Multiple Statistical Tools for Divergence Analysis of Rice (*Oryza sativa* L.) Released Varieties

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Rice released varieties are genetic resources bulked with good genes. To define the potentials of these germplasm, genetic divergence analysis must be done. The study used different statistical tools such as descriptive statistics, Kolmogorov-Smirnov test, Shannon-Weaver diversity index (*H'*), correlation statistics (*r*), principal component analysis (PCA), Dixon's test and clustering statistics in evaluating 29 NSIC (National Seed Industry Council) released varieties based on 11 morphological traits. Descriptive statistics showed significant differences on the traits used while following a normal distribution. Shannon-Weaver diversity derived a range of 0.55 (number of filled grain per panicle, NFGP) to 0.91 (grain yield, GY and number of tillers, NT) that infer moderate to high diversity traits. Correlation statistics among traits showed a range of *r*=-0.55 to 0.84 which GY was noted to positively correlate to all traits. PCA accounted 39.95% and 26.10% for PC1 and PC2, respectively. Notable component loading for the yield component traits such as panicle weight (PW) showed the highest contributor of positive projections in two PCs that explained 66.05% of the variation. PCA also detected two latent traits such GY and spikelet fertility (SF) as confirmed in Dixon's test where outlier was found in SF and to yield contributing traits. Clustering statistics separated varieties into 5 clusters with a range of 5.88 to 106.22 euclidean distance (ED). Among the clusters, 5th cluster composed of one variety, NSIC Rc240 gave the highest GY (7.07 tha-1), NFGP (152.67), one thousand grain weight (24.77 g), PW (5.08 g) and spikelet number per panicle (185.33). The variety could potentially be adapted and a good source of genes for rice improvement localize at General Santos City.

Keywords: clustering statistics, correlation statistics, descriptive statistics, Shannon-Weaver index, rice released varieties, principal component analysis

1. Introduction

Rice (*Oryza sativa* L.) is cultivated on more than 150 million hectares with yearly world production of around 600 million tons cited by several authors (Kondo et al., 2003; Tyagi et al., 2004; Guimarães, 2009; Eckardt, 2009) and likely to increase nowadays. It is undeniably one of the most important food crops among cereals. The success of this crop depends on the adoption of released varieties. In fact, the impact of released varieties is the rapid increment yield production of rice in the Philippines (Umetsu et al., 2003). Despite the yield advantage, few farmers are utilizing released varieties because not all could thrive well in a particular cultivating area.

Rice breeders are interested on the genetic divergence based on the morphological characteristics since they are inexpensive, easy to gather and quick to discriminate genotypes. Understanding these variations especially quantitative data are vital so that variability available will be used directly for rice trait improvement. One method is to apply statistical tools to extract important statistical results. They are useful in defining, describing, correlating and summarizing the inherent differences and relationship between traits, among genotypes and genotype over environment.

Thus, this study aims to use descriptive statistics, Kolmogorov-Smirnov test, Shannon-Weaver diversity index (*H*'), correlation statistics (*r*), principal component analysis (PCA), Dixon's test and clustering statistics to evaluate genetic divergence of 29 NSIC released varieties based on 11 morphological traits.

2. Materials and Methods

Materials and site of the study

Twenty-nine NSIC released varieties (Table 1) were requested from Philippine Rice Research Institute (PhilRice), Central Experiment Station, Maligaya, Science City of Muñoz, Nueva Ecija for the study and were laid out in Randomized Complete Block Design (RCBD) along with three replications. The study was conducted at Purok 6, Katangawan, General Santos (GenSan) City, 6°10'20" N latitude, 125°13'17" E longitude and 38m height above sea level from September 21 to December 28, 2015 with corresponding meteorological data (Figure 1) taken from Department of Science and Technology (DOST)-PAGASA basing at General Santos City.

Table 1. Twenty-nine NSIC released varieties with their corresponding breeding institution were used in the multivariate analysis.

Registration number	Variety name	Variety type	Owner/ Breeder
NSIC Rc132	Mestiso 6 (SL-8H)	Hybrid	SL Agritech Corporation
NSIC Rc148	Mabango 2	Inbred	IRRI
NSIC Rc150	Tubigan 9	Inbred	PhilRice
NSIC Rc154	Tubigan 11	Inbred	PhilRice
NSIC Rc158	Tubigan 13	Inbred	IRRI
NSIC Rc160	Tubigan 14	Inbred	PhilRice
NSIC Rc170	Maligaya Special 11	Special, Inbred	IRRI
NSIC Rc172	Maligaya Special 13	Special, Inbred	IRRI
NSIC Rc204	Mestiso 20	Hybrid	PhilRice, UPLB
NSIC Rc212	Tubigan 15	Inbred	IRRI
NSIC Rc214	Tubigan 16	Inbred	IRRI
NSIC Rc216	Tubigan 17	Inbred	PhilRice
NSIC Rc218	Mabango 3	Special, Inbred	PhilRice
NSIC Rc220	Japonica 1	Special, Inbred	IRRI
NSIC Rc222	Tubigan 18	Inbred	IRRI
NSIC Rc224	Tubigan 19	Inbred	PhilRice
NSIC Rc226	Tubigan 20	Inbred	PhilRice
NSIC Rc238	Tubigan 21	Inbred	IRRI
NSIC Rc240	Tubigan 22	Inbred	PhilRice
NSIC Rc298	Tubigan 23	Inbred	PhilRice
NSIC Rc300	Tubigan 24	Inbred	PhilRice
NSIC Rc302	Tubigan 25	Inbred	IRRI
NSIC Rc308	Tubigan 26	Inbred	PhilRice
NSIC Rc318	Mestiso 48	Hybrid	PhilRice
NSIC Rc322	Mestiso 50	Hybrid	Pioneer Hi-bred Company
NSIC Rc352	Tubigan 27	Inbred	IRRI
NSIC Rc356	Tubigan 29	Inbred	IRRI
NSIC Rc358	Tubigan 30	Inbred	PhilRice
NSIC Rc360	Tubigan 31	Inbred	IRRI

IRRI- International Rice Research Institute; PhilRice- Philippine Rice Research Institute; UPLB- University of the Philippines Los Baños.

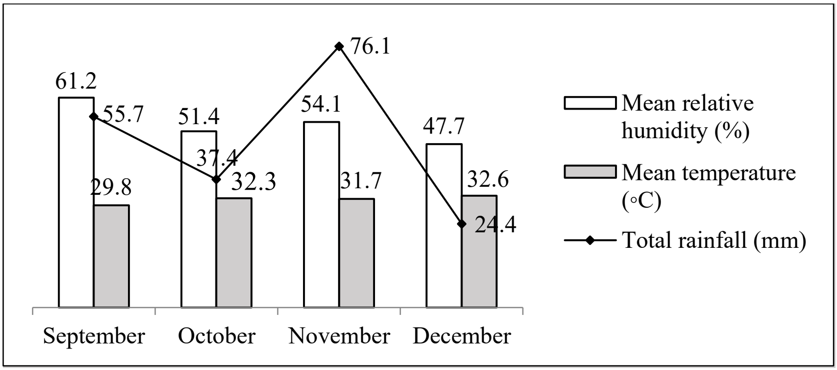


Figure 1. Meteorological data used in the study from September 21 to December 28, 2015.

Crop management

The area was plowed two times using hand tractor and was rotavated until the soil became ready for leveling. It was sprayed with molluscicide to control golden apple snail. Seeds were sown using dry seeding method for seedling preparation. Seedlings were then pulled and transplanted at 2 seedlings hill^{-1} to 10 m^2 plot size and 20cm x 20cm distance hill^{-1} after 21 days. The water was maintained at 3cm deep for a week to suppress the weeds and was increased gradually as the crops grew. Basal fertilizer application afterwards was applied at a rate of 30 kgha^{-1} of 14-14-14. Remaining applications of fertilizer was applied following the recommendation of Soil laboratory, GenSan City: 450 kgha-1 of 14-14-14, 50 kgha^{-1} of 16-20-0 and 450 kgha^{-1} of 21-0-0 at tillering stage and 257 kgha^{-1} of 16-20-0 at panicle initiation stage.

For pest management, spraying of chemicals against pest and diseases protected the experimental materials. Spraying of herbicides, spot weeding and water leveling controlled the weeds. Two weeks before harvesting the water was drained and when the grains become 80-85% straw-colored, the entries were harvested and threshed to avoid grain quality deterioration.

Traits measured

1. Days to flowering (DF) was determined by counting the number of days from transplanting until 50% of the panicle had emerged.
2. Days to maturity (DM) was determined by counting the number of days from sowing up to the 80-85% maturity of grains.
3. Grain yield (GY) was taken using PhilRice (2007) yield computation: GY (tha^{-1}) = [Panicle number per m2 x spikelet number per panicle x filled spikelets % x 1000 GW (g) x 10^{-2}].

4. Number of filled grains per panicle (NFGP) was determined by counting all the filled spikelets (average of 10 panicle samples taken from 10 randomly selected plants per plot).
5. Number of tillers was recorded by counting all the number of tillers from ten randomly selected plants (average of 10 randomly selected plants per plot).
6. One thousand grain weight (OTGW) was determined by weighing 1,000 seeds from each entry in grams (g).
7. Panicle length (PL) was determined by measuring from the last spikelet up to the first spikelet of the panicle in centimeter (cm) (average of 10 panicle samples taken from 10 randomly selected plants per plot).
8. Panicle weight (PW) was recorded by weighing the 10 panicle samples same with panicle length in grams (g) (average of 10 panicle samples taken from 10 randomly selected plants per plot).
9. Plant height (PH) was done by measuring the base up to the tip of the panicle one week before harvesting in centimeters (cm) (average of 10 randomly selected plants per plot).
10. Spikelet fertility (SF) was determined by counting all the number of filled spikelets divided by the total number of spikelet per panicle multiplied by 100 as percentage (%) (average of 10 randomly selected plants per plot).
11. Spikelet number per panicle (SNP) was determined by counting all the spikelet in a panicle (average of 10 randomly selected plants per plot).

Data analyses

Descriptive statistics such as analysis of variance (ANOVA), standard deviation (SD), mean square (MS) and coefficient of variation (CV) was computed using Statistix 9.0 by Analytical software (2009). Each trait was examined for normal distribution through Kolmogorov-Smirnov test using XLStat by Addinsoft (2010) for Microsoft Excel and *H'* index for diversity through the formula from Sotto and Rabara (2007):

$$H' = -\Sigma pi\ (\log_2 pi) / \log_2 N$$

Where pi = frequency proportion of the descriptor state
N = number of classes

Correlation statistics through Pearson's coefficient, PCA with two dimensional scatterplot and outlier test through Dixon (two-tailed test) were done too using XLStat. Clustering statistics on the other hand was derived using Numerical Taxonomy and Multivariate Analysis system for pc, version 2.1 (NTSYS-pc v.2.1) (Rohlf 2002). Cluster analysis followed the sequential agglomerative hierarchical nested (SAHN) technique based on unweighted pair group method arithmetic average (UPGMA) with euclidean distance (ED) as the coefficient. The derived clusters with their means were separated by least significance difference (LSD) of Statistix 9.0.

3. Results and Discussion

Morphological traits have been commonly used for estimating genetic diversity and developing cultivar since they provide an easier way of quantifying genetic variation (Fufa et al., 2005). Sanni et al., (2008) and Seetharam et al., (2009) concluded that these variations will definitely lead to rice improvement by maximizing good offspring combinations and later be selected. Descriptive statistics was used to define variability between traits. All traits showed significant differences for 29 NSIC released varieties evaluated with CV values ranging from 2.77 to 18.95% (Table 2) and followed the normal distribution based on the Kolmogorov-Smirnov test (Table 3). H’ values for the 11 quantitative traits were ranged from 0.55 to 0.91. Values *H’* = 1.00, H’ = 0.76 to 0.99, *H’* = 0.46 to 0.75 and *H’* = 0.01 to 0.45 means maximum, high, moderate and low diversity, respectively (Jamago and Cortes, 2012). High diversity was found on traits such as GY (*H’* = 0.91), NT (*H’* = 0.90), PL (*H’* = 0.87), DF (*H’* = 0.87) and PH (*H’* = 0.77) while moderate diversity for SF (*H’* = 0.74), SNP (H’ = 0.72), OTGW (*H’* = 0.69), DM (*H’* = 0.56) and NFGP (*H’* = 0.55). However, quantitative traits are highly affected by environment and the greater portion of variation may not be genetic.

Table 2. Mean values of 11 quantitative traits with their corresponding standard deviation (SD), mean squares (MS), coefficient of variation (CV) and Shannon-Weaver diversity (H’) used in the study.

Variable	Mean ± SD	MS	CV	H’
DF	64.31 ± 8.38	170.59**	7.08	0.83
DM	112.93 ± 5.67	78.94**	2.77	0.56
GY	4.60 ± 1.45	5.02**	18.04	0.91
NFGP	85.98 ± 17.71	629.17**	14.29	0.55
NT	11.69 ± 3.29	21.94**	18.95	0.91
OTGW	22.87 ± 2.73	15.76**	8.00	0.69
PH	87.12 ± 9.28	207.85**	4.66	0.77
PL	23.44 ± 2.00	8.56**	5.08	0.87

For correlation statistics, it determines the strength of linear association between two traits. When *r* = 0.68 to 0.99, *r* = 0.36 to 0.67 and *r* = 0.01 to 0.35, it means strong, moderate and weak correlations, respectively (Taylor, 1990). Zero, 1 and negative values in the same way imply no correlation, perfect and negative correlation, respectively (Gomez and Gomez, 1983). Table 4 showed a range of -0.55 to 0.84 derived from 55 traits combinations.

Table 3. Kolmogorov-Smirnov test for examining the normal distribution of data in the divergence analysis of rice released varieties

Traits	D	p-value	alpha	Test interpretation:
DF	0.108	0.247	0.05	p-value>alpha, normally distributed
DM	0.160	0.020	0.05	p-value>alpha, normally distributed
GY	0.117	0.174	0.05	p-value>alpha, normally distributed
NFGP	0.136	0.072	0.05	p-value>alpha, normally distributed
NT	0.084	0.554	0.05	p-value>alpha, normally distributed
OTGW	0.131	0.093	0.05	p-value>alpha, normally distributed
PL	0.065	0.841	0.05	p-value>alpha, normally distributed
PH	0.076	0.673	0.05	p-value>alpha, normally distributed
PW	0.098	0.353	0.05	p-value>alpha, normally distributed
SF	0.101	0.324	0.05	p-value>alpha, normally distributed
SNP	0.117	0.175	0.05	p-value>alpha, normally distributed

Table 4. Correlation coefficient between 11 quantitative traits calculated using Pearson's calculation.

Variables	DF	DM	GY	NFGP	NT	OTGW	PH	PL	PW	SF	SNP
DF	1										
DM	**0.79**	1									
GY	**0.49**	**0.51**	1								
NFGP	0.24	0.22	**0.40**	1							
NT	0.25	**0.38**	**0.61**	-0.29	1						
OTGW	0.19	0.25	0.25	0.26	-0.28	1					
PH	**0.53**	**0.58**	**0.42**	**0.37**	-0.11	**0.68**	1				
PL	0.48	0.52	0.27	**0.41**	-0.21	**0.45**	**0.68**	1			
PW	0.29	0.25	0.24	**0.84**	**-0.46**	**0.42**	**0.57**	**0.54**	1		
SF	0.07	**0.44**	0.33	0.14	**0.47**	0.07	-0.11	-0.10	-0.13	1	
SNP	0.13	-0.12	0.14	**0.80**	**-0.55**	0.16	0.35	**0.39**	**0.81**	**-0.48**	1

values in bold are different from 0 with a significance level alpha=0.05

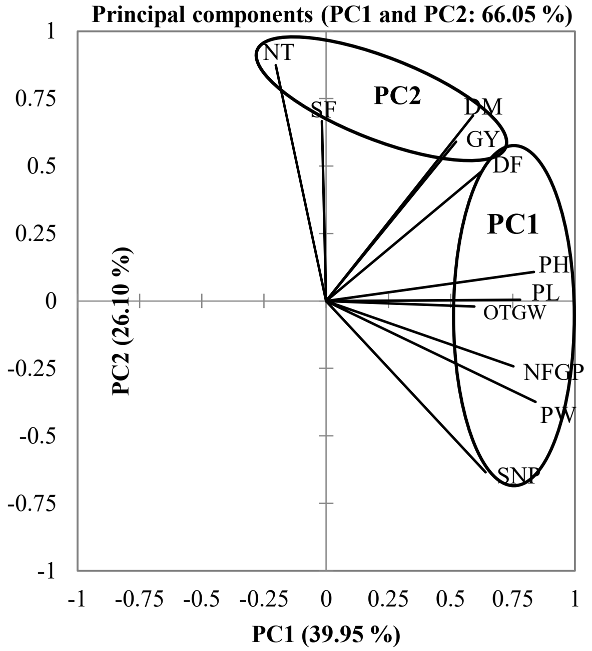
Only 28 combinations however were significant correlations: 6 was strong, 22 was moderate while the remaining combinations was weak correlations. Significant and strong correlations were seen in NFGP and PW ($r = 0.84$), PW and SNP ($r = 0.81$), SNP and NFGP ($r = 0.80$), DF and DM ($r = 0.79$), PH and PL ($r = 0.68$) and PH and OTGW ($r = 0.68$). For NFGP, SNP and PW, it means that the more filled number of grains and spikelets in a panicle, the panicle will be heavy. This result was in parallel to Augustina et al., (2013). PH and PL on the other hand were correlated because data for PH was taken through measuring the plants from the base of the ground to the tip of the panicle. PH and OTGW too were also found to be significantly correlated by Lakshmi et al., (2014) when they studied trait correlations in rice. The remaining significant correlations had moderate, weak and or negative correlations. Negative correlation happens on plants when one component takes advantage over the other according to Adams and Grufius (1971), one example is the competition of nutrient supply as cited by Lakshmi et al. (2014).

Determining the level of association indeed between these 11 traits is important since breeders will identify traits that could be used for rice improvement especially when these traits are related to grain yield. Xiong (1992) previously reported that an increment for GY could be realized through yield component improvement since yield related traits have higher heritability than GY. Ajmal et al. (2013) also added that it could be done by gradual accumulation of favorable genes or elimination of the unfavorable genes in the process of breeding. Grain yield in this study showed positive correlations to all traits but only significant to DF ($r = 0.49$), DM ($r = 0.51$), NFGP ($r = 0.40$), NT ($r = 0.61$) and PH ($r = 0.42$). Though these correlations were moderate, it has confirmed Sohrabi et al. (2012) result's on trait correlation since they found significance too between yield to DF, DM, NFPG, NT and PH. For DF and DM, Fujita et al. (1984) explained that when grain filling is lengthened, higher amount of solar energy is absorbed which results to GY increment. For NFGP, NT and PH on the other hand, it means that having many filled grains, many tillers and taller plants may not necessary confer higher grain yield.

PCA, another tool was used to define the variation level of each traits and complexity reduction of individual data forming a component as stated by Ringer (2008). The total variation from two PCs was 66.05% (Figure 2).

PC1 had 39.95% while PC2 had 26.10% with 4.39 and 2.87 eigenvalues, respectively. Iezzoni and Pritts (1991) previously reported that PCs with eigenvalue >1.0 are considered as intrinsically more informative than any single original data set alone. PC1 was comprised of DF, NFGP, OTGW, PH, PW, PL and SNP. These traits were positively correlated to one another based on computed Pearson results. Most of the variation is summarize on this PC according to Thompson et al. (1998). It was related to flowering, panicle development and plant growth. PC2 on the other hand was comprised of NT, SF, DM and GY. Two latent traits such as SF with 3.17% and GY with 4.03% contribution were in this PC. These traits were positively correlated to one another as well. PC2 can be accounted for the tillering, grain filling and maturity stage. It can be noted too that all traits belong with PC1 had a negative or weak correlation to NT and SF of PC2 based on computed Pearson results.

Outlier test in different manner was done for all traits to detect which variety has extreme phenotypic expression. No outlier was found in traits DF, GY, NT,



Contribution of the variables (%):

Variables	PC1	PC2
DF	**3.52**	2.09
DM	3.16	**4.30**
GY	2.48	**3.17**
NFGP	**5.15**	0.53
NT	0.37	**6.94**
OTGW	**3.23**	0.00
PH	**6.34**	0.10
PL	**5.52**	0.00
PW	**6.43**	1.27
SF	0.01	**4.03**
SNP	**3.74**	3.67
Eigenvalue	4.39	2.87
Variability (%)	39.95	26.10
Cumulative (%)	39.95	66.05

Figure 2. Principal component analysis showing the percentage contribution of principal components (PC) (a) and 11 quantitative traits (b). Values in bold correspond for each trait to the factor for which the contribution is the largest.

PL and PH (Table 5). Only DM, NFGP, OTGW, PW, SF and SNP traits had outlier. NSIC Rc172, Maligaya Special 13 rice type was the outlier in OTGW with 13.87 g as the lowest value, while NSIC Rc220, Japonica 1 special rice on DM with 91 days and SF with 52.86% as the lowest values. In rice, the optimum temperature for the normal development ranges from 27 to 32°C (Yin et al. 1996). The temperature recorded on October and December was beyond 32°C (Figure 1) while having lowest rainfall amounts. The low weight of NSIC Rc172 in OTGW and low percentage in SF and early maturity of NSIC Rc220 can be the specific reaction of their genetic composition over the environment, genotype by environment (GxE) interaction. NSIC Rc240 on the other hand had highest values and the outlier on yield component traits NFGP, PW and SNP with 152.67, 5.08 g and 185.33, respectively. In this study, NSIC Rc240 had the highest yield of 7.07 tha-1 due to the yield component recorded. Padolina et al. (2012) reported that NSIC Rc240 has a maximum potential yield of 10.6 tha^{-1}. Therefore, the genotypic reaction of NSIC Rc240 was better in the study compared to the rest of the released varieties. Latent traits: SF computed from number of filled spikelets over the total number of spikelet per panicle and GY computed from yield components were affected by the environment.

Table 5. Dixon's two-tailed test in the detection of the outlier in the data values of divergence analysis of rice released varieties

Traits	R10 (Observed value)	R10 (Critical value)	p-value (using 1000000 Monte Carlo simulations)	alpha	Test interpretation
DF	0.186	0.301	0.310	0.05	no outliers
DM	0.333	0.301	0.027	0.05	NSIC Rc220
GY	0.105	0.301	0.799	0.05	no outliers
NFGP	0.615	0.301	< 0.0001	0.05	NSIC Rc240
NT	0.138	0.301	0.559	0.05	no outliers
OTGW	0.500	0.301	0.000	0.05	NSIC Rc172
PL	0.005	0.301	0.080	0.05	no outliers
PH	0.246	0.301	0.130	0.05	no outliers
PW	0.447	0.301	0.001	0.05	NSIC Rc240
SF	0.469	0.301	0.001	0.05	NSIC Rc220
SNP	0.403	0.301	0.005	0.05	NSIC Rc240

Clustering statistics an another method assigns individuals of high homogeneity to a cluster and high heterogeneity between each cluster by Jaynes et al. (2003) using genetic distance (GD). The most commonly used GD for morphological data are euclidean distance (ED) as cited by Mohammadi and Prasanna (2003). Figure 3 showed ED ranging from 5.88 to 106.02 and showed no rice varieties were morphologically similar. The closest distances were found

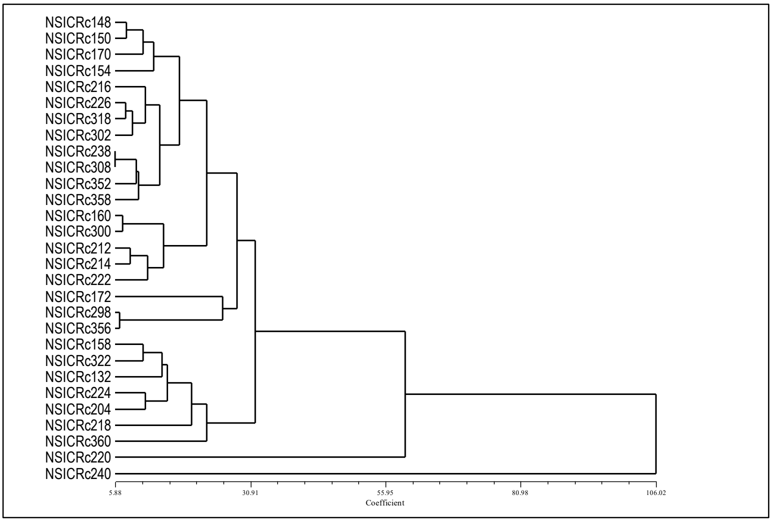


Figure 3. Dendrogram showing the genetic distance between 27 NSIC released varieties generated by UPGMA.

between NSIC Rc238 and NSIC Rc308, NSIC Rc298 and NSIC Rc356, and NSIC Rc160 and NSIC Rc300 with 5.88, 6.71 and 7.23 ED, respectively. Varieties form together because they expressed nearer values of quantitative traits. Five clusters were derived at 23 ED and its corresponding significant mean values and members are presented in Table 6.

Table 6. Six clusters and means for each trait per cluster separated basing on Least Significant Difference (LSD).

Variables	Cluster				
	1	2	3	4	5
Varieties	NSIC Rc148 NSIC Rc150 NSIC Rc154 NSIC Rc160 NSIC Rc170 NSIC Rc212 NSIC Rc214 NSIC Rc216 NSIC Rc222 NSIC Rc226 NSIC Rc238 NSIC Rc300 NSIC Rc302 NSIC Rc308 NSIC Rc352 NSIC Rc358 NSIC Rc318 NSIC Rc218 NSIC Rc224 NSIC Rc360 NSIC Rc204 NSIC Rc322 NSIC Rc132 NSIC Rc298 NSIC Rc356	NSIC Rc158 NSIC Rc172 NSIC Rc220	NSIC Rc240		
DF	64.22b	66.62ab	63.33b	47.67c	69.33a
DM	113.61a	113.86a	113.22a	91b	116a
GY	4.69b	4.51b	4.33b	1.99c	7.07a
NFGP	83.95b	89.17b	71.09b	76.1b	152.67a
NT	11.55b	10.26bc	14.86a	5.67d	8.9c
OTGW	23.22a	23.33a	20.03b	20.17b	24.77a
PH	86.85b	91.95ab	78.23c	73.33c	98.27a
PL	23.43b	24.64ab	21.22c	20.43c	24.97a
PW	2.5bc	2.94b	1.62d	2.29c	5.08a
SF	81.39a	72.11a	81.66a	52.86b	82.21a
SNP	103.22d	123.77c	87.07e	144.98b	185.33a

First cluster was consisted of 17 varieties, 2nd cluster of 7 varieties, 3rd cluster of 3 varieties and both 4th cluster and 5th cluster of 1 variety each. The analysis did not group varieties with the same origin. Zubair et al. (2007), Ahmad et al.

(2008) and Hosan et al. (2010) reported that there is a weak and lack of correlation among morpho-agronomic traits and varietal origin. First and 2nd clusters had varieties that generally flower and mature late. The means for these clusters were statistically not different except to SNP where 2nd cluster had more spikelets than the latter. Third cluster on the other hand were composed of high tillering varieties but with light panicles. Varieties on this cluster can be used for the improvement of more number of tillers. While the 4th cluster had the lowest mean values for most of the traits, 5th cluster had the highest mean values except for NT. Variety of 5th cluster thus could be a good source of genes for varietal improvement specific at Gensan City.

4. Summary and Conclusion

Descriptive statistics detected significant variations for all 11 morphological traits evaluated across the 29 NSIC released varieties. Shannon-Weaver index computed across traits were moderately to highly diverse. Diversity among these traits revealed no varieties were morphologically similar as confirmed in clustering statistics. Correlation statistics showed that 80% of traits combinations were positively correlated. GY, a latent trait as detected by principal component analysis (PCA) and the most important trait was found to be positively correlated to all traits and highest diversity index ($H' = 0.91$). Most of the statistical tools gave parallel results on the important factor that determines potential variety suited at Gensan City. NSIC Rc240, a lone variety of 5th cluster had the highest value of GY with 7.07 tha^{-1} and to several yield components such as NFGP with 152.67, OTGW with 24.77, PW with 5.08 g and SNP with 185.33. This variety performed well however it needs validation to recommend and use the technology.

Acknowledgements

The authors are grateful to the Department of Agriculture Region XII for funding support. Authors are grateful too to the City Agriculturist's office of General Santos City for communicating the farmer in the establishment of the study.

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