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Assessment of Genetic Variability in Upland Rice (*Oryza sativa* L.) Genotypes at Metema, Northwestern Ethiopia

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Abstract

Rice holds promise to meeting food demand and ensuring social stability in Ethiopia. However, the country demand for rice far exceeds its production, with 80% of the deficit being met through imports. Hence, identification and utilization of genetically diverse genotypes is essential for crop improvement programs to develop and deploy high yielding rice varieties. This study was conducted with the objective to assess genetic parameters and determine the extent of genetic diversity among 81 upland rice genotypes. Field study was carried out in Metema during the 2021 main cropping season, using a 9x9 simple lattice design with two replications. Eighteen agro-morphological traits were analyzed using various multivariate techniques and genetic parameter estimation methods. Analysis of variance revealed highly significant differences among genotypes for all the traits studied. Moderate to high genotypic and phenotypic coefficients of variations were found for days to heading, days to maturity, filled grains per panicle, and grain yield. Moderate to high heritability, coupled with genetic advance as a percentage of the mean, were observed for thousand grain weight, grain yield, and filled grains per panicle, suggesting that these traits could be improved through direct selection. Cluster analysis grouped the 81 genotypes into six clusters. The inter-cluster distance was greatest between Cluster IV and Cluster V (D^2 = 261.62), indicating that these clusters could be valuable for hybridization programs. The first five principal components accounted for 75.56% of the total variability in the 18 traits, providing insight into the traits that differentiate the genotypes. The result of this study suggests existences of adequate genetic variability among the genotypes, which could be recommended for future rice improvement programs.

Keywords: Cluster Analysis, Genetic Advance, Genetic Variability, Heritability, Principal component Analysis, Upland Rice.

Introduction

Rice (*Oryza sativa* **L**.) is playing a significant economic role and feeds approximately half of the world's population (Shrestha *et al.* 2021). Among the cereals, rice provides up to 20% of their regular calorie intake for millions of global populations. In Sub-Saharan Africa (SSA), rice serves a fundamental role in food security and social stability (Arouna *et al.* 2017). Its growing significance

in the consumer food basket has made it a political crop, which its price and accessibility influence social stability (Seck *et al*. 2010). Rice has been adopted as a principal staple food crop and consumed in approximately 39 African countries, making it a strategically important crop on the continent (Rodenburg *et al*. 2014).

Rice is considered one of the most important cereal crops in Ethiopia for its role in achieving food security and national economy through its high yield potential in small area, short growing period, its adaptation in water logged areas, and served as animal feed (Hegde & Hegde, 2013; Negussie & Alemu, 2011). In 2020, rice production and area coverage in Ethiopia were 189,649 tons and 62,551 hectares, respectively (FAOSTAT, 2022). Ethiopian rice yield (3.34 t ha^{-1}) was higher than both Africa and east African productivity, however it remains below the global average of 4.6 t ha^{-1} and China's average yield of 7 t ha^{-1} (FAOSTAT, 2022). This low productivity is due to pests, climate change, poor agronomic practices, environmental challenges and limited high-yielding varieties (NRRDSE, 2010). Identifying genotypes with improved yields and agronomic traits is vital to enhancing upland rice yields in Africa (Osman *et al.* 2012).

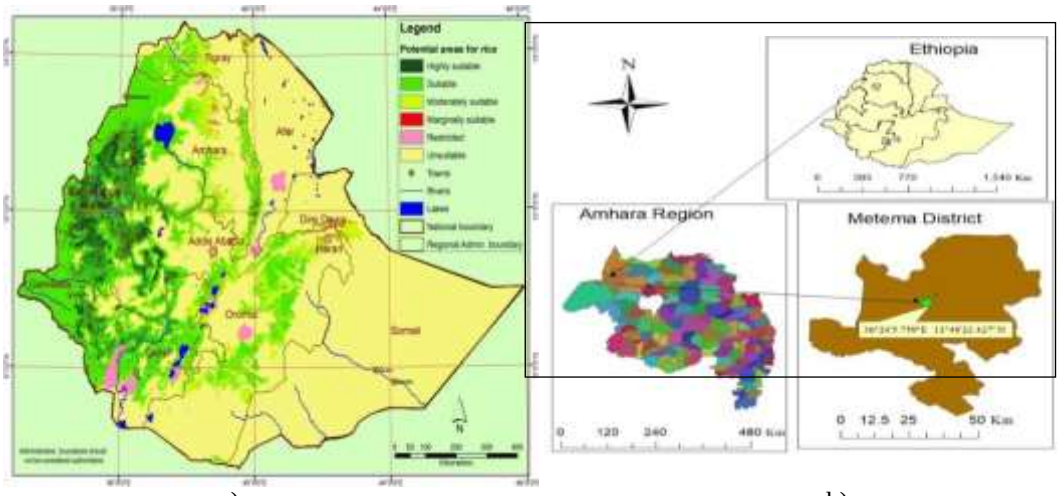
Genetic variation is the foundation of plant breeding, providing a diverse array of genotypes that can be selected to develop new varieties or breeding materials (Swarup *et al.* 2021). Among cereal crops, rice serves as an excellent model for studying genome structure and genetic diversity (IRGSP, 2005). Its diploid genome is relatively small (430 Mb) yet exhibits significant genetic polymorphism and a wealth of well-conserved, genetically diverse material (Latif *et al.* 2011). Variability for agronomic traits is a critical component of breeding programs aimed at broadening the gene pool of rice. This requires reliable estimates of heritability to design effective breeding strategies (Akinwale *et al.* 2011). Sreedhar (2018) highlighted that genetic parameter such as genetic coefficients of variation, heritability, and genetic advance can be utilized to partition the total variation in a population into heritable and non-heritable components. Furthermore, assessing the available genetic diversity is crucial for the effective evaluation and utilization of germplasm (Salgotra & Chauhan, 2023); enabling breeders to explore variability in rice germplasms and identify desirable agronomic traits (Akinwal *et al*. 2011).

In Ethiopia different researchers conducted genetic variability studies on upland rice (Ayenew *et al*. 2019; Tezera, 2021; Tiruneh *et al*. 2021; Demeke *et al.* 2023) and reported significant variation among genotypes for most of the traits studied. The genetic variability observed in introduced rice genotypes is crucial for rice improvement and could be utilized by breeders aiming to enhance yield and other desirable agronomic traits. However, such information remains limited in the Metema area. This study aims to estimate the extent of genetic variability, heritability, genetic advances in key traits of upland rice, and to determine the extent of genetic diversity among rice genotypes for future upland rice improvement programs.

Materials and Methods

Description of the study areas

A field experiment was conducted in 2021 cropping season at Metema sub-center research station, part of the Gondar Agricultural Research Center. The site is located in the northwestern part of the Amhara Region, 875 km from Addis Ababa, Ethiopia's capital. The experimental site lies at 12°48′ N latitude, 36°24′ E longitude, and an elevation of 747 meters above sea level. Based on ten years of meteorological data, the area receives an average annual rainfall of 1000.4 mm, with mean annual minimum and maximum temperatures of 11.5°C and 27.9°C, respectively. The soil is predominantly black *Vertisol* with a pH of 5.9. The area experiences mono-modal rainfall and represents the potential upland rice-producing agro-ecologies of the region (Figure 1a).



a) b)

Figure 1: (a) Suitability map of rice production in Ethiopia (MoARD, 2010), and (b) Map of the study area

Experimental Materials

The study included 81 upland rice genotypes and seven released varieties (Table 1). The genotypes were sourced from the Fogera National Rice Research and Training Center and introduced from the Africa Rice Center and IRR (Table 1).

Table 1. Description of experimental materials (upland rice genotypes)

Code	Genotype designation	Source/origin	Code	Genotype designation	Source/origin
G-1	ARD5-8	Africa Rice	G-42	PCT-4\SA\1\1, Bo\3\1>161-3-2-1-M	Africa Rice
G-2	ARD5-9	Africa Rice	G-43	PCT-4\SA\1\1\2\1>746-1-2-2-1-3-M	Africa Rice
G-3	ARD5-10	Africa Rice	G-44	PCT-4\SA\5\1>1754-5-1-3-2-2-M	Africa Rice
G-4	ARD5-12	Africa Rice	G-45	PCT-4\SA\5\1>1754-5-1-5-3-1-M	Africa Rice
G-5	ARD5-13	Africa Rice	G-46	NM1-29-4-B-P-80-8	Africa Rice
G-6	ART15-16-31-2-1-1-1-B-1-1	Africa Rice	G-47	ART16-9-29-12-1-1-2-B-1-1	Africa Rice
G-7	ART16 5-10-22-4-B-1-B-B-1	Africa Rice	G-48	ART16-9-14-16-2-2-1-B-1-2	Africa Rice
G-8	Fogera 1	Released variety	G-49	ART16-9-122-33-2-1-1-B-1-1	Africa Rice
G-9	ART16 9-29-10-2-B-1-B-B-1	Africa Rice	G-50	ART15-19-5-4-1-1-1-B-1-1	Africa Rice
G-10	ART16-4-13-1-2-1-1-B-1-1	Africa Rice	G-51	ART16-21-4-7-2-2-2-B-2-2	Africa Rice
G-11	ART16-9-5-28-3-13-1-B-2-1	Africa Rice	G-52	ART15-16-45-1-B-1-1-B-1-2	Africa Rice
G-12	ART16-9-9-25-2-1-1-B-2-1	Africa Rice	G-53	ART16-5-10-2-3-B-1-B-1-1	Africa Rice
G-13	ART16-9-19-11-2-2-2-B-1-2	Africa Rice	G-54	ART16-4-1-21-2-B-2-B-1-2	Africa Rice
G-14	ART16-9-25-30-3-2-2-B-1-1	Africa Rice	G-55	PARC.DAT.V-1.2013	Brazil
G-15	ART16-12-28-32-3-B-1-1-2	Africa Rice	G-56	PARC.DAT.V-2.2013	Brazil
G-16	ART34-82-1-7N-1	Africa Rice	G-57	PARC.DAT.V-3.2013	Brazil
G-17	ART34-76-2-8D-2	Africa Rice	G-58	ART15 8-10-36-4-1-1-B-B-1	Africa Rice
G-18	ART35-100-1-7D-1	Africa Rice	G-59	ART15 10-17-46-2-2-2-B-B-2	Africa Rice
G-19	ART35-200-2-2-B-1	Africa Rice	G-60	ART15-16-31-2-1-1-1-B-1-1	Africa Rice
G-20	ART34-86-2-1-B-1	Africa Rice	G-61	ART16 5-10-22-4-B-1-B-B-1	Africa Rice
G-21	ART34-88-1-2-B-1	Africa Rice	G-62	ART16 9-4-18-3-1-1-B-B-1	Africa Rice
G-22	ART34-113-3-2-B-1	Africa Rice	6-63	ART16 9-16-21-1-B-2-B-B-1	Africa Rice
G-23	ART34-256-3-1-B-2	Africa Rice	G-64	ART16 9-29-10-2-B-1-B-B-1	Africa Rice
G-24	ART35-159-1-2-B-1	Africa Rice	G-65	ART16-4-1-21-2-B-2-B-1-1	Africa Rice
G-25	ART35-272-1-2-B-1	Africa Rice	G-66	ART16-4-13-1-2-1-1-B-1-1	Africa Rice
G-26	ART27-58-7-1-2-2-2-2	Africa Rice	G-67	ART16-5-10-2-3-B-1-B-1-2	Africa Rice
G-27	ART27-190-6-4-2-1-1	Africa Rice	G-68	ART16-9-1-9-2-1-1-B-1-1	Africa Rice
G-28	ART27-58-7-2-2-3	Africa Rice	G-69	ART16-9-4-18-4-2-1-B-1-1	Africa Rice
G-29	ART3-7L9P8-3-B-B-2-1	Africa Rice	G-70	ART16-9-4-18-4-2-1-B-1-2	Africa Rice

G-30	ART27-58-8-1-2-3	Africa Rice	G-71	ART16-9-5-28-3-13-1-B-2-1	Africa Rice
G-31	ART27-122-19-3-1-2-1-1	Africa Rice	G-72	ART16-9-6-18-1-1-2-B-1-1	Africa Rice
G-32	PCT-11\0\0\2,Bo\2\1>181-9-1-3-2-M	Africa Rice	G-73	ART16-9-9-25-2-1-1-B-2-1	Africa Rice
G-33	PCT-11\0\0\2,Bo\2\1>32-M-1-1-5-2-M	Africa Rice	G-74	ART16-9-9-25-2-1-1-B-2-2	Africa Rice
G-34	PCT-11\0\0\2,Bo\2\1>404-1-1-1-1-1M	Africa Rice	G-75	ART16-9-19-11-2-2-2-B-1-2	Africa Rice
G-35	PCT-11\0\0\2,Bo\2\1>46-M-3-4-3-2-M	Africa Rice	G-76	NERICA-3	Released variety
G-36	PCT-11\0\0\2,Bo\2\1>487-1-6-2-3-3M	Africa Rice	G-77	NERICA-14	Released variety
G-37	PCT-11\0\0\2,Bo\2\1>82-3-1-1-3-1-M	Africa Rice	G-78	NERICA-4	Released variety
G-38	PCT-11\0\0\2,Bo\2\1>94-1-1-2-1-3-M	Africa Rice	G-79	Getachew	Released variety
G-39	PCT-11\0\0\2,Bo\3\1>1-M-3-1-2-M	Africa Rice	G-80	Tana	Released variety
G-40	PCT-4\0\0\1>295-2-3-1-2-4-M	Africa Rice	G-81	Hidassie	Released variety
G-41	PCT-4\0\0\1>295-2-6-1-3-2-M	Africa Rice			

Experimental design and trial management

The experiment was laid out in a 9×9 simple lattice design with two replications using plot size of 2.4 m^2 (six rows, 2 m length, 0.20 m row spacing). A spacing of 1 m and 0.30 m between blocks and plots respectively was used. Planting was done manually on June 17, 2021, at a seeding rate of 100 kg ha^{-1}. According to Tilahun and Zelalem (2007), the recommended fertilizer rates of 69 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} were applied, with P_2O_5 applied at planting and N applied in three splits: at planting, tillering, and panicle initiation. Weeding was done manually three times, starting 25–30 days after sowing. All other agronomic practices were done as per recommendation.

Collected data

Data were collected from the middle four rows (1.6 m^2). Data for days to 50% heading, days to 85% maturity, grain yield (kg ha^{-1}), thousand-grain weight(g), above-ground biomass yield(t ha^{-1}), harvest index(%), protein Content(%) were recorded on a plot basis, while plant height(cm), leaf Chlorophyll content (LCC, SPAD reading), flag leaf length(cm), Leaf area(cm^2), number of primary branches per panicle, panicle length(cm), number of filled grains per panicle, number of unfilled grains per panicle, grain length(cm), grain width(cm), Grain length-width ratio were recorded on plant basis based on a standard evaluation system (SES) introduced by the IRRI (2013).

Statistical Analysis

The data were subjected to analysis of variance by using SAS software (SAS, 2002).

Estimation of phenotypic and genotypic variances

These genetic parameters were calculated by the formula given by Burton and De Vane (1953), and Johnson *et al.* (1955). These parameters include the following

Genotypic variance: $\sigma^2_g = \frac{MSG - MSE}{r}$

where MSG is the mean square of genotypes, MSE is the mean square of error, and *r* is the number of replications.

Phenotypic variance: $\sigma^2_p = \sigma^2_g + \sigma^2_e$, where σ^2_g is the genotypic variance and σ^2_e is the mean squares of error.

Estimation of genotypic and phenotypic coefficients of variability

Phenotypic coefficient of variance (PCV): **PCV (%)** == $\frac{\sqrt{\sigma^2 p}}{\bar{\chi}} \; x \; 100$

where σ^2_p is the phenotypic variance and $\bar{\chi}$ is the mean of the trait. Genotypic coefficient of variance (GCV): **GCV (%)** = $\frac{\sqrt{\sigma^2 g}}{\bar{\chi}}$ x 100

where $\sigma 2\ g$ is the genotypic variance and $\bar{\chi}$ is the mean of character. The classification for GCV and PCV is given by Deshmukh *et al.* (1986) as low (<10%), moderate (10-20%), and high (>20%).

Estimation of heritability and genetic advance

Heritability (broad sense): $\boldsymbol{h^2B}=\frac{\sigma^2_g}{\sigma^2_p}$

where σ^2_g is the genotypic variance and σ^2_p is the phenotypic variance. As demonstrated by Robinson *et al.* (1949) heritability can be categorized as low (0-30%), moderate (30 -60%), and high (60% and above).

Expected genetic advance (GA) was computed by the formulae described by Johnson *et al.* (1955).

Expected genetic advance$(GA) = H^2b * k * \sigma\rho;$

Genetic advance as percent of mean (GAM) was computed to compare the extent of the predicted advance of different traits under selection using the formula suggested by Johnson *et al.* (1955) and classified as low (<10%), moderate (10-20%) and high (>20%).

Expected genetic advance as percent of mean $(GAM) = \frac{GA}{\mu}$ x 100

Where, $\sigma\rho$ = phenotypic standard deviation on mean basis, H^2b = heritability in broad sense, k = selection differential (where k = 2.06 at 5% selection intensity) and μ = grand mean of the trait under consideration.

Divergence analysis. The patterns of distribution of morphological variation were analyzed using Mahalanobis Generalised Distances (D^2). The D^2 were applied to estimate the distances between and within clusters, using the SAS computer software package as per the following formula:

$D^2ij = (Xi-Xj)' S-1(Xi-Xj)$

Where: - D^2ij is the distance between class i and j; Xi and Xj are the vector means of the traits for the i^{th} and j^{th} groups, and S-1 is the inverse of the pooled covariance matrix. The D^2 analysis was based on the mean values of all morphological traits. The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (χ^2) and were tested for significance at P< 5% against the tabulated value of χ^2 for 'P' degree of freedom, where P is the number of parameters considered (Singh and Chaudhary, 1981)

Principal Component Analysis. Principal components based on correlation matrix, and Euclidian distances were calculated using PAST software (Hammer & Harper, 2001). One of the major reasons that analyses of principal components on correlation matrix was to standardize each variate (by subtracting its mean and

dividing by its standard deviation), which is useful as the parameters considered in this study did not share a common scale of measurement. Principal components having Eigen values >1 was considered significant and presented in the results.

Results and Discussion

Analysis of Variance and performances of genotypes

The analysis of variance (ANOVA) was used to estimate the degree of variation in the studied traits among 81 upland rice genotypes, with the results presented in Table 2. For all 18 traits studied, the ANOVA revealed highly significant differences, indicating substantial variation among the upland rice genotypes. High genetic variability for various yield-contributing traits in rice has also been reported by Tiruneh *et al.* (2021), Demeke *et al*. (2023), Munandar *et al.* (2023), and Lepcha (2023).

Table 2. Mean squares and simple statistics for 18 traits of upland rice genotypes

Traits	Rep (1)	MSg (80)	MSe (64)	Mean	Minimum	maximum	Range	CV (%)
DH	4.84	146.07***	8.14	65.5	56	92.5	36.5	4.36
DM	22.97	186.65***	15.67	92.4	83.5	107.5	24	4.29
FLL	5.19	50.9***	7.45	29.74	22	42.9	20.9	9.18
LA	26.7	147.78***	17.84	31.54	20.9	62.9	42	13.39
PH	203.6	200.03***	18.82	104.9	88.8	138.2	49.4	4.13
PL	1.32	8.25***	1.72	20.49	16.8	26.7	9.9	6.42
FGPP	168.5	1157.58***	162.92	98.46	70.7	195.3	124.6	12.96
UFGPP	5.7	0.271***	0.15	2.81	1.2	5.8	3.6	23.1
LCC	682.6	155.2***	49.89	48.53	35.3	74.3	39	14.49
PBPP	4.63	1.81***	0.57	9.72	7.8	13.3	5.5	7.79
BY	34.1	8.64***	3.84	17.15	12.82	22.9	10.12	11.43
GY	3.8	2.94***	1.01	8.22	4.72	10.6	5.88	12.24
HI	23.2	45.33***	20.97	48.05	35.3	63.8	28.3	9.53
TGW	0.9	16.2***	2.7	29.71	21.9	35.5	13.6	5.53
PC	0.45	0.23***	0.07	8.52	7.6	9.3	1.7	3.14
GL	2.2	1.52***	0.53	9.32	6.8	10.94	4.14	7.87
GW	1.66	0.01***	0.005	1.92	1.7	2.2	0.5	3.69
L/W	2.55	0.48***	0.18	4.86	3.6	5.85	2.25	8.69

where *, ** significant at 0.05 and 0.01 probability levels respectively; NS = non-significant, figures in parenthesis indicate degrees of freedom; MSg = mean squares of genotypes; MSe = mean squares of error; CV = coefficient of variation; DH = days to 50% heading; DM = days to 85% maturity; FLL = flag leaf length; LA = leaf area; LCC = leaf chlorophyll content; PH = plant height; PL = panicle length; FGPP = number of filled grains per panicle; UFGPP = the number of unfilled grains per panicle; NBPP = the number of primary branches per panicle; BY = biomass yield; GY = grain yield, HI = harvest index; TGW = thousand-grain weight; PC = protein content; GL = grain length, GW = grain width and L/W = grain length width ratio.

The mean values and range of variation for the 18 studied traits are presented in Table 2. Significant variation was observed in days to heading among the

genotypes, which ranged from 56 to 92.5 days (mean:65.5 days). Genotype ART35-100-1-7D-1 took the longest (92.5 days) followed by Tana and ART34-76-2-8D-2 (92.2 days) while the shortest (56 days) recorded for ARD5-10 and NERICA 14. Days to 85% maturity varied from 83.5 to 107.5 days, with PCT-4\SA\1\1 (83.5 days), SA\2\1>746-1-2-2-1-3-M (83.5 days) and PCT-4\SA\5\1>1754-5-1-3-2-2-M (84 days) were the earliest; however, Tana was the latest (107.5 days) followed by Getachew and ART35-200-2-2-B-1 (103.5 days). Early-heading and early-maturing genotypes are valuable genetic resources for developing drought-escape varieties. Variations in days to heading and maturity among rice genotypes have also reported by different authors (Akinola *et al.*, 2019; Ayenew *et al*. 2019; Pavankumar *et al.* 2022).

Flag leaf length ranged from 22 to 42.9 cm (mean=29.74 cm). Variability in flag leaf length among genotypes has also observed by Abebe *et al*. (2017) and Pavankumar *et al.* (2021). Genotypes differ significantly in plant height, which ranged from 88.8 to 138.2 cm. According to IRRI (2013), most of the genotypes classified intermediate height (96.3%), which preferred for better lodging resistance. Demeke *et al.* (2023) and Gnaneswari *et al.* (2023) also reported a wide range of variation in plant height ranging from 68 to 110.5 cm in rain-fed rice. Panicle length also showed significant differences among genotypes varied from 16.8- 26.7 cm, this might be due to genotypic difference. Similar results were highlighted by Kafi *et al.* (2021), Pavankumar *et al.* (2022) and Gupta et al. (2022). Primary branches per panicle ranged from 7.8-13.3. Significant variation also was reported by Tiruneh *et al.* (2021) who tested variability of upland rice genotypes under rain fed conditions at Guraferda.

Number of filled-grains per panicle ranged from 70.7 to 195.3, signifying the existence of genotypic variability. This result is in line with Demeke *et al.* (2023) and Gnaneswari *et al.* (2023), who reported significant differences in number of filled grain per panicle in rice genotypes. Biomass yield showed a wide variation ranged from 12.82 tons ha^{-1} to 22.9 tons ha^{-1} (mean =17.5 tons ha^{-1}) Similarly, Pavankumar *et al.* (2022) and Demeke *et al.* (2023) reported significant variations in biomass yield among genotypes. In the present study, grain yield ranged from 4.72 tons ha^{-1} to 10.6 tons ha^{-1}(mean:8.22 tons ha^{-1}), with ART35-200-2-2-B-1 performed the highest yield (10.6 tons ha^{-1}) followed by NM1-29-4-B-P-80-8 (10.15 tons ha^{-1}), ART34-82-1-7N-1 (10.1 tons ha^{-1}), PCT-11\0\0\2,Bo\2\1>82-3-1-1-3-1-M (10 tons ha^{-1}), ART16-9-9-25-2-1-1-B-2-1(9.99 tons ha^{-1}) and ART34-256-3-1-B-2(9.98 tons ha^{-1}) while ARD5-12 the lowest (4.72 ton ha^{-1}). Such significant variation in grain yield among genotypes is crucial for rice breeding, enhancing the potential for effective

crossing and selection. In harmony with the present study, significant variation in grain yield were reported by earlier workers (Kafi *et al.* 2021; Lepcha 2023; Pavankumar *et al.* 2022; Demeke *et al.* 2023 and Gnaneswari *et al.* 2023), who tested rice genotypes under rainfed conditions. Harvest index varied from 35.29% to 63.29% (mean=48.05%). The harvest index is an important trait in determining the photosynthetic efficiency of genotypes and the distribution of photosynthetic products between straw and grain.

Like other traits, thousand grain-weight showed significant differences, which ranged from 21.92g to 35.5g (mean=29.71g). Similarly, Lepcha (2023) and Demeke *et al* (2023), reported significant differences in thousand-grain weight among rice genotypes. Grain length, grain width, and length-width ratio varied from 6.79 to 10.94 mm, 1.92 to 2.19 mm, and 3.64 to 5.84, respectively. Similarley, Haider (2017), Chimthai *et al*. (2021) and Gupta *et al.* (2022), reported significant difference among rice genotypes for these traits. Protein content varied from 7.5% to 9.3% with a mean value of 15.13%. A wide genetic variation in protein content among rice genotypes was also reported by Hossain *et al.* (2015).

Genetic Parameters Among Genotypes

Phenotypic and genotypic coefficient of variation

The estimates of phenotypic coefficient of variation (PCV), genotypic coefficients of variation (GCV), heritability in a broad sense (h^2B), and expected genetic advance as percent of the mean (GAM) are presented in the Table 3. Phenotypic variability represents total observable variation, while genotypic variability, unaffected by environmental factors, is crucial for breeders in selection and hybridization. High PCV and GCV were observed for leaf area and filled grains per panicle, aligning with Kafi *et al.* (2021). Moderate PCV and GCV values for traits like days to heading, maturity, chlorophyll content, and grain yield indicate sufficient genetic variation for selection, corroborated by the findings of Kafi *et al.* (2021) and Tezera (2021). Conversely, the low GCV and moderate PCV for traits such as thousand-grain weight and protein content suggest a narrow genetic base, consistent with that of Srujana *et al*. (2017), Akinola *et al.* (2019) and Pavankumar *et al.* (2021). PCV and GCV values indicate the extent of variability in traits. However, combining them with heritability and genetic advance provides a clearer understanding of potential selection gains in breeding programs.

Heritability and genetic advance

Heritability estimates predict the reliability of phenotypic values and help breeders forecast trait inheritance in future generations. In this study, broad-sense

heritability ranged from 28.85% for unfilled grains to 89.44% for days to heading (Table 3). High heritability (>60%) was observed for traits like days to heading (89.44%), days to maturity (84.51%), flag leaf length (74.46%), leaf area (78.46%), plant height (82.8%), panicle length (65.5%), filled-grains per panicle (75.32%), and thousand-grain weight (71.43%) (Table 3), indicating strong genetic control with minimal environmental influence, making these traits suitable for effective phenotypic selection. The present results, were aligned with the findings of Kafi *et al.* (2021) and Pavankumar *et al.* (2022). Moderate heritability was observed for primary branches per panicle (52.1%), protein content (53.33%) grain length (48.29%), and grain yield (48.86%), as supported by Akinwal *et al*. (2011) and Abebe *et al.* (2017). These traits, less influenced by environmental factors, offer potential for improvement through direct selection based on their phenotypic performance.

Genetic advance is a useful indicator of the expected progress resulting from selection within the population. The range of genetic advance as a percent of mean was ranged from 3.1% for grain width to 46.63% for leaf area (Table 3). Moderate GAM was observed for traits like days to maturity, plant height, panicle length, primary branches per panicle, biomass yield, grain yield, thousand grain weight, grain length, and grain length-to-width ratio (Table 3). This suggested that the inheritance of these traits is governed by additive gene action. In addition, direct selection is effective to improve the traits. similar finding also observed in earlier studies by Sandeep *et al.* (2018), Demeke *et al*. (2023), Bandi *et al.* (2018) and Asante *et al.* (2019). The lowest GAM was noted for grain width, protein content and harvest index, indicating their control by non-additive gene action. Improvement in these traits may be better achieved through heterosis breeding, family selection, and progeny testing. This result supported by Abebe *et al.* (2017) and Asante *et al.* (2019).

Genetic variation, heritability, and genetic advance help predict genetic gain from selection for trait improvement. In this study, high heritability along with high genetic advance as percent of mean was observed for traits such as days to heading and filled grain per panicle, indicates the less influenced by the environment and direct phenotypic selection could be effective. This finding, aligned with reported by Kafi *et al.* (2021) and Demeke *et al.* (2023).

Table 3. Estimates of coefficients of variation and selected parameters for 18 quantitative traits

Traits	σ²g	σ²p	σ²e	GCV	PCV	H²	GA	GAM
DH	68.97	77.11	8.14	12.68	13.41	89.44	16.18	24.70
DM	85.49	101.16	15.67	10.01	10.89	84.51	17.51	18.95
FLL	21.73	29.18	7.45	15.67	18.16	74.46	8.29	27.86
LA	64.97	82.81	17.84	25.56	28.85	78.46	14.71	46.63
PH	90.61	109.4	18.79	9.07	9.97	82.80	17.84	17.01
PL	3.27	4.99	1.72	8.82	10.90	65.50	3.01	14.70
FGPP	497.33	660.3	162.97	22.65	26.1	75.32	39.87	40.49
UFGPP	0.06	0.21	0.15	8.7	16.3	28.5	0.75	26.9
LCC	52.66	102.6	49.94	14.95	20.87	51.35	10.71	22.07
PBPP	0.62	1.19	0.57	8.10	11.22	52.10	1.17	12.05
BY	2.4	6.24	3.84	9.03	14.57	38.46	1.98	11.54
GY	0.97	1.98	1.01	11.95	17.10	48.86	1.41	17.21
HI	12.18	33.15	20.97	7.26	11.98	36.74	4.36	9.07
TGW	6.75	9.45	2.7	8.74	10.35	71.43	4.52	15.22
PC	0.08	0.15	0.07	3.32	4.55	53.33	0.43	4.99
GL	0.5	1.03	0.53	7.55	10.86	48.29	1.01	10.81
GW	0	0.01	0.01	2.60	4.51	33.33	0.06	3.10
L/W	0.15	0.33	0.18	7.97	11.82	45.45	0.54	11.07

where: σ2g = genotypic variance; σ2p = phenotypic variance; σ2e environmental variance; GCV = genotypic coefficient of variation; PCV = phenotypic coefficient of variation; H2 = broad sense heritability; GA = Genetic Advance; GAM = genetic advance as percent of mean; DH = days to 50% heading; DM = days to 85% maturity; FLL = flag leaf length; LA = leaf area; LCC = leaf chlorophyll content; PH = plant height; PL = panicle length; FGPP = number of filled grains per panicle; UFGPP = the number of unfilled grains per panicle; NBPP = the number of primary branches per panicle; BY = biomass yield; GY = grain yield, HI = harvest index; TGW = thousand-grain weight; PC = protein content; GL = grain length, GW = grain width and L/W = grain length width ratio.

Cluster Analysis

Eighty-one upland rice genotypes were grouped into six clusters based on 18 morphological traits through multivariate analysis at a 0.55 similarity coefficient (Figure 2 and Table 4), suggesting that morphological traits can reveal variability existing among rice genotypes. Cluster I contained the highest number of genotypes (31), followed by Cluster III with 26 genotypes. Clusters II, IV, VI and V consisted of thirteen, five, four and two genotypes, respectively (Table 5). Similar results also found by Ayenew *et al.* (2019) and Ibrahim *et al*. (2019) which grouped 87 and 36 genotypes of rice into six and seven clusters, respectively.

Divergence analysis. Pairwise generalized squared distances (D^2) among the six clusters revealed 15 significant inter-cluster distances ($p < 0.01$), while intra-cluster differences were non-significant (Table 4). The maximum inter-cluster distances were observed between Cluster IV and V ($D^2 = 261.62$), Cluster I and V ($D^2 = 238.49$), and Cluster III and IV ($D^2 = 202.49$). The minimum distances were between Cluster I and II ($D^2 = 38.95$), Cluster I and III ($D^2 = 52.51$), and Cluster III and VI ($D^2 = 53.36$). (Table 4).

Table 4. Pair-wise generalized intra (bolded diagonals) and inter-cluster distances (D^2) between six clusters

Cluster	I	II	III	IV	V	VI
I	**7.15**	38.95*	115.38	146.87	238.49	141.48
II		**12.2**	52.51	91.25	128.74	46.32
III			**10.38**	202.49	85.92	53.36
IV				**11.08**	261.62**	124.66
V					**12.76**	60.02
VI						**4.34**

χ2=33.41 at 1% probability level and χ2 =27.59 at 5% probability level

Crossing genotypes from clusters with maximum genetic distance is expected to enhance recombination and variation, increasing opportunities for transgressive segregation and heterosis, as also noted by (Atnaf *et al.,* 2013; Pickup *et al*., 2013). This clustering provides a foundation for upland rice breeding, with further recommendations to select parents based on genetic divergence and specific breeding objectives, considering the unique advantages of each cluster and its members. Previous studies (Hossain *et al.* 2015; Lepcha, 2023) emphasized selecting genotypes from distinct clusters for hybridization to achieve superior hybrids. However, the selection of parents for particular crosses should also consider the special advantages of each cluster and accession within a cluster, depending on specific objectives of hybridization programs. As Habtamu and Million (2013) suggested that members of the same cluster are closely related and may not yield desirable segregates. Future research on selected germplasm will streamline breeding efforts.

Table 5. List and number of genotypes grouped in each cluster based on quantitative traits

Clusters	№ genotypes	Proportion %	Genotypes
C1	31	38..26	ARD5-8, ARD5-9, Fogera1, ART16-9-5-28-3-13-1-B-2-1, ART16-9-25-30-3-2-2-B-1-1, ART34-113-3-2-B-1, ART27-58-7-1-2-2-2-2, PCT-11\0\0\2,Bo\2\1>487-1-6-2-3-3-M, PCT-11\0\0\2,Bo\2\1>82-3-1-1-3-1-M, PCT-11\0\0\2,Bo\2\1>94-1-1-2-1-3-M, PCT-11\0\0\2,Bo\3\1>1-M-3-1-2-M, PCT-4\0\0\1>295-2-6-1-3-2-M, PCT-4\SA\5\1>1754-5-1-3-2-2-M, NM1-29-4-B-P-80-8, ART16-9-14-16-2-2-1-B-1-2, ART16-9-122-33-2-1-1-B-1-1, ART16-21-4-7-2-2-2-B-2-2,ART16-4-1-21-2-B-2-B-1-2,PARC.DAT.V-1.2013, PARC.DAT.V-2.2013, PARC.DAT.V-3.2013, ART15 8-10-36-4-1-1-B-B-1, ART15-16-31-2-1-1-1-B-1-1, ART16 5-10-22-4-B-1-B-B-, ART16 9-4-18-3-1-1-B-B-1, ART16 9-16-21-1-B-2-B-B-1, ART16-4-13-1-2-1-1-B-1-1, ART16-9-6-18-1-1-2-B-1-1, ART16-9-19-11-2-2-2-B-1-2, NERICA 3 and Hidassie.
C2	13	16	ART16-4-13-1-2-1-1-B-1-1, ART16-9-9-25-2-1-1-B-2-1, ART16-9-19-11-2-2-2-B-1-2, ART16-12-28-32-3-B-1-1-2, ART35-272-1-2-B-1, ART27-190-6-4-2-1-1, ART27-58-7-2-2-3, ART3-7L9P8-3-B-B-2-1, ART27-58-8-1-2-3, ART27-122-19-3-1-2-1-1, ART16-5-10-2-3-B-1-B-1-1, ART16-4-1-21-2-B-2-B-1-1 and ART16-5-10-2-3-B-1-B-1-2.
C3	26	32.1	ARD5-10, ARD5-12, ARD5-13, ART15-16-31-2-1-1-1-B-1-1, ART16 5-10-22-4-B-1-B-B-1, ART169-29-10-2-B-1-B-B-1,PCT11\0\0\2,Bo\2\1>32-M-1-1-5-2-M,PCT 11\0\0\2,Bo\2\1>404-1-1-1-1-1-M,PCT-4\0\0\1>295-2-3-1-2-4-M,PCT4\SA\1\1,Bo\3\1>161-3-2-1-M,PCT-4\SA\1\1,SA\2\1>746-1-2-2-1-3-M,PCT-4\SA\5\1>1754-5-1-5-3-1-M,ART16-9-29-12-1-1-2-B-1-1,ART15-19-5-4-1-1-1-B-1-1, ART15-16-45-1-B-1-1-B-1-2, ART15 10-17-46-2-2-2-B-B-2, ART16 9-29-10-2-B-1-B-B-1, ART16-9-1-9-2-1-1-B-1-1, ART16-9-4-18-4-2-1-B-1-1, ART16-9-4-18-4-2-1-B-1-2,ART16-9-5-28-3-13-1-B-2-1, ART16-9-9-25-2 1-1-B-2-1, ART16-9-9-25-2-1-1-B-2-2, NERICA 14 and NERICA 4.
C4	5	6.2	ART34-82-1-7N-1, PCT-11\0\0\2, Bo\2\1>181-9-1-3-2-M, PCT-11\0\0\2,Bo\2\1>46-M-3-4-3-2-M, Getachew and Tana.
C5	2	2.5	ART34-76-2-8D-2 and ART35-100-1-7D-1
C6	4	4.94	ART35-200-2-2-B-1, ART34-86-2-1-B-1, ART34-88-1-2-B-1, ART34-256-3-1-B-2 and ART35-159-1-2-B-1

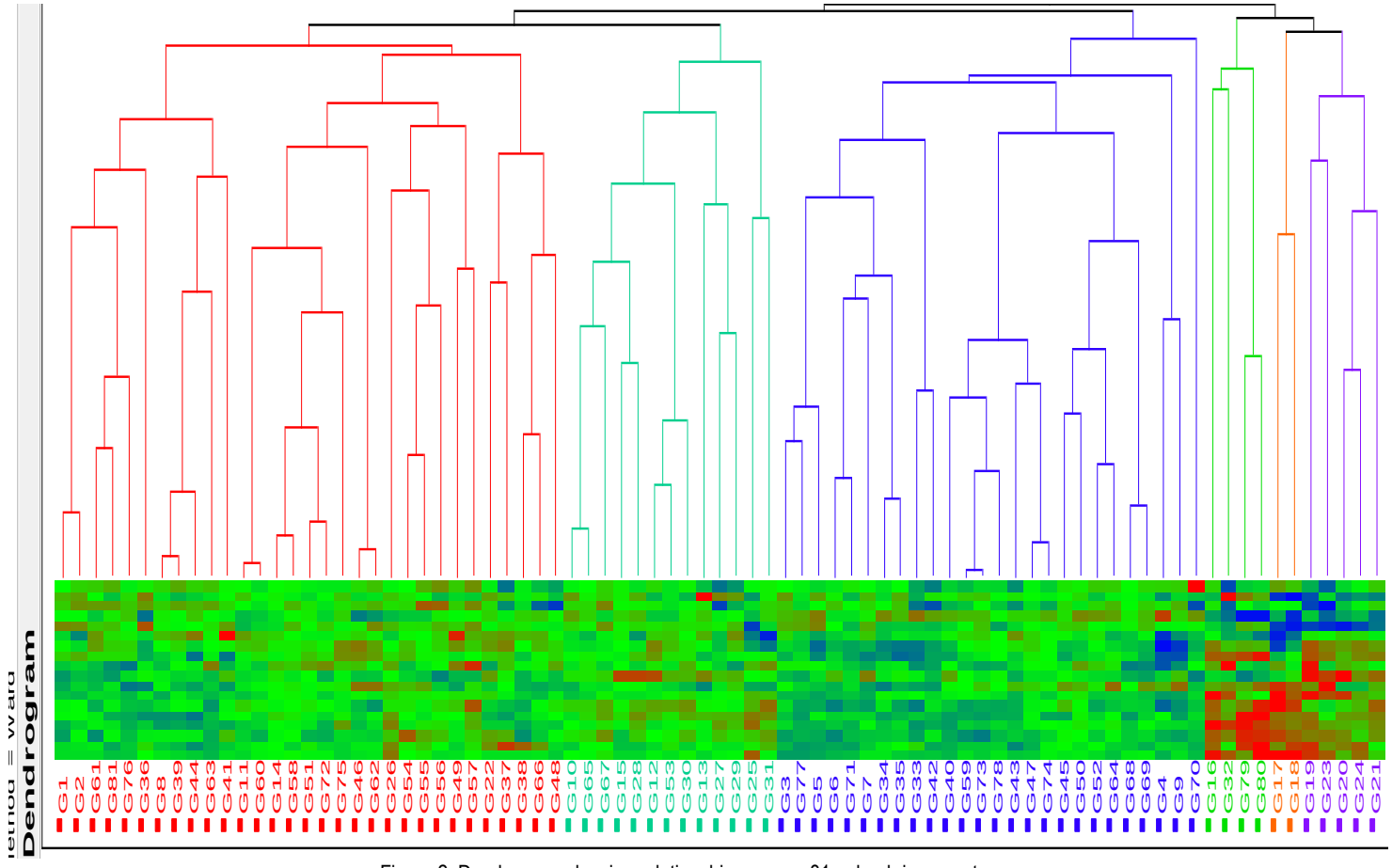


Figure 2: Dendrogram showing relationships among 81 upland rice genotypes

Cluster Means Analysis

The cluster mean performance revealed significant variation among clusters for individual traits. Cluster I (31 genotypes) had the highest means for harvest index and thousand-grain weight (Table 6). Cluster II (13 genotypes) excelled in flag leaf length, while Cluster III (26 genotypes) had high protein content. Cluster IV (5 genotypes) showed the highest means for leaf area and plant height. Cluster V (2 genotypes) stood out for panicle length, days to heading, days to maturity, and filled grains per panicle. Cluster VI (4 genotypes) had the highest means for primary branches per panicle, grain yield, and dry biomass yield. Genotypes in Clusters V and VI were identified as promising candidates for breeding high-yielding rice varieties.

Table 6. Cluster means for 18 parameters of rice genotypes used in a study conducted at Metema

Traits	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5	Cluster-6	mean
DH	63.23	65.27	60.64*	79.30	91.25**	77.50	72.86
DM	90.68	89.81	87.40*	98.80	100.00**	98.20	94.15
FLL	28.96	31.95	25.89*	37.70	40.00**	36.02	33.42
LA	28.21	34.55	27.27*	49.99**	45.59	41.63	37.87
PH	101.58	112.86	98.10*	121.24**	120.80	116.24	111.80
PL	20.19	21.94	18.94*	22.84	25.95**	21.80	21.94
FGPP	92.28	98.15	85.89*	129.08	168.60**	141.74	119.29
UFGPP	2.56	2.32	2.90*	3.66	5.00**	3.52	3.33
FLL	44.63	57.15**	46.17	42.82*	47.85	68.19	51.14
PBPP	9.84	9.74	9.27*	10.36	10.15	10.42**	9.96
BY	17.48	17.34	15.52*	20.04	16.44	20.25**	17.84
GY	8.79	8.07	7.30*	8.82	6.23	9.97**	8.20
HI	50.63**	46.66	46.93	44.40	37.57*	49.18	45.89
TGW	30.91**	30.18	29.90	29.35	22.59*	23.30	27.71
PC	8.46	8.57	8.81**	8.00	7.93*	8.11	8.31
GL	9.72**	9.27	9.04	9.01	9.23	8.23*	9.08
GW	1.93	1.96	1.92	2.01**	1.71*	1.82	1.89
LW	5.04	4.75	4.97	4.54	5.56**	4.53*	4.90

Where: * and ** are the lowest and highest cluster mean, and all other traits name listed in the above; DH = days to 50% heading; DM = days to 85% maturity; FLL = flag leaf length; LA = leaf area; LCC = leaf chlorophyll content; PH = plant height; PL = panicle length; FGPP = number of filled grains per panicle; UFGPP = the number of unfilled grains per panicle; NBPP = the number of primary branches per panicle; BY = biomass yield; GY = grain yield, HI = harvest index; TGW = thousand-grain weight; PC = protein content; GL = grain length, GW = grain width and L/W = grain length width ratio.

Principal Component Analysis

The first five PCAs accounted for 75.56% of the total variation among 81 upland rice genotypes, with eigenvalues greater than 1. PC1, PC2, PC3, PC4, and PC5 contributed 39.92%, 11.64%, 10.2%, 7.9%, and 6.96% of the variation, respectively (Table 6). Similar observations were made by Sinha and Mishra (2013), Tiruneh *et al.* (2021) and Ibrahim *et al.* (2019), reported that the first five PCAs accounted for 75% the variability among 34, 36, and 87 rice genotypes based on 11, 15, and 17 traits, respectively. PC1 was primarily influenced by plant

height, days to maturity, filled-grains per panicle, flag-leaf length, leaf area, and days to heading, making it key for genotype discrimination. PC2 was driven by grain yield, harvest index and biomass yield, while PC3 was dominated by grain length and thousand-grain weight. PC4 was characterized by grain width and length-width ratio PC5 was influenced by panicle length, unfilled-grain per panicle, leaf chlorophyl content and primary branches per panicle. Similar findings also reported by various researchers (Worede *et al.* 2014; Tuhina-Khatun *et al.* 2015; Tiruneh *et al.* 2021 and Ibrahim *et al.* 2019), highlights the importance of grain yield and other traits for rice breeding and selection.

Table 6. Eigen vectors, explained variance and Eigen values of the first five significant principal component axes for 18 parameters of rice genotypes.

	Eigen vector values				
Traits	PCA1	PCA2	PCA3	PCA4	PCA5
Days to heading	0.34	-0.12	-0.02	-0.02	-0.07
Days to maturity	0.29	0.04	-0.03	-0.06	-0.05
Flag leaf length	0.32	-0.02	0.10	-0.09	0.13
Leaf area	0.33	-0.06	0.09	-0.17	0.04
Plant height	0.31	-0.04	0.13	-0.17	0.24
Panicle length	0.29	-0.07	0.20	0.01	0.31
Filled-grains per panicle	0.32	-0.09	-0.13	0.15	0.07
Unfilled-grain per panicle	0.19	-0.14	0.04	-0.05	-0.63
Leaf chlorophyl content	0.11	0.10	-0.39	0.03	0.45
Primary branches per panicle	0.17	0.09	0.02	0.18	0.23
Biomass yield	0.27	0.31	0.13	0.02	-0.21
Grain yield	0.15	0.56	0.05	0.28	-0.14
Harvest index	-0.10	0.51	-0.05	0.37	0.08
Thousand-grain weight	-0.18	0.20	0.48	-0.24	0.03
Protein content	-0.27	-0.16	-0.16	-0.03	0.19
Grain-length	-0.08	-0.06	0.54	0.15	0.20
Grain-width	-0.09	0.33	0.12	-0.62	0.12
Length-width ratio	-0.05	-0.29	0.41	0.44	0.06
Eigenvalue	**7.18**	**2.10**	**1.80**	**1.41**	**1.09**
Explained variance	**39.92**	**11.64**	**10.20**	**7.90**	**6.96**
Cumulative variance	**39.92**	**51.56**	**61.78**	**69.68**	**75.56**

The first two principal components, PC1 (39.92%) and PC2 (11.64%), were plotted on a two-dimensional plane(biplot), with trait and genotype distributions visualized in Figure 3. Among the 81 upland rice genotypes, the highest principal component scores were observed across five components (Table 16), which can serve as indicators for selection based on the variability explained by each PC. According to Dehghani *et al*. (2008), the correlation between any two traits is approximated by the cosine of the angle between their vectors. The most prominent relationships shown in Figure 3 are a strong positive association grain yield with biomass yield, leaf chlorophyll content, primary branch per panicle, days to maturity and flag leaf length (Figure 3). Moreover, the prominent strong relationship between harvest index and grain width; grain length and length width

ratio; days to 50% heading with leaf area, panicle length and filled-grain per panicle each other (Figure3).

The bi-plot gave more opportunity to assess which genotypes were good for which traits that would help as excellent baseline information for upland rice improvement. In this PC biplot analysis, the aggregation of traits and genotypes has presented in Fig 4. Genotypes G-37, G-29, G-75, G-12, G-49 and G-53) could be directly selected for grain yield improvement; whereas G-53, G-54 and G-14 are good for biomass yield improvement. For improving days to heading, plant height, and panicle length filled-grain per panicle, G-27, G-28, G-55 and G-65, while G-26, G-57 and G-67 are key for enhancing days to maturity, such information highlighted key traits that can be exploited using principal component analysis.

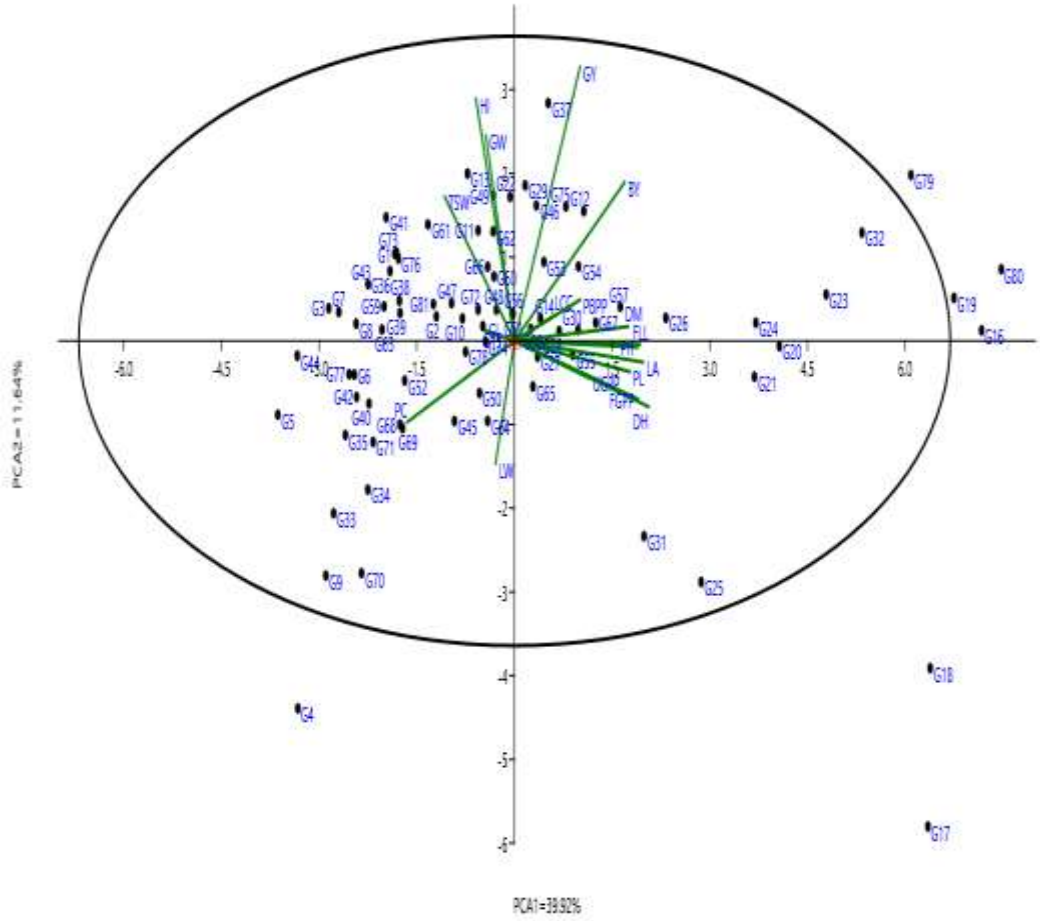


Figure 3. Bi plot of PC1 and PC2 showing the relationships of genotypes by traits. The blue color of the dot represents genotypes (n=81) and the blue color of the vector represents the traits under study. The number representation is as indicated in Table 1.

Conclusion

The result affirms considerable variability among the 81 tested upland rice genotypes for traits such as days to heading, days to maturity, plant height, panicle length, primary branches per panicle, filled grains per panicle, biomass yield, thousand grain weight and grain yield. Moderate to high GCV, PCV, heritability, and genetic advance as a percentage of the mean for traits such as days to heading, days to maturity, filled grains per panicle, leaf area, and grain yield indicate strong potential for improvement through phenotypic selection. Cluster analysis grouped the genotypes into six clusters, with the highest genetic distance between clusters IV and V, highlighting promising gene sources for hybridization. Clusters VI and IV were identified as valuable for breeding high-yielding rice genotypes. Principal component analysis showed that the first five components accounted for 75.56% of total variability across 18 traits, emphasizing their importance in explaining diversity and guiding trait improvement. In the present study, promising genotypes were observed and could be considered for improving upland rice production, particularly in Metema area. Further molecular analysis is recommended to enhance the reliability and utility of the results of the current study.

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