of the mean indicates that additive genetic effects largely govern the expression of leaf yellowing under cold stress. Owing to its wide variability and strong genetic control, leaf yellowing score represents a valuable phenotypic indicator for the effective selection of cold-tolerant rice genotypes at the seedling stage. Comparable observations were reported by Li *et al.* (2022) ^[11].

Table 3: Estimates of Mean, Range (Maximum and Minimum), Genetic Variability, Heritability and Genetic Advance for Seedling Traits under Cold Conditions in Rice

Trait	Mean	Minimum	Maximum	GCV (%)	PCV (%)	Heritability (%)	Genetic Advance as % of Mean
Germination%	66.67	50.00	94.00	17.98	18.54	94.09	35.94
Seedling Vigour Index (SVI)	731.19	483.14	1185.65	22.46	23.40	92.11	44.40
Leaf Yellowing Score (1-9)	3.73	1.00	7.00	49.89	50.73	96.72	50.08
Shoot Length (cm) - 8-10 DAS	7.25	5.50	8.79	12.85	13.72	87.80	24.82
Root Length (cm) - 8-10 DAS	3.64	2.50	8.33	18.64	20.15	85.60	35.47
Chlorophyll Content (SPAD)	21.12	15.77	29.21	10.72	11.85	81.90	19.96
Survival Rate (%)	66.00	49.50	92.50	16.42	17.58	87.30	31.24

Note: GCV = Genotypic Coefficient of Variation, PCV = Phenotypic Coefficient of Variation H^2 = Broad Sense Heritability, GA = Genetic Advance, SPAD = Soil Plant Analysis Development (chlorophyll meter reading)

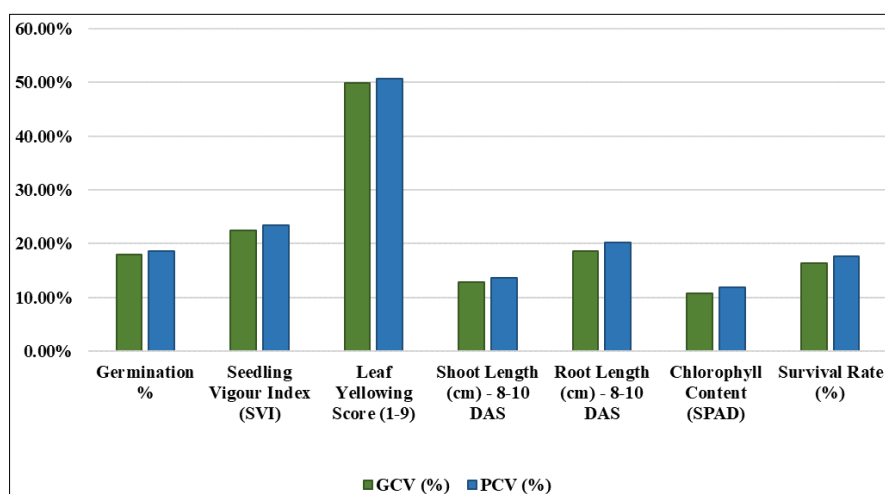


Fig 1: Illustration of GCV% and PCV% for Seedling Traits Under Natural Cold Conditions in Rice

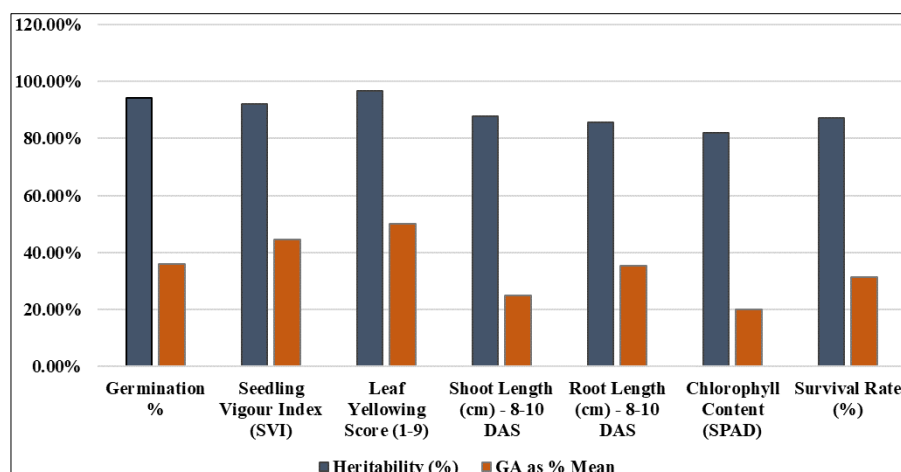


Fig 2: Illustration of Heritability (%), Genetic Advance as % Mean for Seedling Traits Under Cold Conditions in Rice

3.3 Estimates of genetic variance components, heritability and genetic advance for seedling traits under cold conditions in rice (line × tester analysis)

The present investigation demonstrated substantial genetic variability for all seedling traits evaluated under natural cold stress conditions. The estimates of additive genetic variance (σ^2A), dominance genetic variance (σ^2D), degree of dominance, phenotypic variance (σ^2P), narrow-sense heritability and genetic advance obtained from the line x tester analysis are presented in (Table 4). The consistently narrow differences between GCV and PCV across traits indicate that environmental effects on trait expression were relatively small, thereby enhancing the reliability of phenotypic selection. High heritability associated with high

genetic advance was observed for Germination%, Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Root length (cm) -8-10 DAS and Survival Rate (%), suggesting that these traits are predominantly governed by additive gene effects and are likely to respond effectively to direct selection. In contrast, Shoot length (cm) - 8-10 DAS and Chlorophyll Content (SPAD) exhibited high heritability but comparatively moderate genetic advance, indicating that both additive and non-additive gene effects contribute to their inheritance. Overall, the results highlight the usefulness of these seedling traits for identifying cold-tolerant rice genotypes and provide valuable information for developing improved rice cultivars adapted to low-temperature environments.

Table 4: Estimates of Genetic Variance Components, Heritability and Genetic Advance for Seedling Traits under Cold Conditions in Rice (Line × Tester Analysis)

Genetic Parameter	Germination%	Seedling Vigour Index (SVI)	Leaf Yellowing Score (1-9)	Shoot length (cm) - 8-10 DAS	Root length (cm) - 8-10 DAS	Chlorophyll Content (SPAD)	Survival Rate (%)
σ^2 Error	3.784	721.795	0.067	0.015	0.008	0.113	3.779
σ^2A (F = 1)	39.664	5072.977	1.946	0.101	0.892	4.796	36.877
σ^2D (F = 1)	59.449	7201.554	1.858	1.046	1.484	6.313	73.173
σ^2A / σ^2D (F = 1)	0.667	0.704	1.047	0.097	0.601	0.760	0.504
Degree of Dominance (F = 1)	1.224	1.191	0.977	3.213	1.290	1.147	1.409
σ^2A (F = 0)	79.329	10145.950	3.893	0.203	1.784	9.593	73.755
σ^2D (F = 0)	237.795	28806.210	7.434	4.185	5.937	25.251	292.693
σ^2A / σ^2D (F = 0)	0.334	0.352	0.524	0.048	0.300	0.380	0.252
Degree of Dominance (F = 0)	1.731	1.685	1.382	4.543	1.824	1.622	1.992
Phenotypic Variance (σ^2P)	102.898	12996.330	3.871	1.162	2.384	11.223	113.830
Heritability (Narrow Sense, %)	38.547	39.034	50.276	8.722	37.413	42.738	32.397
Genetic Advance (5%)	8.055	91.668	2.038	0.194	1.190	2.949	7.120

Note: σ^2A = Additive genetic variance; σ^2D = Dominance genetic variance; σ^2P = Phenotypic variance; Chlorophyll Content (SPAD) = Soil Plant Analysis Development (chlorophyll meter reading); F = Inbreeding coefficient.

4. Conclusion

The present study demonstrated the existence of significant genetic variability among the evaluated rice genotypes for all seedling-stage traits under natural low-temperature conditions, indicating ample opportunity for the genetic

improvement of cold tolerance in rice. The moderate to high values of both the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) observed for most traits reflect the presence of substantial inherent genetic variation. Furthermore, the narrow differences

between PCV and GCV estimates suggest that environmental effects on trait expression were minimal, indicating that the observed phenotypic variation was largely governed by genetic factors.

High estimates of broad-sense heritability coupled with moderate to high genetic advance and genetic advance as per cent of mean for most of the seedling traits indicate the predominance of additive gene action. These results suggest that direct phenotypic selection would be effective for improving seedling-stage cold tolerance in rice breeding programmes.

Among the traits evaluated, Germination %, Seedling Vigour Index (SVI), Leaf Yellowing Score (1-9), Root Length (cm) - 8-10 DAS and Survival Rate (%) proved to be the most reliable indicators for identifying cold-tolerant genotypes because they exhibited comparatively higher GCV, PCV, heritability and expected genetic advance. Although Shoot Length (cm) - 8-10 DAS and Chlorophyll Content (SPAD) recorded relatively lower genetic advance, these traits remain valuable as supplementary indicators for the comprehensive evaluation of seedling performance under cold stress.

Overall, the findings confirm the availability of sufficient exploitable genetic variability for enhancing seedling-stage cold tolerance in rice through appropriate selection and breeding strategies. The information generated from this investigation provides a useful foundation for identifying superior parental lines and promising hybrids for future breeding programmes. In addition, the important seedling-stage traits

identified in this study can be effectively integrated with advanced molecular breeding approaches, including marker-assisted selection (MAS) and QTL-based breeding, to accelerate the development of high-yielding, cold-tolerant rice cultivars adapted to low-temperature environments.

Competing interests

Authors have declared that no competing interests exist

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