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Genotype by Environment Interaction Using AMMI, GGE Biplot and Multivariate Analysis of Nepalese Aromatic Rice Landraces

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ABSTRACT

Aromatic group of rice comprises fine, medium, long and extra-long grain that has rich in agro-biodiversity, carries historical and cultural significance in Nepalese society. The objective was to identify stable, high-yielding aromatic rice landraces for Nepal Thirty aromatic rice landraces including two released varieties as standard checks were evaluated at three locations during 2020 and 2021. The experiment was laid out in a Randomized Complete Block Design with three replications. The combined analysis of variance indicated differences among genotypes and genotype-environment interactions for grain yield. The additive main effects and multiplicative interaction (AMMI) analysis revealed that genotypic effects for 69%, genotype × environment interaction effects for 28% and environmental effects accounted for 3% of the total sum of squares. Similarly, GGE biplot analysis showed that the principal components (PC1 and PC2) accounted for 53% and 47% of the GGE sum of squares, respectively. Based on AMMI1, AMMI2 biplots, and GGE biplots, the genotypes Samba Masuli Sub-1 and Sugandhit Dhan-1 were identified as the high yielding and stable fine and aromatic rice landraces. Other promising genotypes are Damari Dhan, Tilki, Hiupuri, Kalo Basmati, Gouriya, and Brahmamusi Dhan. Specifically, Samba Masuli Sub-1 demonstrated the highest grain yield performance at the NRRP, Hardinath, while Sunaulo Sugandha and Balamsari Dhan excelled in the Tarahara and Khajura environments, respectively. In PCA analysis, four PCs showed significant eigen value >1.0 contributing more than 84% of total variance. Biplot analysis reflected that 1000 grain weight, panicle length, heading and maturity days, and grain yield showed greater variation among the landraces. The results provide a robust basis for the further evaluation and verification of this findings in advance stage trials. The superior and outstanding landraces with higher yields and aroma are potential future varieties that can be either use directly as varieties after selection or utilize in future breeding programs for the development of new aromatic rice varieties.

Keywords: Aromatic rice, AMMI, GGE biplot, landraces, PCA, stability analysis

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INTRODUCTION

Rice (*Oryza sativa L., 2n=24*) is the principal food grain of Nepal, contributing 20% to the agricultural gross domestic product (AGDP) and 53% to total cereal food production (MoALD 2023). It is cultivated on 1.4 million hectares, with a production of 5.4 million metric tons and a productivity of 3.78 metric tons per hectare (AITC 2024). The Tarai region, along with other areas in the Himalayan foothills, is considered a center of rice diversity (Khush 1997). Many aromatic cultivars continue to be grown in this diversity hub. Nepal boasts a rich repository of aromatic rice landraces due to its diverse agro-ecological zones (Khush 1997). These landraces have been traditionally cultivated for centuries, adapting to specific local conditions, and are often preferred over improved varieties for their superior grain quality and aroma (Joshi et al. 2007). Among the numerous types of rice landraces, aromatic rice is particularly valued for its distinct aroma and flavor, holding immense cultural and economic significance in Nepal and beyond (Joshi et al. 2021, Islam et al. 2016).

Landraces have a diverse genetic base, yet many remain unexplored, characterized, and utilized in breeding programs. The genetic improvement of any crop largely depends on the amount of genetic variability present in the population (Tiwari et al. 2019). Aromatic rice landraces often display remarkable diversity in their adaptability to different environments (Joshi et al. 2007). However, their performance can vary significantly across different environments due to variations in edaphic, climatic, and biotic factors. The performance of any character is a combined result of the genotype (G) of the variety, the environment (E), and the interaction between genotype and environment (GE). To evaluate the consistency of rice grain yield across years and geographical regions, it is essential to research yield stability and GE interactions (Blanche et al 2009). Yield, being a complex quantitative trait, is greatly influenced by environmental changes (Tiwari et al. 2019). From the viewpoint of conservation and utilization in breeding programs, understanding genotypes' diversity, stability, and adaptability is crucial.

The AMMI model was employed to understand the genotypic and environmental interactions among the genotypes for grain yield. The model provides a comprehensive perspective on G × E interaction by analyzing it across multiple dimensions. Compared to ANOVA, the AMMI model offers a better approach for studying and understanding G × E interactions (Hassani et al. 2018). Generally, multi-environment trials (METs) are conducted to evaluate the stability of crop varieties across diverse environments. These trials generate valuable data on genotype-environment interactions (GEI), which are critical for identifying stable and high-yielding genotypes (Yan and Kang 2003). Several statistical methods analyze MET data to identify genotypes with stable performance.

Among these methods, the Additive Main Effects and Multiplicative Interaction (AMMI), and Genotype and Genotype × Environment (GGE) biplot analysis help in understanding the stability of genotypes and aid in selecting those with consistent performance (Gauch 2006). AMMI is particularly useful for visualizing G × E effects graphically (Nayak et al. 2008; Bose et al. 2014). The AMMI model is a hybrid model combining the additive component from analysis of variance (ANOVA) and the multiplicative component from principal component analysis. Additionally, the biplot display provides a visual interpretation of genotype-environment interaction components. Biplot displays and stability statistics together enable genotypes to be grouped based on similarity of performance across environments (Thillainathan and Fernandez 2001). The results of AMMI analysis are useful for decision-making in breeding programs for the selection of specifically and broadly adapted genotypes as well as environments (Gauch and Zobel 1997).

The application of multivariate analysis method is a to measure the magnitude of variation among the genotypes. Principal component analysis (PCA) is generally used to define the variation present as multivariate methods (Salem et al. 2021). The major objective of PCA is used to assess variability among the various traits in the germplasm and to explore the distinguishing characteristics of genotypes to offer the opportunity for selection. It also applied to assess the variation between the genotypes collected from various sources. The agro-morphological markers provide great information for the assessment of diversity in rice (Chakravorty et al. 2012). Furthermore, PCA takes in account the importance and contribution of every component to total variance (Sinha and Mishra 2013), while particular value indicates the extent of each variable contribution associated with every main component (Salem et al. 2021). Thus, characterization of germplasm based on the phenotypic traits has huge significance in rice breeding that provides valuable information for selection of functional traits (Sinha and Mishra 2013).

In this study, we analyzed the stability and GEI of selected aromatic rice landraces across three locations in Nepal. By integrating phenotypic data from multi-environment trials and employing AMMI statistical models, we aim to elucidate the genotype-environment interactions for the performance of these landraces. Our investigation will contribute to understanding the adaptability and stability of Nepalese aromatic rice and provide valuable insights for aromatic rice breeding programs.

MATERIALS AND METHODS

Experimental location

Geographically, NRRP Dhanusa, Nepal, is situated at 93 meters above mean sea level, with coordinates of

26°49' N latitude and 86°01' E longitude. It has a subtropical climate with maximum temperatures reaching 44℃ in the summer and minimum temperatures dropping to 4.8℃ in the winter. The average annual rainfall is 1281 mm, with maximum precipitation occurring in July and 80% of the total annual rainfall falling between June and September (NRRP 2020).DoAR Tarahara is situated at 26°42'16.85" N latitude and 87°16'38.43" E longitude, at an elevation of 136 meters above sea level. The farm's climate is subtropical, with soil textures predominantly clay loam, varying from sandy loam to loam within the farm. The soil pH ranges from 6.5 to 7.0, indicating a slightly acidic to neutral status (DoAR Tarahara 2020). Similarly, DoAR Khajura is located at 181 meters above mean sea level, with coordinates of 28°06' N latitude and 81°37' E longitude. The average annual rainfall at this station ranges from 1000 to 1500 mm. The maximum temperature is 46℃, and the minimum temperature is 5.4℃, with relative humidity varying between 27% and 94%. The soil at the station is sandy to silty loam, low in organic carbon and available nitrogen but medium in available phosphorus and potassium (DoAR Khajura 2020).

Field experiment

Field evaluations were conducted at three locations: the National Rice Research Program (NRRP) Hardinath, Dhanusa in Madhesh Province; the Directorate of Agricultural Research (DoAR) Tarahara, Sunsari in Koshi Province; and the DoAR Khajura, Banke in Lumbini Province over two consecutive years of 2020 and 2021.

Table 1. List of aromatic rice landraces used in multi-environment trial and quality test in 2020/21

Entry	Genotypes	Local name	Description
1	NGRC 05866	Karia Kamod	Gene-bank accession (landrace)
2	Sunaulo Sugandha	Sunaulo Sugandha	Variety
3	NGRC 03096	Kalo Masino Dhan	Gene-bank accession (landrace)
4	NGRC 01849	Balamsari Dhan	Gene-bank accession (landrace)
5	Karia Kamod 1	Karia Kamod	Local collection (Landrace)
6	NGRC 05813	Tilki	Gene-bank accession (landrace)
7	NGRC 05914	Kalo Nunia	Gene-bank accession (landrace)
8	NGRC 08137	Bayani Masino	Gene-bank accession (landrace)
9	NGRC 05869	Brahmamusi Dhan	Gene-bank accession (landrace)
10	NGRC 05915	Basmati Jhapa	Gene-bank accession (landrace)
11	NGRC 05873	Malbhog	Gene-bank accession (landrace)
12	NGRC 05816	Jhuse Basmati	Gene-bank accession (landrace)
13	NGRC 05827	Gouriya	Gene-bank accession (landrace)
14	NGRC 05018	Hansaraj Dhan	Gene-bank accession (landrace)
15	NGRC 03284	Suhpat Dhan	Gene-bank accession (landrace)
16	NGRC 02113	Damari Dhan	Gene-bank accession (landrace)
17	NGRC 05870	Ujarka Basmati	Gene-bank accession (landrace)
18	Karia Kamod 2	Karia Kamod	Local collection (Landrace)
19	NGRC 03110	Jhinuwa Dhan	Gene-bank accession (landrace)
20	NGRC 05829	Hiupuri	Gene-bank accession (landrace)
21	Sugandhit dhan-1	Sugandhit dhan-1	Variety
22	NGRC 05919	Chananchur	Gene-bank accession (landrace)
23	Lalka Basmati	Lalka Basmati	Variety; landrace selection
24	NGRC 05801	Kalo Jira	Gene-bank accession (landrace)
25	NGRC 05878	Tulsiful	Gene-bank accession (landrace)
26	NGRC 05832	Kalo Basmati	Gene-bank accession (landrace)
27	NGRC 05865	Lalka Basmati Agahani	Gene-bank accession (landrace)
28	NGRC 02137	Gaure Dhan	Gene-bank accession (landrace)
29	Kalanamak	Kalanamak	Variety; used as aromatic check
30	Samba Masuli Sub-1	Samba Masuli Sub-1	Variety; used as non-aromatic check

Note: NGRC is an abbreviated form of National Agriculture Genetic Resources Center used in each accession of its collection

In total thirty rice genotypes, comprising of gene-bank accessions (n=23) preserved at National Agricultural Genetic Resource Center, Khumaltar, local collection from the vicinity of National Rice Research Program (NRRP), Dhanusa (n=2), released varieties- aromatic (n=3) and two standard check varieties, Samba Masuli Sub-1 (non-aromatic) and Kalanamak (aromatic) were included in the study. The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications across the three locations (Hardinath, Tarahara, and Khajura). At each location, plot size was 6.0 m², with inter-row and intra-row (plant-to-plant) spacing of 20 cm × 20 cm was maintained. Seed sowing was performed during the second week of June, and 22-days-old seedlings were transplanted into the main field. The recommended cultural practices of NRRP were followed for all intercultural operations throughout the crop season. Thirty aromatic accessions (**Table 1**) identified from the gene pool after a preliminary evaluation of grain aroma at NRRP, Hardinath, using a 1.7% KOH solution treatment were used in this study.

Quantitative traits

The major quantitative traits observed in each location were days to heading and maturity, plant height, panicle length, filled and unfilled grains per panicle, tillers per square meter, thousand grain weight, filled grain and its weight, straw yield, and grain yield. The standard protocol of NRRP was followed in recording each trait during field observation.

Statistical analysis

Analysis of variance (ANOVA) was computed to estimate the variance of genotypes, environment, and genotypes by environment using the R package. The combined analysis of variance proceeded to derive at G × E and the stability of the genotypes across all environments. The AMMI model, which combines standard analysis of variance with PC analysis was used to investigate G × E interaction (Zobel et al. 1988). AMMI analysis uses ANOVA and PCA for estimating stability and the G × E interactions (Gauch 1988; Yan et al. 2007). The AMMI model used for the stability analysis is as follows:

$$Y_{ij} = \mu + \delta_i + \beta_j + \sum_{K=1}^{K} \lambda_k \delta_{ik} \beta_{jk} + \varepsilon_{ij}$$

Where, Yij = mean of the given trait of ith genotype in jth environment; μ = is the grand mean; δ_i = denotes the genotypic effect; β_j = is the environmental effect; λ_k = is the eigenvalue of Interaction Principal Components Axes (IPCA) k; δ_{ik} = is the eigenvector of genotype k for PC k; β_{jk}= denotes eigenvector for environment j for PC k; and ε_{ij} = referred to as error associated with genotype i in environment j.

The model AMMI uses the biplot obtained through the principal components generated by the interaction of environment-genotype. If there is such interaction, the percentage of the two principal components would explain more than 50% of the total variation; in such a case, the biplot would be a good alternative to study the interaction environment-genotype as mentioned by Crossa (1990). Biplot analysis is the most powerful interpretive tool in AMMI models. Biplots are graphs where aspects of both genotypes and environments are plotted on the same axis so the inter-relationships can be visualized. There are two basic AMMI biplots, the AMMI1 biplot where the main effects (genotype mean and environment) are plotted against each other, and the AMMI2 biplot, where scores for IPCA1 and IPCA2 are plotted.

GGE biplot analysis

The effect of genotype and genotype-environment interaction generated bi-plot are based on multi environment trial dataset provides information on which-won-where pattern, relationship between the environments and ranking of the genotypes according to mean performance and stability traits. To understand the bi-plot model, dissecting of singular values between genotype and environment eigenvectors is required. The GGE bi-plot model utilizes Singular value decomposition (SVD) of the first two principal components (Yan 2002).

$$Yij = \mu + \beta j + \lambda_1 \zeta_{i1} \eta_{j1} + \lambda_2 \zeta_{i2} \eta_{j2} + \varepsilon ij$$

Where, Yij is the population mean of trait for genotype i in environment j, μ is the population mean, βj refers to the main effect of environment j, μ + βj implies the mean yield of all genotypes in environment j, λ_1 and λ_2 are the singular values for the first and second PCs, respectively, ζ_{i1} and ζ_{i2} are eigenvectors of genotype i for PC1 and PC2, respectively, η_{j1} and η_{j2} are eigenvectors of environment j for PC1 and PC2, respectively, εij is the residual effect of genotype i in environment j. In GGE bi-plot analysis, PC1 scores were plotted against PC2 (Yan and Tinker 2006).

Multivariate analysis

The combined mean data from three location over the two years for all the genotypes were subjected to multivariate analysis. Principal component analysis is the fundamental method to measure the variation in traits among the genotypes (Everitt and Dunn 1992; Salem 2021). Similarly, another important tool within the multivariate analysis is biplot analysis that is used to identify the desirable genotypes. PCA is reduction techniques of many variables into few sets of variables called factors or components (Cattell 1965). The extent of variance explained by the combined variables was derived by the highest correlation coefficient (Seiller and Stafford 1985). Principal components analysis, eigenvalues and 2D biplots were obtained using FactoMineR and factoextra packages (Dhakal et al. 2020). PCA can be undertaken for identifying the sources of morphological diversity. PCA had been used to determine the optimum number of clusters (Sinha and Mishra 2013).

Statistical analysis

The statistical analysis was performed to derive the analysis of variance (ANOVA) using the GenStat 15 edition software. Data analysis was carried out following randomized incomplete block design within the package general analysis of variance. From the ANOVA, mean sum of square values with their respective probability level for genotypes, environment, genotype and environment interaction along with residual variance was tabulated to show their main effects and its interaction.

RESULTS AND DISCUSSION

The results obtained from the experiment were analyzed and presented with the help of tables and figures below.

Mean sum of square of the evaluated traits

The analysis of variance (ANOVA) with mean sum of square revealed difference for main effect of the genotypes (G), locations (L), Year (Y), environments (E, here referred to as L × Y), and their respective interactions including G × E for several key agronomic traits of aromatic rice landraces (**Table 2**). Genotypes showed variation for heading days (HD) with a mean square value of 3895 ($p < 0.001$). The environment also had a significant effect in heading days with mean sum of square value of (2830 $p < 0.001$), and the G × E interaction was also significant (54.4, $p < 0.001$). A variation was observed for mean sum of squares among genotypes (3599, $p < 0.001$) and environments (2481, $p < 0.001$) and G × E interaction (74.64, $p<0.001$) for maturity days (MD). Genotypes and environments had a significant effect on panicle length (43.89 and 75.02 respectively, $p < 0.001$). Differences were found among genotypes (4299, $p < 0.001$) and environments (12513, $p < 0.001$) for plant height. However, the G × E interaction was not significant. Both genotypes and environments significantly influenced the number of filled grains (79403 and 281786 respectively, $p < 0.001$). Genotypes (141.16, $p < 0.001$) and environments (18.8, $p < 0.05$) significantly affected thousand-grain weight, whereas the G × E interaction was not significant. Similarly, genotypes (9514, $p < 0.001$) and environments (269572, $p < 0.001$) significantly influenced the number of tillers per square meter. Grain yield exhibited differences among genotypes (3995897, $p < 0.001$), environments (18663020, $p < 0.001$), and their interactions (573039, $p < 0.001$) (**Table 2**).

Table 2. Mean square values of different main effects (Genotype, Location, Year) and their respective interactions (G × L, G × Y, Environment (E, as interaction of L and Y) and G × E for various quantitative traits

Source of Variation	**DF**	**Heading Days**	**Maturity Days**	**Panicle Length (cm)**	**Plant height (cm)**	**Tillers per meter square (no.)**	**1000 grain weight (g)**	**Filled Grain (no.)**	**Grain yield (t ha^{-1})**
Rep	2	12.95	15.54	27.45	186.8	6100	1.857	30440	819532
Genotype (G)	29	3835**	3599**	43.88**	4299**	9514**	141.0**	79403**	3995897**
Location (L)	2	9736**	8182**	4.475	31428**	46222**	146.84**	9135	3393263**
Year (Y)	1	12130**	11934**	0.639	35424**	670796**	9.206	1152453**	3203748**
G × L	58	81.85**	84.4**	5.939	401.1**	1513	11.852**	27931*	923250**
G × Y	29	90.13**	100.7**	10.318**	212.5	4241*	0.959	31426**	999622**
Environment (E)	2	2830**	2481**	75.02**	12513.1**	269572**	18.79*	281786**	18663020**
G × E	58	54.4**	74.64**	8.966*	190.1	1327	1.093	13723	572039**
Residual	352	26.72	31.26	5.519	160.8	2654	4.102	13646	262551

Note: *, ** indicates the level of significance at 5% and 1% probability.

The significant variation observed among genotypes for all agro-morphological traits suggested the presence of substantial genetic diversity in the rice germplasm under study. The significant effects of location and year indicated the influence of environmental factors on the performance of these traits. This aligns with previous studies that emphasize the importance of genotype-by-environment interactions in determining the stability and adaptability of crop varieties (Kang 2002; Yan and Tinker 2006). The significant genotype and environment interactions for heading and maturity days suggested that these traits are sensitive to environmental conditions. This finding is consistent with reports by Dingkuhn et al. (1995), who noted that temperature and photoperiod significantly affect the phenological development of rice. The non-significant G × E interaction for plant height, tillers per square meter, 1000 grain weight and filled grain suggested that this trait are relatively less influenced by environments indicating stable performance across environments, making it a reliable criterion for selection. The differential main effects of year, location and genotypes and their interaction could be due to variable environmental situations and genetic constitution of particular genotype or plants was explained by Hassani et al. (2023). Therefore, the G × E interaction study is pre-requisite to confirm the possible interactions exist among the different field of agriculture research.

Combined ANOVA across the location over the years

The ANOVA was performed to show the individual effects of genotypic, location, year, environment, and genotype-by-environment interaction for different quantitative traits (**Table 3**). The results showed that the genotypes were highly significant ($p<0.001$) for all the traits considered in this study. Highly significant location differences ($p<0.001$) were observed in the majority of traits except panicle length and 1000 grain weight. Similarly, the year effect was also found to be significant ($p<0.001$) for all the traits measured except panicle length and filled grains. The result indicated significant differences ($p<0.001$) for the environmental effects (herein Location × Year) for all the traits in this study. However, the overall interaction of genotype, environment, and year was only significant in heading and maturity days, and grain yield ($p<0.01$). The results indicated that the pooled mean grain yield of genotypes across locations and over the years ranged from 1.26 t/ha (Kalanamak) to 3.35 t/ha (Samba Masuli Sub-1). Similarly, heading and maturity days ranged from 78 to 108 days, and 128 to 156 days, respectively. Hiupuri recorded the earliest maturity while Kalo Nunia was the latest among the evaluated landraces. Furthermore, Hiupuri (13.1 g TGW) and Tulsiful (13.1 g) had the finest grains among the landraces, while Sugandhit Dhan-1 recorded the highest grain boldness (23.1 g). The 1000 grain weight ranged from 13.1 g to 23.1 g among the evaluated landraces (**Table 3**).

Table 3. Combined mean of quantitative traits recorded from aromatic rice landraces across the location over the years

Genotype	Heading Days	Maturity Days	Panicle Length (cm)	Plant Height (cm)	1000 Grain Weight (g)	Tillers Per meter square (no.)	Filled Grain (no.)	Grain Yield t ha^{-1})
Karia Kamod	125	152.00	26.28	155.70	16.51	209.00	566.30	1.36
Sunaulo Sugandha	111	141.00	27.52	115.50	20.47	256.00	511.10	2.47
Kalo Masino Dhan	102	131.00	30.06	158.30	17.84	213.20	444.60	1.44
Balamsari Dhan	81	109.00	24.63	131.70	19.10	267.60	579.10	2.42
Karia Kamod	123	150.00	26.93	140.60	14.24	242.30	580.20	1.32
Tilki	105	135.00	24.81	169.90	14.90	213.80	627.70	2.15
Kalo Nunia	128	156.00	25.73	144.70	15.06	265.90	536.90	1.40
Bayani Masino	112	143.00	25.97	167.30	16.56	224.10	439.40	1.49
Brahmamusi Dhan	113	142.00	25.44	155.30	13.53	216.80	618.00	1.73
Basmati Jhapa	126	154.00	25.90	146.80	17.28	267.10	413.10	1.29
Malbhog	122	151.00	26.32	149.10	16.77	269.10	499.80	1.56
Jhuse Basmati	95	124.00	29.69	155.50	19.55	222.10	456.30	1.82
Gouriya	103	132.00	25.91	145.30	19.34	233.20	495.90	1.80
Hansaraj Dhan	89	119.00	28.45	152.20	22.47	273.30	514.40	1.69
Suhpat Dhan	111	140.00	26.07	147.90	20.95	205.80	501.50	1.91
Damari Dhan	82	112.00	23.70	126.70	15.95	257.30	549.80	2.21
Ujarka Basmati	122	152.00	27.73	158.70	19.98	236.70	444.20	1.67
Karia Kamod	122	152.00	26.65	141.80	14.03	247.20	575.50	1.33
Jhinuwa Dhan	90	121.00	25.75	152.70	17.46	229.20	464.00	1.31
Hiupuri	78	108.00	25.46	114.10	13.10	288.30	487.00	1.98
Sugandhit dhan-1	90	120.00	25.54	122.20	23.10	287.00	490.80	3.10
Chananchur	123	153.00	25.77	148.40	13.06	239.00	656.10	1.35
Lalka Basmati	123	152.00	25.24	143.80	18.61	248.00	452.70	1.78
Kalo Jira	105	135.00	28.56	162.80	16.28	223.60	390.20	1.33
Tulsiful	126	155.00	26.35	147.30	13.10	243.10	586.50	1.34
Kalo Basmati	103	132.00	26.54	148.60	18.66	228.10	462.60	2.11
Lalka Basmati Agahani	121	150.00	26.96	147.20	19.46	263.50	420.50	1.60
Gaure Dhan	108	138.00	25.76	164.50	15.77	228.30	538.20	1.53
Kalanamak	125	151.00	26.08	141.60	14.90	228.90	436.10	1.26
Samba Masuli Sub-1	109	138.00	21.95	100.10	13.28	277.30	562.30	3.35
Mean	109	138	26.26	145	17.04	243.5	510	1.76
Genotype (G)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Location (L)	<.001	<.001	0.9	<.001	0.1	<.001	<.001	0.002
Year (Y)	<.001	<.001	0.36	<.001	<.001	<.001	0.2	<.001
Environment (E)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
G x E	<.001	<.001	0.005	0.18	1	0.9	0.4	0.002
LSD (0.05)	8.37	9.05	3.81	20.55	3.28	83.5	195.7	0.93
CV, %	4.7	4.04	8.95	8.7	11.9	21.16	22.8	28.3

GY-grain yield (t/ha), HD-heading days, MD-maturity days, PL-panicle length (cm), PH-plant height (cm), FG-filled grains (total of five panicles), FGW-filled grains weight, TGW-1000 grain weight (g), SY-straw yield (t/ha), TPMS-tillers per square meter, LSD- least significant difference

Significant results from the combined analysis of variance for the genotype × environment interaction for days to heading, maturity, panicle length, and grain yield indicate that genotypes responded differently across the environment and over the years for these traits. This result suggests that there is an opportunity for the selection of genotype(s) suitable for specific locations as well as stable genotype(s) across locations. Our results are in harmony with the findings of Akter et al (2015), who studied G × E interaction effects in different hybrid rice genotypes at different locations. Similarly, Xu et al (2014) revealed similar results from genotype × environment interaction studies for agronomic traits of rice.

The large variation in heading and maturity dates among genotypes indicated the potential for selecting early- or late-maturing varieties based on specific agro-ecological needs. The non-significant G × E interaction for 1000 grain weight suggested that this trait is less influenced by environmental factors, supporting its use as a stable selection criterion. However, the significant G × E interaction for grain yield underscores the complexity of yield performance, which is influenced by both genetic and environmental factors (Blum 2011). The significant effects of genotype and environment on tillers per square meter and filled grain reflect the importance of both genetic potential and environmental conditions in determining yield components. The stability of tillers per square meter across environments suggests that this trait could be a reliable indicator of yield potential, while the variability in filled grain emphasizes the need for careful selection to enhance grain filling under different environmental conditions. In conclusion, the significant G × E interactions for key traits such as grain yield, heading days, and maturity days emphasize the importance of environment-specific genotypes. The identification of stable genotypes with consistent performance across diverse environments, or those with specific adaptation, will be crucial for improving rice productivity in varying agro-ecological zones.

Additive Main effects and Multiple Interactions (AMMI) Analysis

In this study, AMMI analysis of variance for grain yield (t/ha) showed that the yield variance was significantly ($p<0.001$) influenced by the environment, genotypes, and genotype-by-environment interaction (**Table 4, Table 5**). Similarly, both PCs (PC_1 and PC_2) showed significant difference ($p <0.001$) for the grain yield. The result reflected that the genotype had the dominant contribution to the total variation, which accounted 69% of sum of squares. The second most important contribution was due to G × E interaction which accounted 28% of sum of squares of total variation. while only 3% of the total sum of squares was attributed to environmental effects (Table 4). Furthermore, it was revealed that PC_1 and PC_2 accounted for 53% and 47% of GGE sum of squares, respectively, over the years. In the AMMI analysis, variance components were computed by Fisher's F-test using the sum of squares, mean sum of squares value obtained from the ANOVA result. The analysis of variance exhibited significant difference ($p<0.001$) for genotype, environment and its interaction effects. It showed that genotype explained 15.93% of variation followed by environment 10.29% and 3.21% by genotype × environment. Similarly, PC_1 and PC_2 also explained 3.27% and 3.15% of variation in the population.

Table 4. AMMI analysis of variance for grain yield of 30 fine and aromatic rice landrace tested in three environments over the years 2020- 2021

Source of variation	Sum of Squares	%Sum of Square	Cumulative %	Degree of Freedom	Mean Square	F-Fishers test	Probf
Env	3199832	3	3	2	1599916	10.29	<0.001
Gen	72000000	69	72	29	2478017	15.93	<0.001
Env x Gen	29000000	28	100	58	499688	3.21	<0.001
PC_1	15000000	53	53	30	508725	3.27	<0.001
PC_2	14000000	47	100	28	490006	3.15	<0.001
Residuals	28000000	0	0	180	155556	NA	NA

Note: ENV- environment, GEN- genotype, PC_1, PC_2, - indicates respective principal components, SS- sum of squares, DF- degree of freedom, MS- mean sum of squares, F- Fisher's test

The presence of GEI was clearly demonstrated by the AMMI model when the interaction was partitioned among the first two principal component axes (PCA), as they were significant ($p<0.001$) based on Gollob's F-test (Gollob 1968). This implies that the interaction of the 30 rice genotypes with three environments was predicted by the first two components of genotypes and environments. Gauch and Zobel (1996) also recommended that the most accurate model for AMMI can be predicted using the first two PCAs.

Genotype and environment additive main effects against first principal component (PC_1) are depicted as AMMI1 biplot (**Figure 1a**). From the AMMI1 biplot, Samba Masuli Sub-1, Sugandhit Dhan-1, Kalo Basmati, Gouriya, Kariya Kamod-2, Brahmamusi Dhan, and Kariya Kamod-1 with their relative PC_1 scores close to zero, had less response to the interaction and showed general adaptation to the test environments. Among them, Samba Masuli Sub-1 and Sugandhit Dhan-1 with the highest mean yield over the test environments and low PC_1 scores (**Figure 1a**) were considered as the most stable genotypes with relatively less variable yield performance across environments. Sunaulo Sugandha and Balamsari Dhan demonstrated large PC_1 score and found better adaption to Tarahara and Hardinath respectively (**Figure 1a**).

Table 5. AMMI analysis of variance for pooled mean grain yield of 30 fine and aromatic rice landrace tested at three environments and over the years 2020- 2021

Type	Genotypes	Yield	DIM1	DIM2
GEN	Karia Kamod	1.53	0.081	0.064
GEN	Basmati Jhapa	1.31	0.203	0.226
GEN	Malbhog	1.47	0.051	0.14
GEN	Jhuse Basmati	2.01	0.213	0.125
GEN	Gouriya	1.95	-0.01	0.009
GEN	Hansaraj Dhan	1.64	0.116	0.196
GEN	Suhpat Dhan	1.89	0.269	-0.081
GEN	Damari Dhan	2.11	-0.274	0.045
GEN	Ujarka Basmati	1.67	-0.088	-0.135
GEN	Karia Kamod	1.27	-0.002	0.083
GEN	Jhinuwa Dhan	1.30	-0.095	0.344
GEN	Sunaulo Sugandha	2.53	0.667	-0.16
GEN	Hiupuri	2.06	-0.197	-0.094
GEN	Sugandhit dhan-1	3.06	0.117	0.237
GEN	Chananchur	1.58	-0.144	0.113
GEN	Lalka Basmati	1.85	0.225	-0.098
GEN	Kalo Jira	1.36	-0.254	-0.062
GEN	Tulsiful	1.55	-0.066	-0.083
GEN	Kalo Basmati	2.05	0.039	-0.138
GEN	Lalka Basmati Agahani	1.50	0.131	0.134
GEN	Gaure Dhan	1.59	-0.088	-0.298
GEN	Kalanamak	1.29	0.15	0.176
GEN	Kalo Masino Dhan	1.68	-0.104	0.232
GEN	Samba Masuli Sub-1	3.49	0.129	-1
GEN	Balamsari Dhan	2.45	-0.802	-0.144
GEN	Karia Kamod	1.54	-0.005	0.082
GEN	Tilki	2.09	-0.201	-0.015
GEN	Kalo Nunia	1.31	0.171	0.075
GEN	Bayani Masino	1.37	-0.219	0.081
GEN	Brahmamusi Dhan	1.81	-0.013	-0.051
ENV	Hardinath	1.77	-0.377	-0.912
ENV	Tarahara	1.96	1	0.138
ENV	Khajura	1.70	-0.623	0.774

Note: GEN- genotype, ENV- environment

Similarly, AMMI2 biplot of the second principal component axis (PC_2) against the first principal component axis (PC_1) scores was generated to cross-validate the interaction pattern of the thirty rice genotypes within three locations (Figure 1b). AMMI2 helps in detection of genotypes with either broad or narrow spectrum adaptability. The PCA scores of genotypes in the AMMI analysis is an indication of stability over environments (Gauch and Zobel 1996). The greater the PCA scores, the more specifically adapted is a genotype to certain environments whereas the PCA scores approximate to zero, the stable over the tested environments (Sanni et al. 2009). As seen in AMMI2 biplot, Sunaulo Sugandha, Samba Masuli Sub-1 and Balamsari Dhan expressed either positively or negatively high interactive behavior and contributed to the exhibited GE interaction (**Figure 1b**). Genotype-environment interaction was portrayed as orthogonal projections of the genotypes on the environmental vectors to identify the best genotypes with respect to environments. The best genotype in NRRP, Hardinath was Samba Masuli Sub-1, in Tarahara were Samba Masuli Sub-1 and Sunaulo Sugandha and in Khajura was Balamsari Dhan. Landraces Gouriya, Brahmamusi Dhan, Sugandhit Dhan, Kariya Kamod-1, Karia Kamod-2, Kalo Basmati and Tulsiful are more stable since they are near to origin. Their yield is more or less similar in all locations. Among them Kalo Basmati, Gouriya, and Brahmamusi yielded higher than average yield (**Table 4**, **Figure 1b**). All the three test locations have higher PCA scores as represented by long environmental spokes. This indicates that all the environments had potential for large GEI. Similar results on AMMI analysis were reported by Oroian et al (2023), Sharifi et al (2017), Akter et al (2015) and Tariku et al (2013).

Ranking landraces relative to the ideal genotype

An ideal genotype exhibits highest mean performance and be absolutely stable (performs well in all environments). It is the one with a large PC_1 score- indicating high yielding ability, and small PC_2 score- high stability (Yan et al 2000). Such an ideal genotype is represented by having the greatest vector length of the high yielding accessions and with zero GEI (**Figure 2a**). The center of the concentric circles in this figure represents the position of an ideal or stable genotype, which is defined by a projection on to the mean-environment axis that equals the longest vector of the genotypes that had above-average mean yield and by a zero projection on to the perpendicular line (zero variability across years for genotypes) (Yan and Hunt 2002). Although the ideal genotype might not exist in reality, it could be used as a reference for genotype evaluation (Anothai et al., 2009). A genotype is more desirable if it is located closer to the ideal genotype. Thus, using the ideal genotype as the center, concentric circles were drawn to visualize the distance between each genotype and the ideal genotype. Based on the grain yield, Samba Masuli Sub-1 and Sugandhit Dhan-1 which fell into the centre or adjoining of concentric circles were the ideal genotypes in terms of stability and higher yield, compared with the rest of the accessions (**Figure 2a**). Likewise, Kalo Basmati, Tilki, Hiupuri, Damari Dhan, Gouriya and Brahmamusi Dhan, located on the next concentric circle, may be regarded as desirable accessions in terms of higher and stable yielding ability across locations (**Figure 2a**).

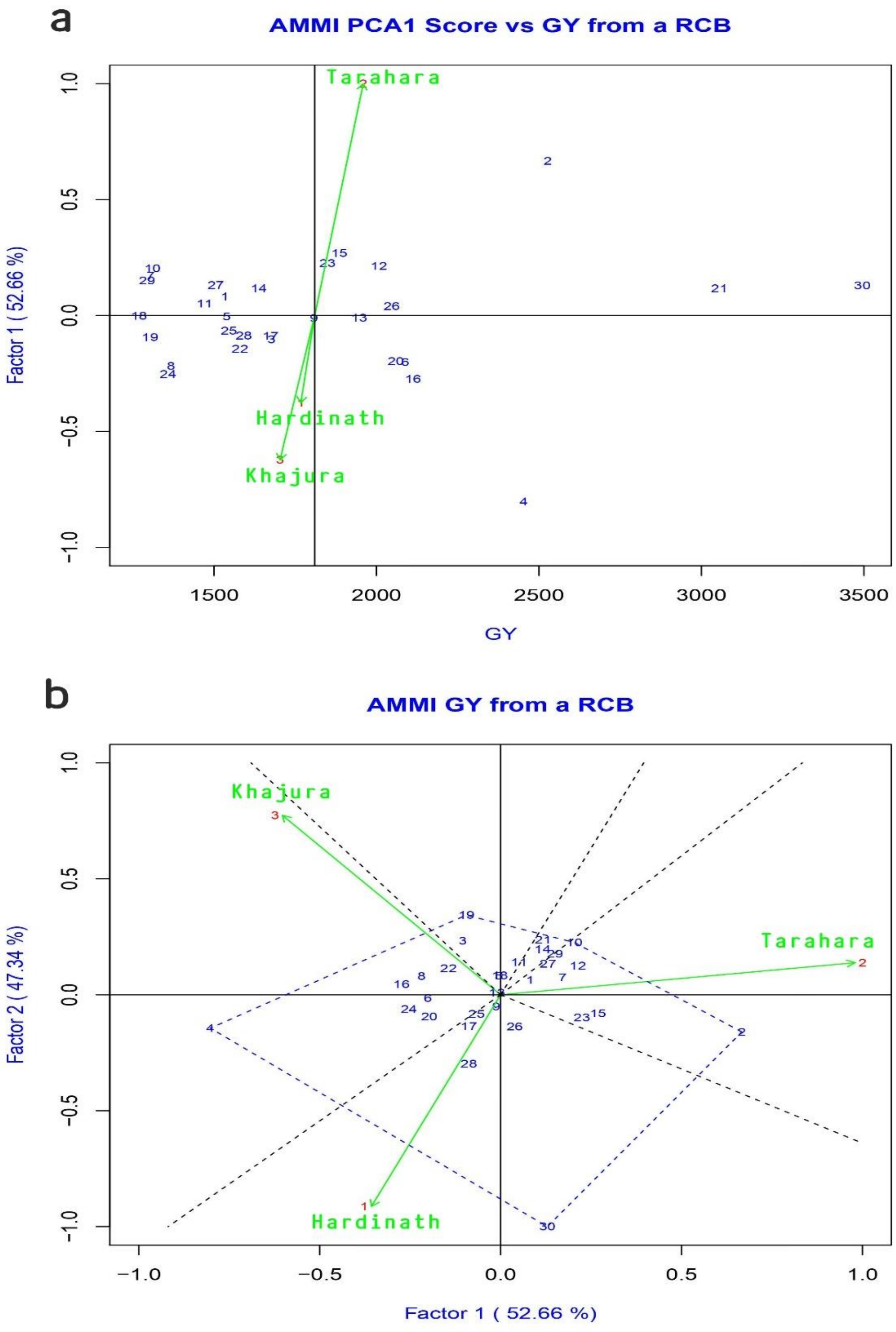

Figure 1. Additive Main effects and Multiple Interactions (AMMI) Analysis a) AMMI1 score vs grain yield (GY- t/ha), b) AMMI1 vs AMMI2 scores. In both the figures (a and b), numbers (blue color) within the plotted graph represent entry number and related information of each can be obtained from table 4

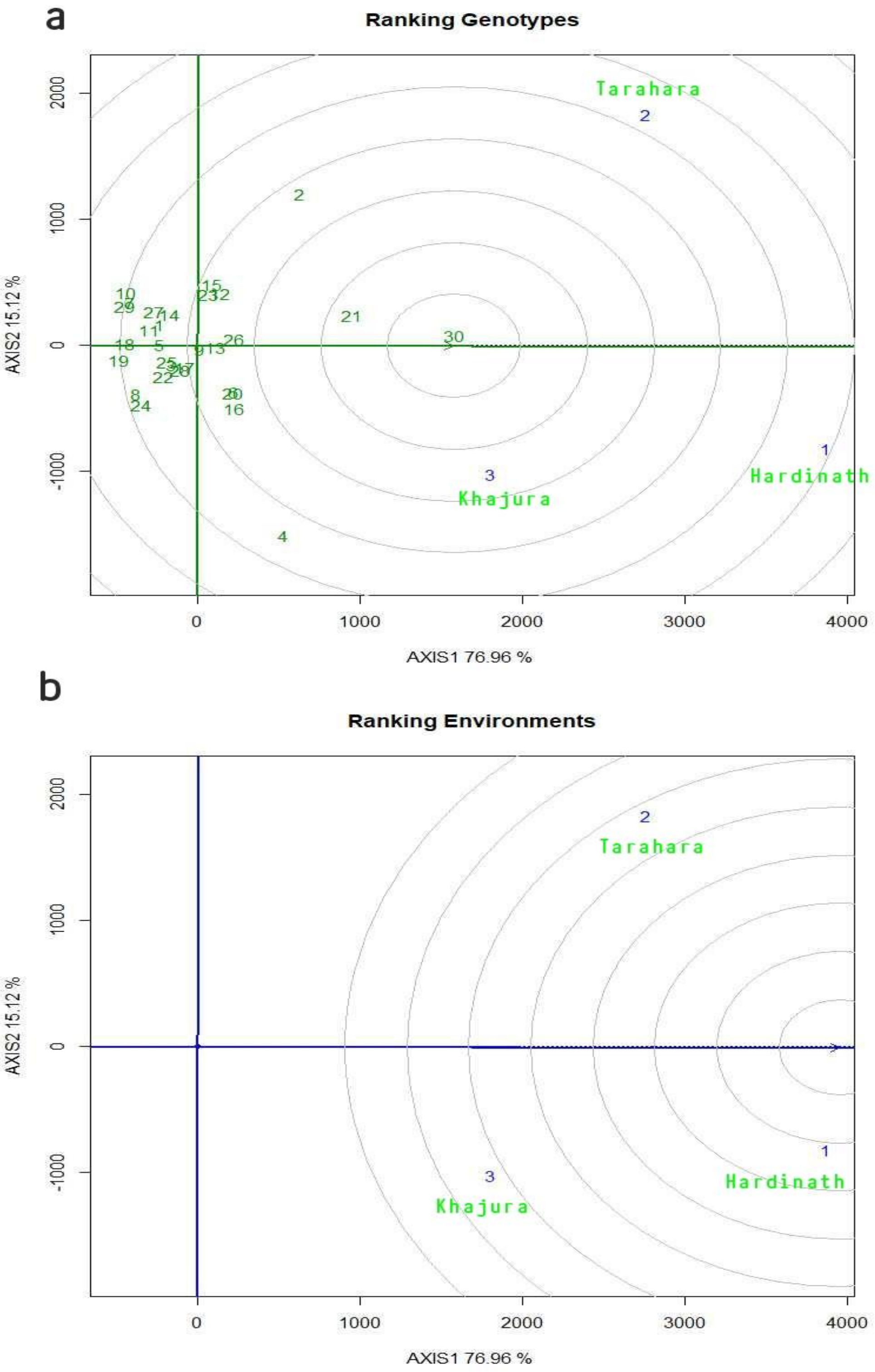

Figure 2. **GGE biplot analysis based on genotype-focused scaling for comparison of the landraces with ideal genotype (a), and GGE biplot analysis based on environment focused scaling for comparison of environment with ideal environment (b). In figure (a), numbers (green color) within the plotted graph represent entry number and related information of each can be obtained from table 4**

Ranking of environments relative to the ideal environments

Considering the ideal environment for ranking, NRRP, Hardinath is located in the first concentric circle and has been regarded as the most ideal environment (**Figure 2b**). Therefore, it is evident that NRRP, Hardinath environment was representing the ideal environment and such environment has been referred to as desirable environments. In addition, Tarahara environment was close to the second concentric circle which is represented by NRRP environment and has been good for grain yield performances, but Khajura environment was distantly located from the centre therefore it suggests to identify the location specific accessions (**Figure 2b**). This difference between environments attributed due to rainfall pattern during the crop season, soil fertility variation, and other environmental variations in different years.

Principal Component Analysis (PCA) of aromatic rice landraces

The PCA stand as the important tool to understand the contribution of each trait in variation between the different genotypes that would be useful for selection of suitable material for crop breeding and subsequent improvement programs. Thus, PCA was performed for the 11 quantitative traits across the genotypes. The eigenvalue, proportion of variation percentage and cumulative variation percentage explained of different morphological traits of aromatic rice landraces using multivariate analysis is presented in the **table 6**. The first four components in PCA analysis (PC1, PC2, PC3 and PC4) were considered to exhibit variation in the given traits that was reflected by the eigenvalue >1.0 (4.04, 2.22, 1.7 and 1.24) respectively (**Table 6**). The four PCs collectively represented 83.64% of total variance explained of which first PC showed the highest variability of 36.74% followed by 20.16%, 15.45% and 11.27% by PC2, PC3 and PC4 respectively. The current finding is very consistently confirmed by the earlier study in which two PCs representing 66.1% variance (Salem et al. 2021) and five PCs explaining 86.9% variance (Sinha and Mishra 2013) as indicated by eigenvalue >1.0.

The study reflected that first principal component contributes 36.75% to total variance. In PC1, four variables grain yield (1.0), filled grain weight (0.87), tillers per square meter (0.84), and 1000 grain weight (0.67) provided highly and positive loadings (**Table 6**). On the other hand, six variables namely heading days (-0.93), maturity days (-0.94), plant height (-0.87), panicle length (-0.55), unfilled grains (-0.75), and straw yield (-0.84) contributed negatively to the total variance. The second principal component PC2 accounted for more than 20% variation and only one variable filled grains (1.0) was positively and high loadings while panicle length (-0.9), 1000 grain weight (-0.86), filled grain weight (-0.33), and plant height (-0.43) showed negative loadings. Furthermore, PC3 also accounted 15.45% to total variation is more associated with heading days (0.61), maturity days (0.59), tillers per square meter (0.86), and straw yield (0.87) with positive and high loadings. In the same manner, five variables namely plant height (-0.57), panicle length (-0.39), filled grains (-0.74), filled grain weight (-0.6), and unfilled grain (-1.0) gave high but negative loadings. Additionally, PC4 possessed 11.27% of variation, in which two variables unfilled grains (0.85) and tillers per square meter (0.31) play positive role and five variables maturity days (-0.3), panicle length (-0.51), filled grains (-1.0), filled grain weight (-0.93), and straw yield (-0.65) were high but negative role for expression of traits. The findings revealed that some PCs identified agro-morphological variables with positive and negative loadings respectively. It indicates that both the vegetative and reproductive traits contribute to each component separately. This type of positive and negative relationship by vegetative and reproductive traits was mentioned by (Sinha and Mishra, 2013) during their study in rice landraces using quantitative traits in West Bengal, India. Meanwhile, two principal components explained contribution of five and two characters to respective components as reported by (Salem et al. 2021). Similar results obtained in PCA analysis where six components with eigenvalue >1.0 (Tripathi et al. 2013) identified that accounted 76.6% of variation while five PCs out of 13 PCs with significant (eigenvalue >1.0) contributing 84.67% variation (Dhakal et al. 2020) during investigation on agro-morphological traits in Jhinuwa rice populations and upland rice landraces using quantitative traits respectively in Nepal. Likewise, investigation on rice genotypes showed five principal components with >1.0 eigenvalue accounting 87.35% of variation in yield attributing traits that is in agreement with the findings in current study of 30 aromatic rice landraces (Kasanaboina et al. 2022). In addition, first four PCs accounted 83.76% of genetic variation with eigenvalue >1.0 during the study of colored rice (Ahmad 2015). Thus, use of PCA as multivariate tool assists us to know the traits of interest that has great significance and provides a basis for selection of important traits in breeding program.

The biplot diagram shows the graphical visualization of association between traits and genotypes with outstanding performance. The vector length indicates direct contribution to differentiation of the traits. It explicitly implies higher the vector length, greater will be the contribution of particular traits. The biplot constructed from first two PCs reflects the distribution and magnitude of diversity of various quantitative traits for aromatic landraces are clearly presented in the biplot diagram (**Figure 3**). The variable 1000 grain weight followed by panicle length, heading and maturity days as well as grain yield exhibited longer vector length suggesting greater contribution to the total divergence i.e. variability. It illustrated that these traits have greatest significance to variation as explained by (Kasanaboina et al. 2022). The angle between the trait vectors explains the nature of correlation between the traits. It is evident that an angle (>90º) denotes a negative correlation and a right angle (90º) denotes no correlation. The biplot analysis showed that the traits heading and maturity days are highly significant (<0.001) and positively correlation (**Figure 3**). Similarly, plant height and panicle length, 1000 grain weight, and filled grain weight as well as grain yield and tillers per square meter are have significant (<0.05) and positively correlated. Similar findings were reported by (Kasanaboina et al. 2022) in their study of rice germplasm using biplot analysis.

Table 6. Eigenvalue, proportion and cumulative variation of major morphological traits of aromatic rice landraces using multivariate analysis

Trait	PC1	PC2	PC3	PC4
Heading days	-0.93	0.13	0.61	-0.26
Maturity days	-0.94	0.13	0.59	-0.30
Plant height (cm)	-0.87	-0.43	-0.57	-0.01
Panicle length (cm)	-0.55	-0.90	-0.39	-0.51
Tillers per meter square	0.84	0.16	0.86	0.31
Filled grains (No)	-0.10	1.00	-0.74	-1.00
Filled grain weight (g)	0.87	-0.33	-0.60	-0.93
Un filled grains	-0.75	0.19	-1.00	0.85
1000 grain weight (g)	0.67	-0.86	0.03	-0.04
Grain yield (t/ha)	1.00	0.21	0.09	-0.24
Straw yield (t/ha)	-0.84	-0.17	0.87	-0.65
Eigen value	4.042	2.22	1.7	1.24
Proportion of variation (%)	36.75	20.16	15.45	11.27
Cumulative variation (%)	36.74	56.91	72.36	83.64

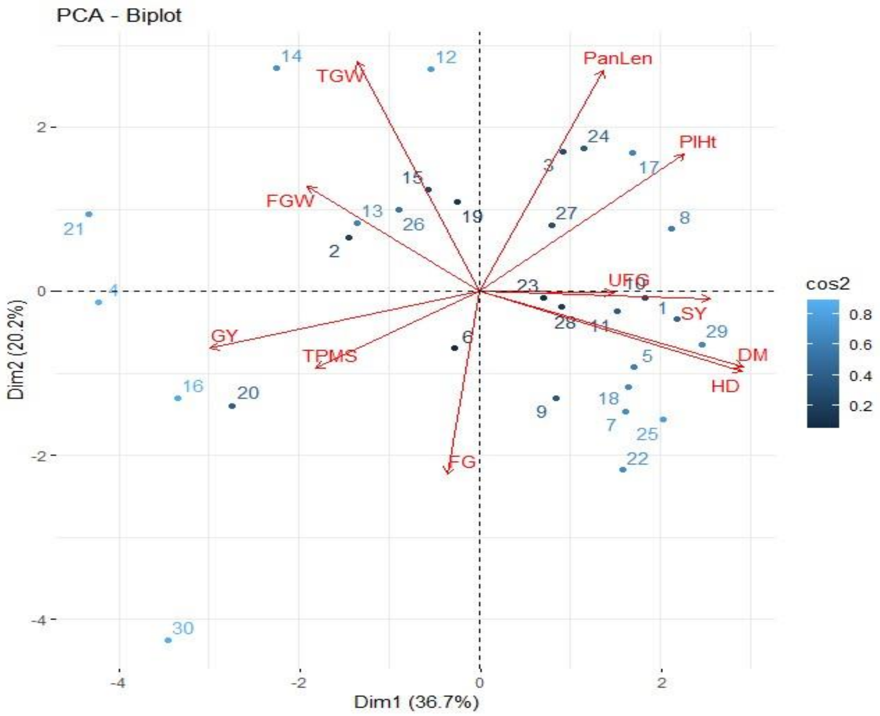

Figure 3. **PCA biplot from the pooled mean of 11 quantitative traits of aromatic rice landraces**

CONCLUSIONS

In our study, a combined analysis of variance revealed significant differences among genotypes, environments, and genotype-by-environment interactions for grain yield. This suggests that genotypes can be selected for adaptation to specific environments. Based on AMMI1, AMMI2 biplots, and GGE biplots, the genotypes Samba Masuli Sub-1 and Sugandhit Dhan-1 were identified as the best fine and aromatic rice varieties. Other promising genotypes with higher yield and stability included Damari Dhan, Tilki, Hiupuri, Kalob Basmati, Gouriya, and Brahmamusi Dhan. Meanwhile, Sunaulo Sugandha and Balamsari Dhan were more responsive due to their greater distance from the origin. Samba Masuli Sub-1 was the best genotype in NRRP Hardinath, Sunaulo Sugandha in Tarahara, and Balamsari Dhan in Khajura. PCA analysis reflected that four PCs were found significant with eigen value >1.0 contributing more than 84% of variance from the 11 quantitative traits of 30 aromatic landraces. It was also highlighted that the vegetative and reproductive traits both were predominant in all the PCs with their respective positive and negative contribution. Biplot analysis also explained that the traits 1000 grain weight, panicle length, heading and maturity days and grain yield showed greater divergence among the landraces. These identified genotypes could be directly released as varieties or used as a parent for future rice breeding programs. Among the tested locations, Hardinath was found to be the most suitable for aromatic rice cultivation.

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AUTHORS' CONTRIBUTION

Dev Nidhi Tiwari and Madhav Prasad Pandey conceived and designed the experiments; Dev Nidhi Tiwari performed the experiments; analyzed and interpreted the data; wrote the paper. Madhav Prasad Pandey, Hira Kaji Manandhar and Tej Narayan Bhusal assisted in research analyzed and interpreted the data; Dev Nidhi Tiwari wrote the final manuscript. Hira Kaji Manandhar and Tej Narayan Bhusal: Guidance, analyzed and interpreted the data; Dev Nidhi Tiwari wrote the paper and Tej Narayan Bhusal monitoring of experiment, reviewed initial draft of manuscript. Madhav Prasad Pandey assisted in manuscript writing and reviewed final draft of manuscript. Hira Kaji Manandhar: Reviewed final draft of manuscript

CONFLICTS OF INTEREST

The authors have no any conflict of interest to disclose.

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