



Morphometric Analysis of Upland Rice Phenotypes in Lowland Condition

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Article published on April 23, 2017

Key words: Clustering, Diversity coefficient, Morphometric analysis, Principal component analysis, Upland rice.

Abstract

Knowledge about upland characters expressed in an unfavorable environment will direct to appropriate utilization of upland rice varieties (URVs) for breeding and improvement. Morphometric analysis of 55 URVs in lowland condition was done using different statistical parameters such as basic statistics (standard deviation, coefficient of variation and ANOVA), Shannon-Weaver diversity coefficient (H'), principal component analysis (PCA) and clustering analysis based on 14 characters. H' values were ranged from 0.69 (flag leaf width, FLW) to 0.95 (grain yield, GY), indicating a medium to high diversity characters. PCA captured 84.78% variation for six principal components (PC), retained using proportion of variance and eigenvalues >1.0 . Grain length (GL), grain width (GW) and grain size ratio (GSR) formed PC1 and days to 50% flowering (DF), days to maturity (DM) and thousand grain weight (TGW) formed PC2. PCA found that grain attributes (GL, GW, GSR and TGW) followed by DF and DM were highly affected. Clustering analysis grouped varieties into four. The results therefore could be used especially on deciding what URV is to be utilized for any rice breeding program in lowland condition.

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Introduction

Philippines is a good source of upland rice varieties (URVs) since this crop is undeniably the most important food crop in the country. Country's numerous URV is due to farmers' ability to keep, select, maintain and develop varieties along the years. URVs could give rare genes for biotic and abiotic stresses to the modern varieties. These genetic resources indeed are vital elements for a rice breeding and development program. That is why URVs' variation and morphometric should be studied and understood. Phenotypic measurement is the usual method in analyzing morphometry; in fact it could assess relationship between genotypes (Bajracharya *et al.*, 2006). This method was done to evaluate diversity on Philippine ancestral lines of rice cultivars (Caldo *et al.*, 1996), phylogenetic diversity and relationship of rice accessions in Nigerian germplasm (Ogunbayo *et al.*, 2005), genetic diversity of upland rice cultivars in Saranggani Province, Mindanao (Lasalita-Zapico *et al.*, 2010) and genetic divergence of Philippine registered varieties (Cantila *et al.*, 2016). Therefore, the present study was done to have morphometric analysis of 55 URVs using different statistical parameters.

Materials and methods

Materials and site characterization

Fifty-five URVs (Table 1) were seed increased for a season at Philippine Rice Research Institute, Midsayap Experimental Station (PhilRice-MES). URVs were laid out in RCBD for three replications at the following season in PhilRice-MES, North Cotabato, Philippine sat 7° 10' 45.43"N latitude, 124° 30' 16.34" E longitude. Weather Link 5.9.2, Vantage PRO2, Davis Weather Station on the other hand, provided the meteorological data from January to April 2016 (Figure 1).

Crop establishment and data gathered

The site was space isolated for 200 meters so that it will be rain fed. It was prepared using hand tractor and applied with molluscicide. Dry seeding was used for seedling preparation. Seedlings were then transplanted after 21 days in a plot size of 12 m²per entry.

Fertilizers were applied using the rate of 70 kg ha⁻¹ of 14-14-14 and 8.33 kg ha⁻¹ of 0-0-60 at basal, 38 kg ha⁻¹ of 46-0-0 at tillering and 83 kg ha⁻¹ of 21-0-0-24 at panicle initiation. Water supplication was done only when applying fertilizer. The water then was drained after three days. Spraying of chemicals on the other hand, protected the experimental materials. Spraying of herbicides and spot weeding in the same way controlled the weeds. Lastly, harvesting was done when the grains become 80-85% straw-colored.

For gathering the data of characters, measurement was done in an average of 10 random plants per entry. Characters were culm length (CL) in cm, days to 50% flowering (DF), days to maturity (DM), flag leaf length (FLL) in cm, flag leaf width (FLW) in cm, flag leaf ratio (FLR), grain length (GL) in mm, grain width (GW) in mm, grain yield (GY) in t/ha, productive tillers (PT), panicle length (PL) in cm, panicle weight (PW) in grams, plant height (PH) in cm and thousand grain weight (TGW) in grams.

Data analyses

Statistix 9.0 (Analytical software, 2009) computed basic statistics like range, mean, standard deviation (SD), coefficient of variation (CV) and ANOVA. Diversity was based on Shannon-Weaver coefficient ($H' = -\sum p_i (\log_2 p_i) / \log_2 N$), where p_i = frequency proportion of the descriptor state and N = number of classes (Sotto and Rabara, 2007). XLSt at (Addin soft, 2010) on the other hand, computed principal component analysis (PCA) to group the characters and clustering analysis to group the URVs. The derived groups from clustering analysis with their mean for a character were separated through least significance difference (LSD) by Statistix 9.0.

Results and discussion

Scope of variation

All characters had high significant variation among 55 URVs (Table 2). SD had a range from 0.32 to 63.31 while CV was 3.89 to 31.87%. Also, H' values were in a range from 0.69 to 0.95 (Table 2). H' implies the level variation for a character according to Bisht *et al.* (1998).

H' values of 14 characters arranged from highest to lowest values were as follows: GY ($H'=0.95$), PT ($H'=0.93$), PH ($H'=0.92$), GSR ($H'=0.89$), CL ($H'=0.88$), DF ($H'=0.84$), GL ($H'=0.84$), GW ($H'=0.84$), TGW ($H'=0.78$), FLR ($H'=0.75$), DM ($H'=0.74$), PL ($H'=0.74$), FLL ($H'=0.72$) and FLW ($H'=0.69$).

Jamago and Cortes (2012) defined maximum diversity when $H'=1.00$, high diversity when H' values is within a range of 0.76 to 0.99, medium diversity of 0.46 to 0.75 and low diversity from 0.01 to 0.45. High diversity therefore was found in characters related to grain (GL, GW, GSR and TGW), yield (GY), flowering (DF), growth (CL and PH) and tillering (PT).

Table 1. List of 55 upland rice varieties used in the morphometric analysis at PhilRice-MES.

Name	Code	Name	Code	Name	Code
1. Arabon	PRRI004381	20. Hinomay	PRRI006370	37. Minarugon	PRRI003818
2. Aritao-Cagayan	PRRI002428	21. Hinumay	PRRI006471	38. Murado	PRRI006539
3. Awot	PRRI001757	22. Inamos	PRRI000876	39. P1-2-2 (Mimis)	PRRI001932
4. Balibod	PRRI002464	23. Inipot-Ibon	PRRI000807	40. Palawan	PRRI002684
5. Batangueño (Glut)	PRRI006360	24. Kawatil Gold	PRRI002723	41. Parirutong	PRRI006378
6. Binatang	PRRI002716	25. Kinaboag	PRRI000848	42. Pe-2	PRRI004127
7. Binato	PRRI000662	26. Kutivos	PRRI003287	43. Penantad	PRRI004132
8. Binisaya	PRRI006361	27. Langangan	PRRI002543	44. PilitTapol	PRRI004756
9. BuntotKabayo	PRRI002908	28. Linangka	PRRI000612	45. Pinalwa	PRRI003236
10. C 2	PRRI002815	29. MagsanayaSeln (Ci 12039)	PRRI002881	46. PinilingBaybay	PRRI002901
11. C 22	PRRI002812	30. Magsanaya	PRRI006530	47. Pingkitan	PRRI004150
12. C 43	PRRI002818	31. Malagkit (Inaku-Neneng) Dinakit	PRRI002267	48. Pinursigi	PRRI003108
13. Camuros	PRRI001856			49. Pirurutong	PRRI002993
14. Chumi-I-Tinawon	PRRI003495			50. Pokkali	PRRI004177
15. Daludo	PRRI003282	32. Maligaya 2	PRRI002832	51. Ranan	PRRI004222
16. Dinit-An	PRRI002749	33. Maliket	PRRI000311	52. Sang-Laya	PRRI001799
17. Dinorado	PRRI006439	34. Mangglutus	PRRI003091	53. Sinaguing	PRRI002897
18. Dukpayon	PRRI006446	35. Milbuen 3	PRRI002833	54. Sinampablo	PRRI002931
19. Guinata	PRRI002934	36. Milpal 18	PRRI002835	55. Wagwag	PRRI005699

Cantila *et al.* (2016) found high diversity only to characters related to yield, flowering and tillering when studying the divergence of Philippine registered rice varieties. Sanni *et al.* (2008) and Seetheram *et al.* (2009) pointed out that variations are necessary to any rice improvement since it allows maximum selection of better genotypes. Medium diversity on the other hand was found in characters related to flag leaf (FLR, FLL and FLW), maturity (DM) and panicle growth (PL).

Grouping of characters

PCA computes the value of variation for a character and derives information while grouping it.

PCA retained six PCs (Table 3) using proportion of variance (O'Rourke and Hatcher, 2013) with eigenvalues >1.0 (Iezzoni and Pritts, 1991). PC1 explained 21.72% (Figure 2), PC2 to 16.89%, PC3 to 14.62%, PC4 to 13.19%, PC5 to 9.71% and PC6 to 8.66% totalling to 84.78% of the variation with corresponding 3.04, 2.36, 2.05, 1.85, 1.36 and 1.21 eigenvalues, respectively. Grain-related characters such as GL, GSR and GW (Figure 3) formed PC1 based on the largest squared cosines. Phenological characters such as DF and DM formed PC2 while grain related character, TGW and growth characters such as CL and PH formed PC3. Bon *et al.* (2005; 2011) used only three PCs to define genetic variability of *Oryza* species in the Philippines.

Grain-related characters are important in rice (Maji and Shaibu, 2012) due to its direct association to grain quality and quantity (Zheng *et al.*, 2007). These characters will determine the shape and exterior quality of the rice grains (Shi *et al.*, 2000; Iwata *et al.*, 2010).

Fujita *et al.* (1984) on the other hand, cited that when crops mature late, the longer is the time to grow, develop and fill the grains. That is why phenological and growth characters affect grain yield.

Table 2. Fourteen characters and their corresponding range, mean, standard deviation (SD) and Shannon-Weaver diversity coefficient (H') used in the morphometric analysis.

Variables	Range	Mean	SD	CV (%)	H' coefficient
CL	61.43-128.93	104.90**	14.93	10.41	0.88
DF	43.00-77.00	55.95**	7.06	5.38	0.84
DM	78.00-112.00	91.72**	6.72	4.14	0.74
FLL	279.67-584.67	380.13**	63.31	10.3	0.72
FLR	16.29-44.96	26.54**	5.65	16.14	0.75
FLW	8.40-24.23	14.77**	3.11	11.75	0.69
GL	6.69-9.97	8.65**	0.7	3.89	0.84
GSR	2.23-4.01	2.98**	0.47	7.69	0.89
GW	2.13-3.57	2.96**	0.32	7.5	0.84
GY	0.21-3.18	1.90**	0.87	31.87	0.95
PH	84.78-148.78	123.41**	15.02	5.19	0.92
PL	19.78-35.58	26.59**	3.47	12.64	0.74
PT	2.67-12.56	6.68**	2.34	10.09	0.93
TGW	14.10-26.50	21.28**	2.63	9.98	0.78

** means high significant variation ($p = 0.00$).

Table 3. Six PCs with their corresponding squared cosines, eigenvalues and proportion of variance used in grouping of characters.

Variables	Principal components					
	PC1	PC2	PC3	PC4	PC5	PC6
CL	0.12	0	0.7	0.05	0.04	0.02
DF	0.03	0.72	0.04	0.02	0.06	0.01
DM	0.05	0.67	0.04	0.02	0.1	0
FLL	0.1	0.04	0.12	0.01	0.38	0.27
FLR	0.11	0.06	0.07	0.43	0.22	0.01
FLW	0.25	0	0	0.4	0	0.27
GL	0.55	0.2	0	0.05	0.04	0.05
GSR	0.87	0.01	0.01	0.01	0.01	0.01
GW	0.66	0.03	0.02	0.06	0.08	0
GY	0.1	0.1	0.19	0.29	0.03	0
PH	0.13	0	0.72	0.05	0.06	0
PL	0.05	0	0.1	0.14	0	0.52
PT	0.02	0.11	0.01	0.22	0.16	0
TGW	0.01	0.41	0.01	0.09	0.19	0.04
Eigenvalue	3.04	2.36	2.05	1.85	1.36	1.21
Variability (%)	21.72	16.89	14.62	13.19	9.71	8.66
Cumulative (%)	21.72	38.61	53.22	66.42	76.13	84.78

Numbers in bold correspond for each observation to the factor for which the squared cosine is the largest.

Grouping of URVs

Clustering analysis groups URVs of high similarity using genetic distance (Jaynes *et al.*, 2003) like Euclidean distance (ED). Figure 4 showed a range of 6.75 to 211.5 ED. The closest distances were observed between Magsanaya Seln and Dukpayon,

Inamos and Awot, and Sang-Laya and Ranan with 6.75, 8.56 and 9.68 ED, respectively. Four groups were derived among 55 URVs. Group 2 (35 URVs) was the biggest (Figure 4, Table 4) and group 3 (only one URV) was the smallest.

Table 4. Four groups with their means for a character separated based on Least Significant Difference (LSD).

Groups	1	2	3	4
URVs genotypes	Arabon, Batangueño (Glut), Binatang, Binato, Binisaya, C22, C43, Dinorado, Hinomay, Kutivos, Mangglutus, Milbuen 3, P1-2-2 (Mimis), PilitTapol, Pinalwa, Pinursigi and Sinaguing	Aritao Cagayan, Awot, Balibod, BuntotKabayo, Camuros, C2, Daludo, Dinit-An, Dukpayon, Guinata, Inamos, InipotIbon, Kawatil Gold, Kinaboag, Langanan, Linangka, MagsanayaSeln, Magsanaya, Malagkit (I.D.N), Maligaya 2, Maliket, Milpal 18, Minarugon, Murado, Palawan, Pe-2, Penantad, Pingkitan, PinilingBaybay, Pirurutong, Pokkali, Ranan, Sang-Laya, Sinampablo and Wagwag	Chumi-I-Tinawon	Hinumay and Parirutong
CL	109.22 ^a	104.26 ^a	77.27 ^c	93.33 ^b
DF	54.98 ^b	56.37 ^{ab}	53.33 ^b	58.17 ^a
DM	92.08 ^a	91.47 ^a	91.67 ^a	93.17 ^a
FLL	433.96 ^b	342.33 ^b	387.67 ^b	580.34 ^a
FLR	27.51 ^{ab}	25.54 ^b	28.37 ^{ab}	34.87 ^a
FLW	16.24 ^{ab}	13.95 ^b	13.67 ^b	17.29 ^a
GL	8.81 ^b	8.53 ^c	8.89 ^b	9.39 ^a
GSR	3.01 ^{ab}	2.91 ^b	3.61 ^a	3.59 ^a
GW	2.97 ^a	2.98 ^a	2.50 ^b	2.62 ^{ab}
GY	1.94 ^b	1.79 ^b	2.45 ^{ab}	3.14 ^a
TGW	22.09 ^a	20.94 ^a	19.10 ^b	21.52 ^a
PH	126.74 ^a	123.67 ^a	84.78 ^c	109.89 ^b
PL	26.24 ^a	26.86 ^a	24.48 ^b	25.88 ^{ab}
PT	6.61 ^b	6.63 ^b	9.67 ^a	6.61 ^b

Numbers with similar superscript letters are means that are not significantly different.

Group 1 and 4 had 17 and 2 URVs, respectively. Significant differences between means of each group were also computed (Table 4). Group 4 had the highest average values compared to the other 3

groups based on DF (58.17 days), DM (93.17 days), FLL (580.14 mm), FLR (34.87), FLW (17.29 mm), GL (9.39 mm) and GY (3.14 t/ha).

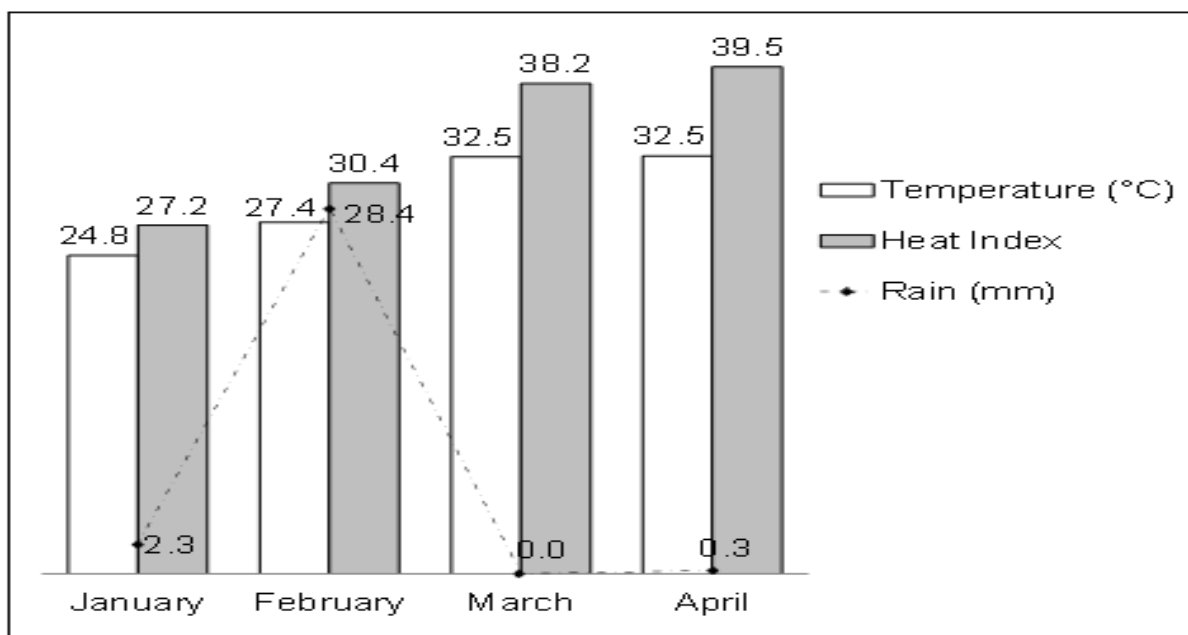


Fig. 1. Temperature (°C), heat index and rainfall amount from January to April, 2016 were recorded in the study.

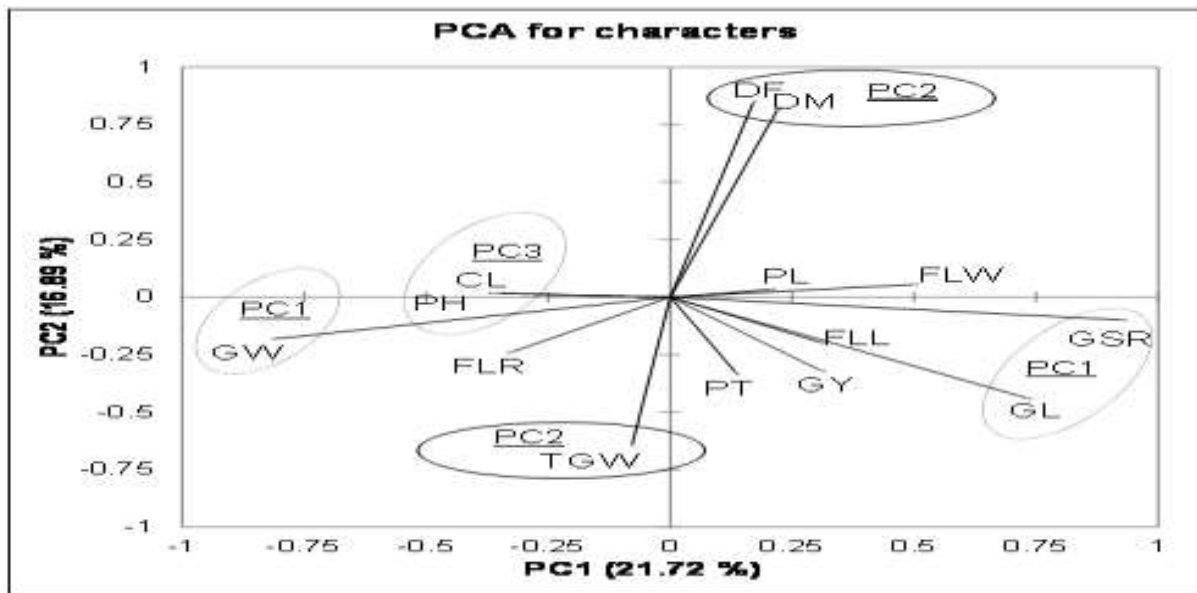


Fig. 2. Scatterplot of PC1 and PC2 with their corresponding variation values used in grouping of 14 characters.



Fig. 3. Grains of some upland rice varieties showing diversity.

This group had URVs of late-maturing, bigger flag leaves, longer grains and higher grain yields. Indeed, yield will even increase due to lengthened days of filling the grains and bigger leaves where large amount of solar energy is absorbed (Fujita *et al.*, 1984). Group 3 on the other hand had the lowest average values in characters such as CL (77.27 cm), DF (53.33 days),

FLW (13.67 mm), GW (2.5 mm), TGW (19.1 g), PH (84.78 cm) and PL (24.48 cm) but highest in PT (9.67). This group was generally short in stature, early-maturing, more number of tillers, and thinner leaves and grains. For this reason breeding URVs from different groups is more advantageous since it will lead in developing good varieties.

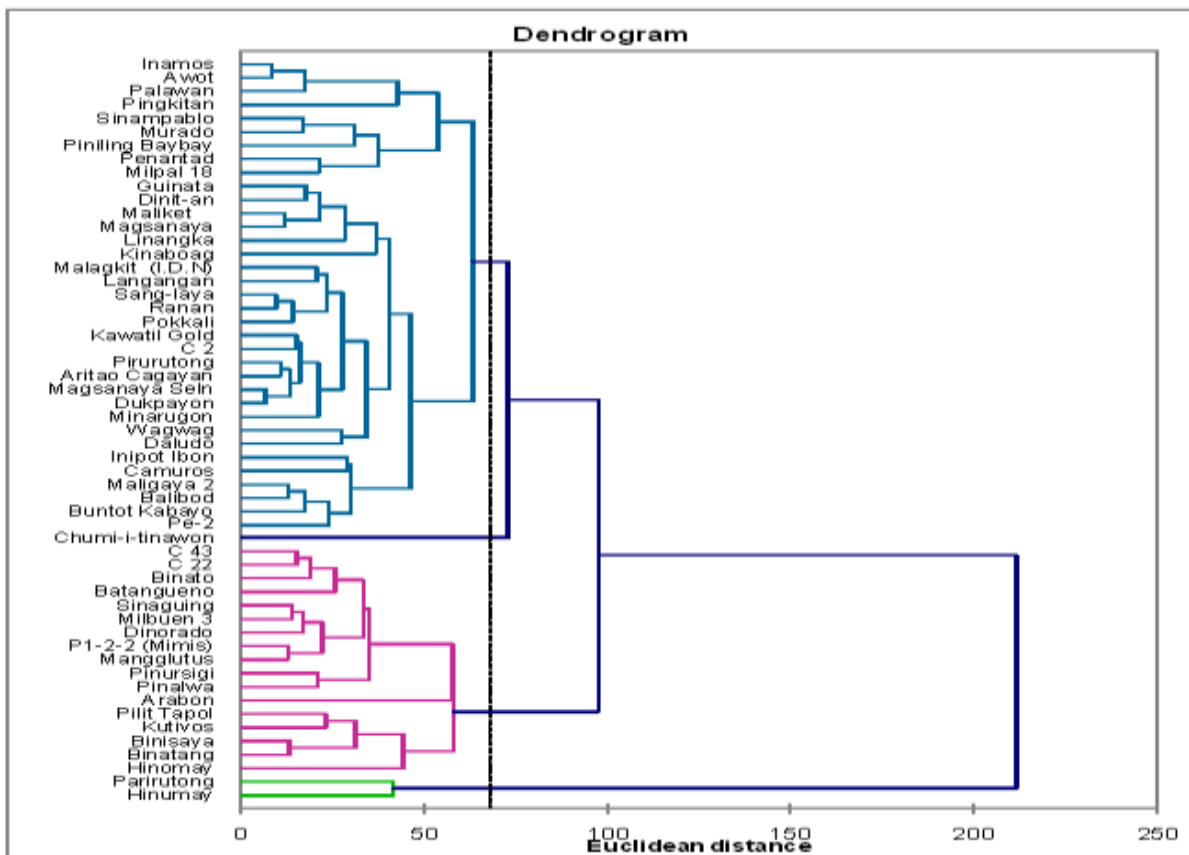


Fig. 4. Dendrogram showing 55 upland rice varieties used in the clustering analysis.

Conclusion

Significant variation based on 14 characters explained URVs’ morphometric. Diversity coefficient (*H'*) revealed that URVs’ maturity, panicle and flag leaf development were moderately diverse while the rest of the characters were highly diverse. First three PCs of PCA explained 53.22% of the variation. Characters on grain (grain length, size ratio and width) in PC₁ contributed 21.72%, which implied as highly affected by the environment. Clustering analysis grouped 55 URVs into four and breeding URVs from different groups will result to a better selection opportunity due to broad genetic make-up progenies. Results will be used as basic information upon exploiting URVs for any rice varietal development.

Acknowledgement

The authors are grateful to the Department of Agriculture, Philippine Rice Research Institute for the funding support.

The authors are thankful too to Miss Irish Mae B. Cantila for the few comments and grammar editing.

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