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**Genetic Variability and Multivariate Study in Indica Rice (*Oryza sativa* L.) Germplasm
of the Tungabhadra Basin, Karnataka**

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Abstract

In the present study, 214 rice germplasm accessions were evaluated along with four check varieties using an augmented randomized complete block design to study the genetic variability, correlation, path analysis and genetic diversity studies for yield and yield attributing traits. Analysis of variance revealed significant differences among the germplasm for different traits. The high genotypic coefficients of variation, and phenotypic coefficients of variation were observed for biological yield (46.86%, 46.92%), harvest index (36.44%, 36.47%), High broad-sense heritability across all studied traits, high estimates of genetic advance in percent of the mean were observed for many traits except days to maturity and days to 50% flowering. biological yield, harvest index, number of spikelets per panicle, exhibit significant positive correlation with grain yield. Principal component analysis revealed that the first five principal components with eigenvalues >1 explained 64.34% of the total variation. Cluster analysis grouped the genotypes into five distinct clusters, with the maximum inter-cluster distance observed between Cluster I and Cluster V (6.00), indicating the highest genetic divergence between these clusters. Overall, the substantial genetic variability and divergence observed among the rice germplasm accessions provide ample of scope for the selection and hybridization of genetically diverse parents to develop high-yielding rice varieties.

Keywords: Rice Germplasm, Genetic Variability, Genetic Advance, Multivariate analysis, Genetic Divergence

Introduction

Rice (*Oryza sativa*. L) the most important staple food crop that feeds the more than half of the world population (Sen et al. 2020). In India rice cultivation spans 47.83 million hectares, the production approximately 137.82 million tonnes with productivity of about 2,882 kg/ha. Karnataka, a predominant rice producing state, contribute significantly to national rice production, it is cultivated over an area of 1.3 million ha with a production of 3.4 million tonnes (<https://www.upag.gov.in>). Projections indicate a substantial increase in food demand, several studies suggest that the world will need 70 to 100% more food by 2050; however, yield trends are insufficient to double global crop production to meet this demand (Godfray et al. 2010; Ray et al. 2013).

The Tungabhadra Command Area (TCA) of Karnataka, includes the 4 districts viz., Raichur, Koppal, Ballari, and Vijayanagara, referred as the "Rice Bowl of Karnataka" (Fig 1). due to its substantial contribution to the state's total rice output (50% to 75%). TCA harbours a

rich diversity of locally adapted rice germplasm however, highly vulnerable to various climate induced stresses, including water scarcity, which can severely impact the rice productivity. Furthermore, recent trend of decreasing paddy yields in the region raises concern. Despite the challenges, the genetic potential of local germplasm remains uncharacterized. The foundation of any successful crop improvement program lies in the availability and effective utilization of genetic resources with a broad genetic base, serve as crucial reservoirs of novel genes/QTLs (Mondal et al. 2023). A comprehensive understanding of the genetic diversity within this germplasm is the essential step for its systematic conservation and strategic use in breeding programs (Nachimuthu et al. 2015). The spectrum of genetic variability present among germplasm offers greater scope for effective selection (Arunachalam 1981; Hill et al. 2008; Akinwale et al. 2011; Tuhina-Khatun et al. 2015).



Fig 1. Annotated map of the Tungabhadra Command Area showing the command districts of Karnataka, India.

The available genetic variability can be explored by GCV, and PCV, (Pratap et al. 2012; ONYIA et al. 2017). Study of heritability, and genetic advance are important to elucidate the influence of environmental factors on trait expression (Dutta et al. 2013; Ogunbayo et al. 2014; Nirmaladevi et al. 2015; Chacko et al. 2023). Correlation and path coefficient analysis crucial for revealing the relative contribution of various yield components to the overall grain yield (Augustina et al. 2013; Karim et al. 2014; Wright 1934; Bagheri et al. 2011; Tuhina-Khatun et al. 2015; Satturu et al. 2023). Principal component analysis (PCA) enable the quantification of

genetic divergence and assist in the selection of suitable parents for hybridization (Maji and Shaibu 2012; Mahalanobis 2018; Rao et al. 2020; Talekar et al. 2022). These studies facilitate the selection of diverse parental lines for hybridization, thereby accelerating the development of superior, climate-smart cultivars.

In this context, the objective of the present study was to conducted to assess the untapped genetic variability for yield and yield related traits among 214 rice germplasm of TCA of Karnataka.

Materials and methods

Plant Material

The material for the present study comprised 214 rice germplasm, including four checks (Table S1). These genotypes were sourced from the rice germplasm collection maintained under the All India Coordinated Rice Improvement Project (AICRIP) at the Agricultural Research Station (ARS), Gangavathi. The experiment was conducted in the F3-B1 block at ARS, Gangavathi, located at 15.43° N latitude and 76.53° E longitude, with an altitude of 406 m (1332 ft) above mean sea level. The crop was raised under puddled irrigated conditions (Fig 2). The trial was laid out in an augmented randomized complete block design (ARCBD) in four blocks (Federer 1955). Data were recorded as per the standard evaluation system (IRRI SES, 2015) for the key agronomic traits viz., plant height (PHT, cm), number of tillers per plant (NTP), days to 50% flowering (DFF), number of panicles per plant (NPP), panicle length (PL, cm), grain yield per plant (GY, g), number of spikelets per panicle (NSP), days to maturity (DTM), biological yield per plant (BY, g), harvest index (HI, %), and test weight (TW, g).



Fig 2. Overview of experimental plot of rice germplasm at the ARS, Gangavathi. (I). Vegetative stage (II). Reproductive phase (III). Maturity phase (arrow indicates late duration genotype (IV). Layout of the experiment.

Statistical analysis

Statistical analysis was done by using R software version 4.3.1. The augmented (Federer 1956) analysis of variance (ANOVA) was carried out using augmented RCBD packages in Rstudio 1.3 (Aravind et al. 2020). The correlation was visualized with metan

100 package (Olivoto and Lúcio 2020) and Multivariate analyses such as diversity analysis and
101 PCA were carried out using ‘factoextra’ package in R packages (Kassambara and Mundt 2016).
102 Heatmap and Hierarchical clustering based on Euclidian matrix distance was performed
103 through the “NbClust” package using the wardD method in RStudio as done by Amongi et al.
104 (2023).
105

Results and Discussion

Analysis of variance and mean performances of the genotypes

In the present study, ANOVA both under treatment-adjusted and block-adjusted revealed highly significant differences among the genotypes for all the traits (Table 1). Similar results were obtained in studies of (Ashfaq et al. 2012; Mural et al. 2012; Jambhulkar and Bose 2014; Sumanth et al. 2017; Deepika et al. 2021; Jyothi et al. 2018). The critical difference (CD) of checks confirms the uniform contrast expression of traits across the blocks. This indicates that differences among the germplasm. Thus, the possibility genetic improvement through selection is highly promising (Oladosu et al. 2018).

Mean, range and standard deviation for all the traits revealed a wide range of phenotypic variation was found (Table S2). PHT ranged from 52.56 cm in G-39 to 161.56 cm in G-175. The NTP varied from 10 in G-145 to 28 in G-208, while days to 50 per cent flowering ranged from 72 days in G-169 to 140 days in G-65. The NPP ranged from 8 in G-60 to 26 in G-208, and PL varied from 11.75 cm in G-33 to 31.75 cm in G-117. GYP exhibited wide variability, ranging from 173.88 g in G-178 to 2120.63 g in G-16, with a mean value of 1160.63 g. The NSP ranged from 43 in G-189 to 234 in G-195. The earliest maturity was recorded in G-169 (107 days), whereas G-65 was the late maturing at 174 days. BY varied markedly, with the lowest value of 85.31 g observed in G-178 and the highest value of 1495.31 g in G-206. HI ranged from 53.14% in G-69 to 81.34% in G-26. TW also showed substantial variation, with the lowest value of 9.28 g recorded in G-131 and the highest value of 30.61 g in G-170, indicating ample scope for selection and genetic improvement of these traits. In the study, Violin plots (Fig 3 and Fig S1) were used for depicting the variation of yield and yield attributing traits.

Skewness and kurtosis analysis provides the information about number of genes governing the trait (Fisher et al. 1932; Robson 1956; Shrestha et al. 2021; Kumari et al. 2024). The distribution pattern of the studied traits was assessed through kurtosis estimates (Table S2).

Genetic variability parameters

In the present study, the coefficient of variation (CV) revealed differential levels of experimental variability among the traits (Table 1). NTP (9.52%) recorded the highest CV values, indicating relatively greater variability and wider scope for selection. GYP (6.67%) and TW (4.73%) exhibited moderate variability. The range of variability for a particular trait is evident through PCV and GCV estimates (Table S2 and Fig 4). High phenotypic and genotypic

coefficients of variation were recorded for BY (46.92% and 46.86%), HI (36.47% and 36.44%), NSP (35.22% and 35.09%), and GYP (28.52% and 27.73%), Similar such inferences were drawn by (Bagudam et al. 2018; Kishore et al. 2008) (Fig 4). The close correspondence between phenotypic and genotypic coefficients of variation across all traits indicates minimal environmental influence (Ahmad et al. 2011).

High heritability accompanied by high genetic advance as a percent of the mean was observed for BY (99.71% heritability; 96.52% genetic advance over mean), HI (99.83%; 75.11%), NSP (99.27%; 72.12%), GYP (94.52%; 55.62%), PHT (98.35%; 44.74%), and TW (94.78%; 40.25%), indicating the predominance of additive gene action and greater effectiveness of direct selection for these traits (Prapakaran et al. 2025) (Table S2 and Fig S2) (Abebe et al. 2017).

Heatmap (Fig 5) also revealed the genetic variability among rice germplasm. Traits such as GYP, BY, and HI showed pronounced variability, with several genotypes exhibiting high intensity (red) values, suggesting their major contribution to genetic differentiation. The heat map analysis demonstrates that GYP, BY, HI, NSP, and tillering traits are the principal contributors to genetic variability among the rice germplasm.

155 Table 1. Analysis of variance from augmented block design for the eleven yield and yield-related traits of rice germplasm.

		Mean sum of squares									
Source	Df	PHT	NTP	DFF	NPP	PL	GYP	NSP	DTM	BY	
Treatment (Ignoring Blocks)	213	463.42 **	10.68 **	78.06 **	10.15 **	7.90 **	113119.78 **	1672.58 **	76.05 **	73391.38 **	44
Treatment: Check	3	80.06 **	12.56 **	598.92 **	3.42 ^{ns}	3.33 **	394513.08 **	4051.31 **	318.75 **	145944.73 **	2
Treatment: Test	209	460.88 **	10.53 **	66.85 **	10.01 **	8.00 **	109604.29 **	1641.59 **	66.49 **	72621.20 **	44
Treatment: Test vs. Check	1	2145.77 **	35.91 **	857.93 **	60.11 **	1.06 ^{ns}	3677.42 ^{ns}	1015.15 **	1346.15 **	16699.61 **	97
Block (Eliminating Treatments)	3	4.40 ^{ns}	0.23 ^{ns}	0.42 ^{ns}	0.42 ^{ns}	0.50 ^{ns}	5453.42 ^{ns}	9.39 ^{ns}	3.75 ^{ns}	89.23 ^{ns}	
Residuals	9	7.62	1.78	4.42	2.25	0.28	6006.03	12.01	5.81	208.78	
Coeff. Var. (CV)		2.85	7.53	2.00	9.52	2.37	6.67	3.02	1.72	2.51	

156

157 ^{ns}P > 0.05; * P ≤ 0.05; ** P ≤ 0.01 *Significance at 5%. **Significance at 1%. **PHT**=Plant height (cm), **NTP**= Number of tillers per plant,
158 **DFF** = days to 50% flowering, **NPP**= Number of panicles per plant, **PL**= Panicle length (cm), **GYP**=Grain yield per plant (g), **NSP**=Number
159 of spikelets per panicle, **DTM**= Days to maturity, **BY**=Biological yield (g), **HI**=Harvesting index (g), **TW**= Test weight (g).

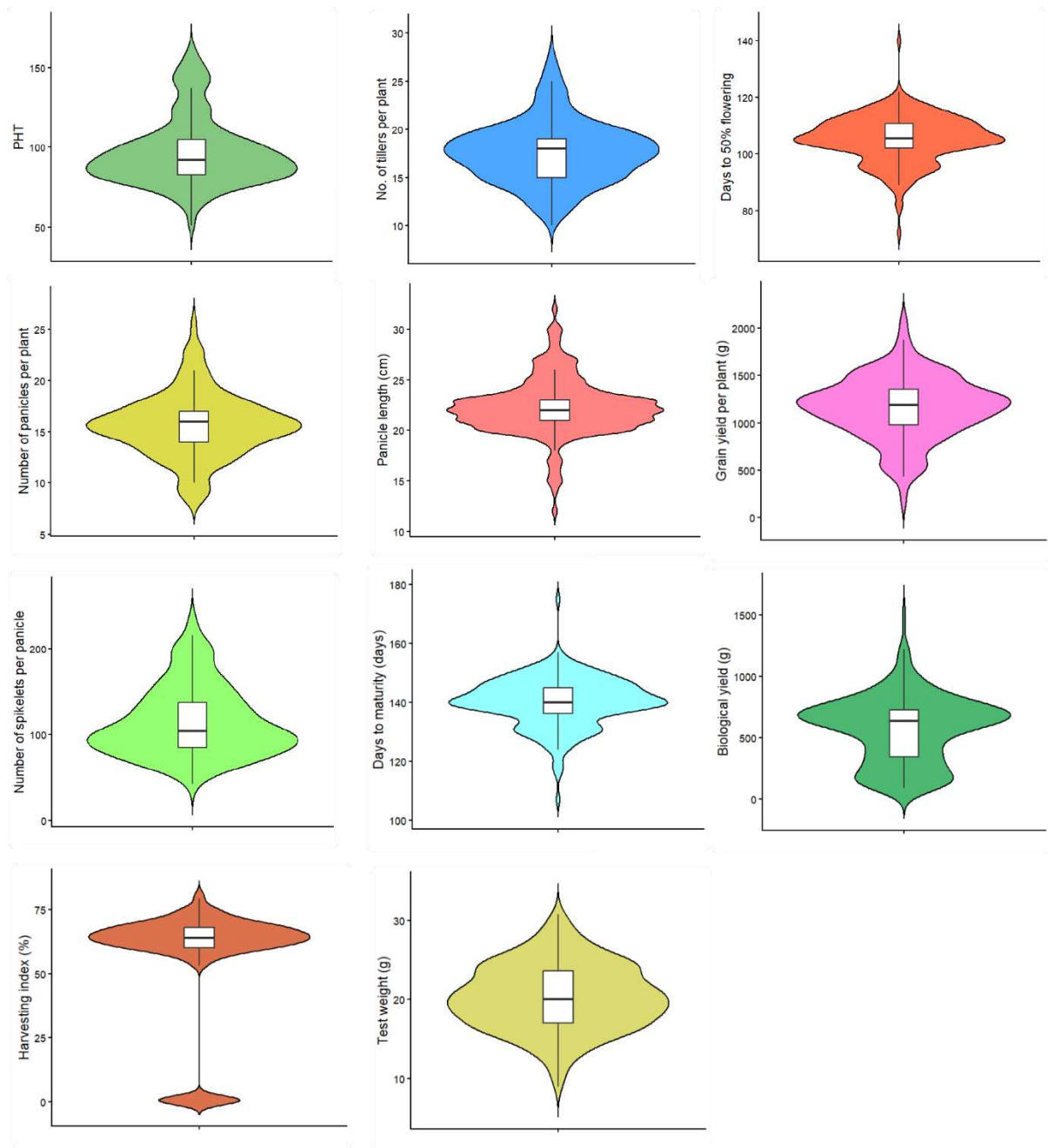


Fig 3. Violin-plots showing variation in the data of 11 yield and yield related traits in the studied rice germplasm.

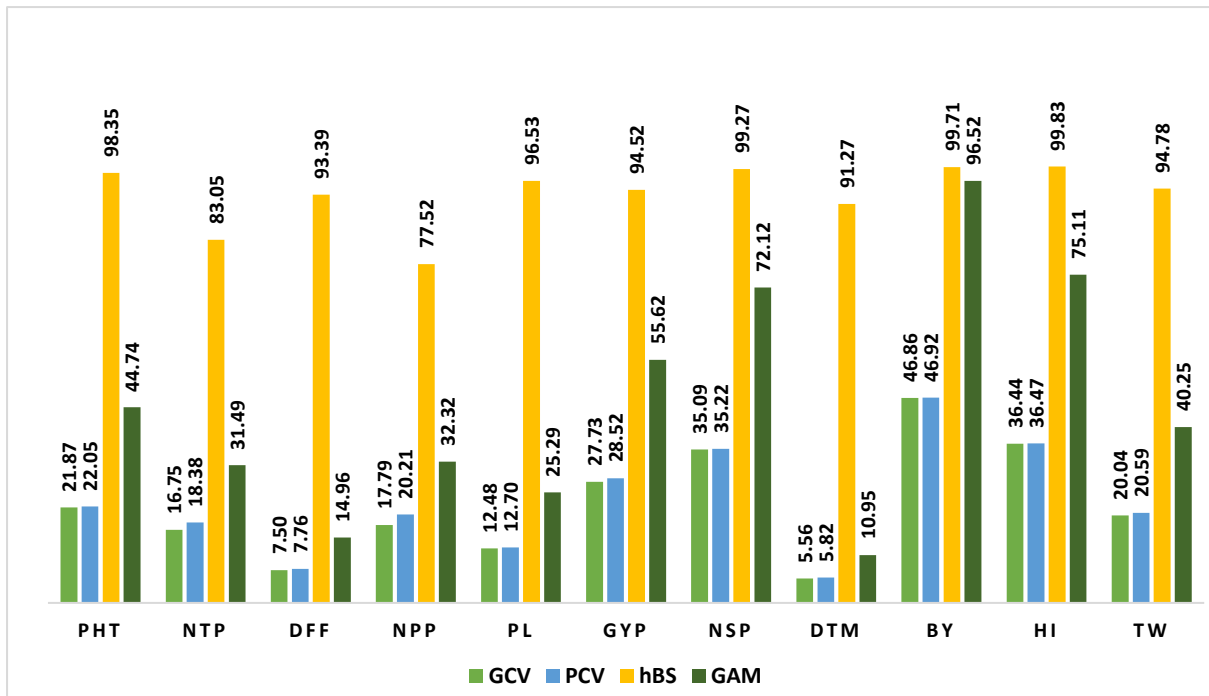


Fig 4. Heritability broad-sense (hBS), genetic advance (GD), and genetic advance as percent of mean (GAM) for yield and yield-related traits in rice germplasm.

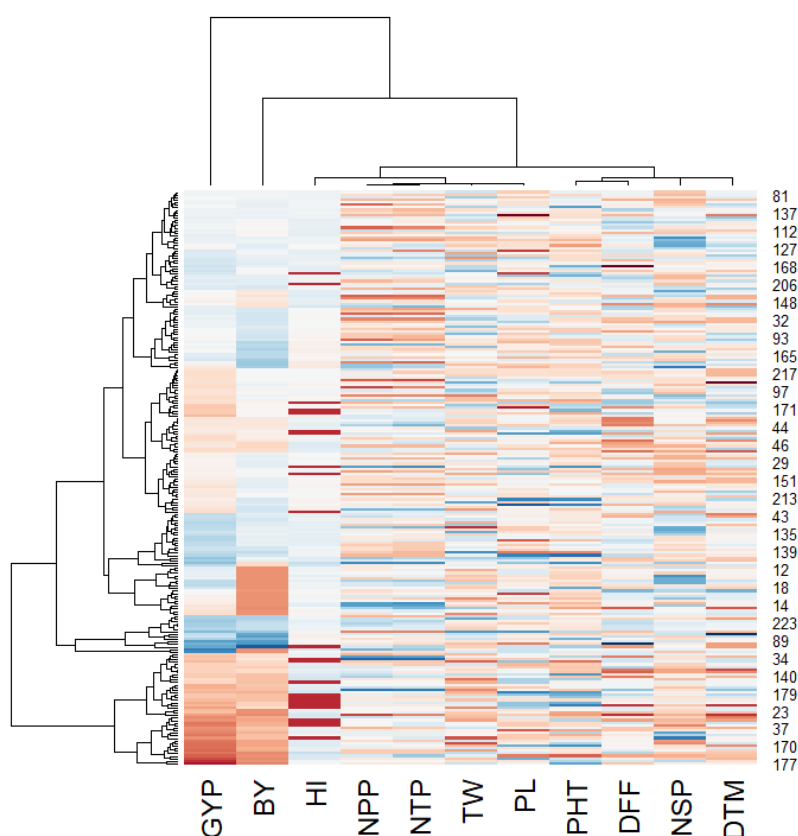


Fig. 5 Heatmap depicting the genetic variability in 214 rice genotypes for yield and yield attributing traits.

Pearson correlation and path analysis

The correlation analysis revealed, GYP exhibited a highly significant and positive correlation with BY (0.54), indicating that genotypes with higher biomass production tended to produce higher grain yield (Fig 6). Indicates that partitioning of assimilates and heavier grains contribute substantially to yield improvement. Similar results have been reported by Lakshmi et al. (2019). Among component traits, DFF exhibited a strong positive association with DTM (0.51), reflecting close linkage between flowering and maturity. PHT showed a significant positive correlation with PL (0.48), suggesting that taller plants tend to bear longer panicles (Mohammad et al. 2021). The correlation results emphasize that BY, HI, NSP, and TW are the most important traits contributing to grain yield (Anwar et al. 2022).

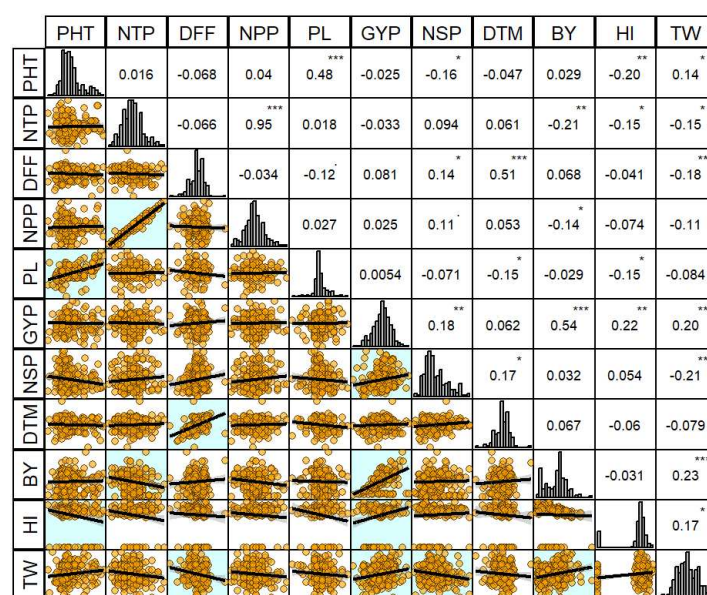


Fig 6. Correlation matrix showing relationships among grain yield and yield-related traits in rice germplasm.

Path coefficient analysis revealed BY exerted the highest positive direct effect on GYP (0.553), followed by HI (0.249) (Table S3). These results indicate that improvement in grain yield can be effectively achieved through direct selection for these traits, particularly BY and HI (Peng et al. 2008; Bagheri et al. 2011). The path analysis highlights biological yield and harvest index as the most important selection criteria for yield improvement in the evaluated rice germplasm, supported by their strong direct effects and substantial indirect contributions through other traits (Gour et al. 2017).

Cluster analysis

Hierarchical cluster analysis based on Ward's method grouped the rice germplasm into five distinct clusters, indicating the presence of substantial genetic diversity among the genotypes for yield and yield related traits (Table S4 and Fig 7). The average intra-cluster distances were relatively low compared to the inter-cluster distances, indicating close genetic resemblance among genotypes within clusters and substantial divergence between clusters (Maji and Shaibu 2012; Akter et al. 2018).

Among the clusters, Cluster V exhibited the highest intra-cluster distance, suggesting greater variability within this group, while Cluster IV showed the lowest intra-cluster distance, indicating relatively higher homogeneity. The maximum inter-cluster distance was observed between Cluster I and Cluster V, reflecting wide genetic divergence and highlighting the

potential of selecting parents from these clusters for hybridization programmes (Hoque et al. 2015). The dendrogram clearly depicts the genetic relationships among genotypes, with well-defined cluster separation, emphasizing the effectiveness of multivariate analysis in discriminating genetically diverse groups (Table 2). Particularly Cluster I and Cluster V, would be most effective for broadening the genetic base and improving yield and yield-related traits in rice breeding programmes (Ata-Ul-Karim et al. 2022; Yeshitila et al. 2023).

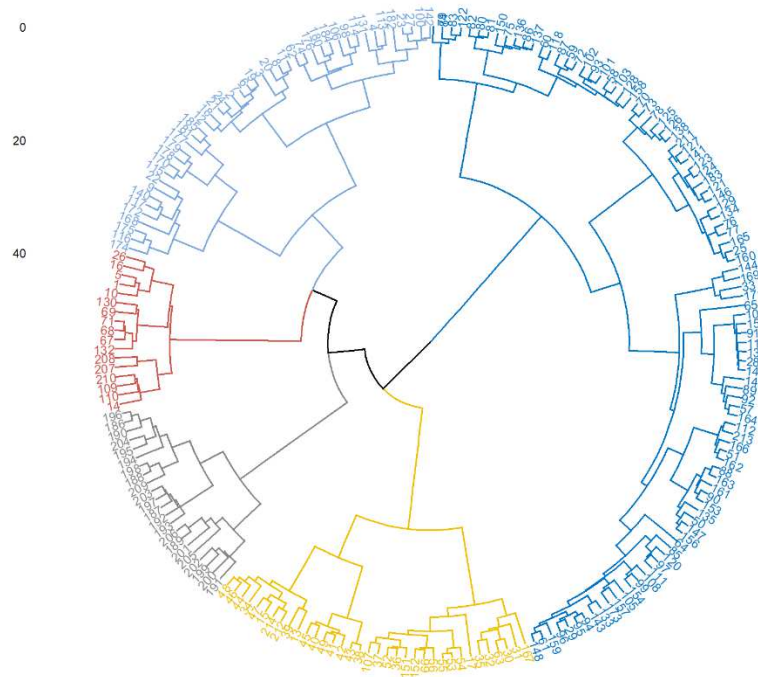


Fig 7. Dendrogram illustrating genetic divergence among 214 rice germplasm based on hierarchical cluster analysis (Ward's method)

212 **Table 2. Grouping of 214 rice genotypes into various clusters based on genetic distance**

Cluster	No of genotypes	Genotypes
I.	17	G-1, G-5, G-10, G-14, G-16, G-26, G-67, G-68, G-69, G-71, G-109, G-110, G-130, G-132, G-207, G-208, G-210
II.	44	G-2, G-3, G-4, G-6, G-7, G-8, G-9, G-11, G-12, G-18, G-19, G-20, G-21, G-22, G-23, G-24, G-27, G-62, G-74, G-98, G-100, G-106, G-113, G-114, G-115, G-116, G-117, G-131, G-140, G-142, G-168, G-170, G-172, G-174, G-175, G-176, G-177, G-178, G-179, G-180, G-181, G-182, G-184, G-185
III.	35	G-13, G-15, G-17, G-30, G-31, G-32, G-34, G-35, G-36, G-37, G-38, G-39, G-40, G-41, G-42, G-43, G-44, G-45, G-46, G-47, G-48, G-49, G-52, G-53, G-54, G-55, G-56, G-63, G-66, G-107, G-151, G-152, G-167, C-2, C-4
IV.	96	G-25, G-28, G-29, G-33, G-50, G-51, G-57, G-58, G-59, G-60, G-61, G-64, G-65, G-70, G-72, G-73, G-75, G-76, G-77, G-78, G-79, G-80, G-81, G-82, G-83, G-84, G-85, G-86, G-87, G-88, G-89, G-90, G-91, G-92, G-93, G-94, G-95, G-96, G-97, G-99, G-101, G-102, G-103, G-104, G-105, G-108, G-111, G-112, G-118, G-119, G-120, G-121, G-122, G-123, G-124, G-125, G-126, G-127, G-128, G-129, G-133, G-134, G-135, G-136, G-137, G-138, G-139, G-141, G-143, G-144, G-145, G-146, G-147, G-148, G-149, G-150, G-153, G-154, G-155, G-156, G-157, G-158, G-159, G-160, G-161, G-162, G-163, G-164, G-165, G-166, G-169, G-171, G-173, G-183, C-1, C-3
V.	22	G-186, G-187, G-188, G-189, G-190, G-191, G-192, G-193, G-194, G-195, G-196, G-197, G-198, G-199, G-200, G-201, G-202, G-203, G-204, G-205, G-206, G-209

213

214

Principal component analysis

Principal component analysis (PCA) was performed for eleven traits of the 214 genotypes and extracted 11 Principal components (PCs) and it revealed first five most informative PCs with Eigen values of 2.17, 1.88, 1.56, 1.44 and 1.04 which account for 64.34% of the cumulative variance (Table 3). Similar amount of cumulative variance with two major PCs were drawn by (Worede et al. 2014; Deepika et al. 2021) Fig 8(a). As per the PCA-variables plot (Fig. 8(b)) and PCA Bi-plots Fig. 8(c), six traits *viz.*, NTP, NPP, NSP, PL, DTM, and PHT contributed maximum variations in PC1. While eight traits *viz.*, DTM, DFF, NSP, GYP, BY, HI, NPP, and NTP contributed maximum variations in PC2. Genotypes with high scores in the first few principal components may be effectively utilized in breeding programs to exploit maximum genetic divergence (Soe et al. 2019).

Table 3. Eigen values, contribution of variability and factor loading for the principal component axis in 214 rice genotypes

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11
Plant height (cm)	0.04346	- 0.38061	- 0.26393	0.45902	0.0654 4	0.2928 0	- 0.2320 6	0.3645 4	- 0.53302	- 0.10652	0.0261 4
No. of tillers per plant	0.60760	0.06986	- 0.27368	- 0.14237	0.1299 2	- 0.0171 0	0.0500 6	- 0.0032 9	0.02391	0.03093	0.7138 3
Days to 50% flowering	- 0.01010	0.45254	0.14209	0.42169	0.1878 8	0.2145 5	0.2384 8	0.5557 0	0.36143	0.13309	0.0418 0
Number of panicles per plant	0.57921	0.09095	- 0.33421	- 0.14649	0.1187 7	0.0364 4	0.0745 4	0.0621 8	0.00386	0.14272	- 0.6912 7
Panicle length (cm)	0.08170	- 0.38067	- 0.19764	0.41350	- 0.3649 2	0.2765 6	0.1809 6	- 0.3671 8	0.47005	0.20033	0.0086 5
Grain yield/ plant (g)	- 0.22219	0.26793	- 0.57415	- 0.01564	- 0.1881 7	0.0083 0	0.2275 9	0.0077 8	0.08305	- 0.67415	- 0.0153 9

Number of spikelets per panicle	0.09762	0.37680	- 0.05207	0.00889	- 0.6221 5	0.0257 9	- 0.6527 7	0.1181 3	0.05133	0.12267	0.0149 8
Days to maturity (days)	0.05443	0.45896	0.05991	0.37577	0.3283 3	0.2192 3	- 0.1774 3	- 0.6347 1	- 0.21146	- 0.07686	- 0.0279 0
Biological yield (g)	- 0.32335	- 0.17524	- 0.47641	0.14778	0.0040 0	0.4188 9	0.1597 4	0.0431 5	- 0.23283	0.59554	0.0647 4
Harvesting index (%)	- 0.20282	- 0.13459	- 0.06222	- 0.44445	- 0.1208 1	0.7369 5	0.2215 3	- 0.0169 3	- 0.23343	0.27074	0.0670 8
Test weight (g)	- 0.28127	- 0.13801	- 0.34308	- 0.19456	0.5013 9	0.1509 4	- 0.5142 8	0.0244 8	0.44808	0.07804	0.0127 3
Eigen value	2.17285	1.88619	1.56932	1.44904	1.0402 2	0.8926 8	0.6824 4	0.4660 8	0.43725	0.36160	0.0423 4
Percentage of variance	19.7531 4	17.1471 4	14.2665 8	13.1730 6	9.4565 8	8.11524	6.2039 7	4.2370 9	3.97502	3.28723	0.3849 5
Cumulative per cent of variance	19.7531 4	36.9002 8	51.1668 6	64.3399 2	73.796 5	81.9117 3	88.1157 1	92.352 8	96.3278 2	99.6150 5	100

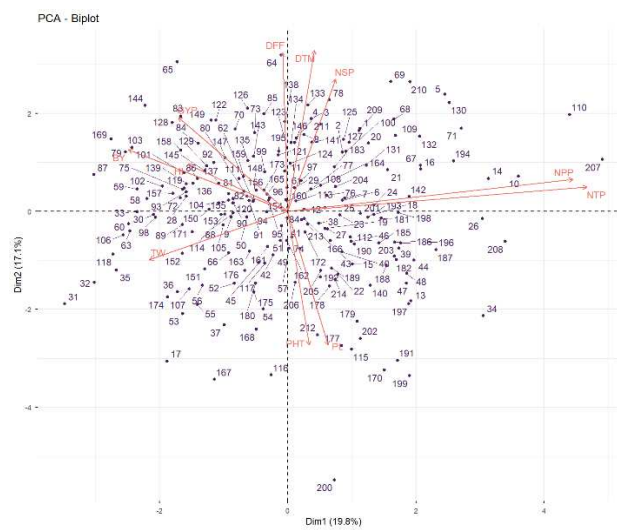
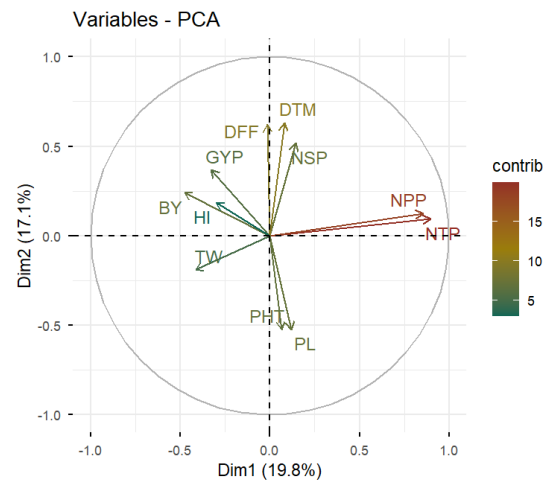
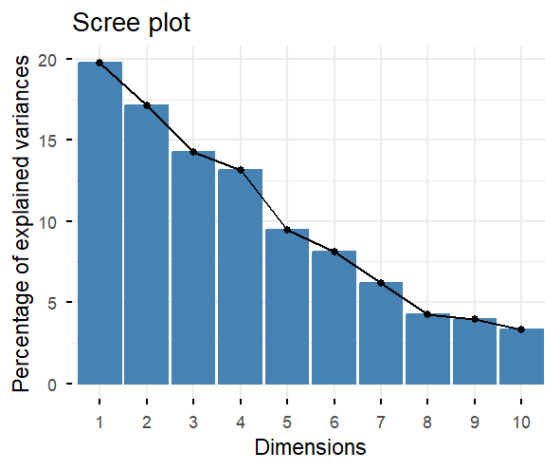


Fig 8. (a) Scree Plot explaining the contribution of 11 various principal components, (b) PCA-Variables Plot, (c) PCA-Biplots explaining the contribution of 11 traits to the total variation in the 214 rice genotypes.

Conclusion

The present investigation revealed significant differences among the indica rice germplasm for most of the traits studied, indicating the existence of substantial genetic variability. Estimates of mean, range, and standard deviation demonstrated a wide spectrum of phenotypic variation across genotypes. The high phenotypic and genotypic coefficients of variation, coupled with high heritability and genetic advance, particularly for BY, HI, NSP, and GYP, suggest the predominance of additive gene action and the effectiveness of direct selection for these traits. Correlation analysis indicated that BY, NSP, and TW were positively and significantly associated with grain yield, emphasizing their importance as reliable selection criteria for yield improvement. Multivariate analyses, including PC and cluster analysis, revealed clear genetic divergence among the genotypes and facilitated the identification of genetically diverse and high-yielding pools suitable for use as parents in hybridization programmes. In conclusion, the results confirm the availability of ample genetic variability in the indica rice germplasm of the Tungabhadra Command Area of Karnataka and highlight the potential of exploiting key yield-contributing traits for the development of high-yielding smart rice varieties to the region.

253 **Statements and Declarations**

254 **Competing Interests**

255 The authors have no relevant financial or non-financial interests to disclose.

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258 of this manuscript.

259 **Data availability**

260 The original contributions presented in the study are included in the article/Supplementary
261 Material, further inquiries can be directed to the corresponding author.

262 **Author Contribution**

263 LR conceptualized & supervision the thematic framework of the original article, NRR
264 conducted methodology, data collection, and writing original draft. BSO contributed to data
265 analysis, visualization, curation. MI & GMK writing and revising the manuscript. All authors
266 read and approved the final version.

267 **Conflict of Interest Statement**

268 The authors declare that the research was conducted in the absence of any commercial or
269 financial relationships that could be construed as a potential conflict of interest.

270 **Ethics, Consent to Participate, and Consent to Publish declarations:** not applicable

271 **Clinical Trial Registration:** not applicable

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