



Genetic variation in twenty Philippine traditional rice varieties

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Abstract

The study of genetic variation (GV) in 20 Philippine traditional rice varieties (TRVs) were determined in grain morphology such as length (Gl), width (Gwd), and weight (Gwt), grain yield (Gy), and grain micronutrients such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Analysis of variance obtained highly significant GV among TRVs in all traits. The mean for Gl, Gwd, Gwt, Gy, Cu, Fe, Mn, and Zn contents was 8.56mm, 2.8mm, 20.01g, 2.13t/ha, 8.64ppm, 14.56ppm, 20.79ppm, and 30.26ppm, respectively. The micronutrients in TRVs followed an order as Zn > Mn > Fe > Cu. For the correlation in traits, 11 pairs were significantly correlated; however, only Mn and Zn content in grains was positively and strongly correlated. Other significant pairs were either in positive or negative and moderately correlated. Cluster analysis, on the other hand, revealed five clusters and showed distinct TRV in two clusters. In conclusion, the presence of higher grain micronutrient content in TRVs indicates a better opportunity for breeders to determine parental genetic resource in breeding rice with high grain micronutrients.

Received: 27 January 2019

Revised: 16 June 2019

Accepted: 01 July 2019

DOI: <https://doi.org/10.3329/bjisir.v55i1.46730>

Keywords: Grain-related traits; Indigenous rice varieties; Grain micronutrients

Introduction

Rice is one of the Green Revolution's priority crops that had been called as the "global grain" since it is consumed about half of the world's population (FAO, 2013). Their intervention made a significant increment in world's crop production (Ortiz, 2011) and prevented uncontrollable global famines (Khush, 1999). From then on, high yielding rice varieties had been the most output in any rice research. Yield is the product of multiple effects of secondary traits (Yoshida, 1983). This means that yield increment can also be attained once genetic variation in secondary traits is fully understood. For instance, grain related traits were observed as the determinants of having good quality and quantity of grains (Shi *et al.*, 2000; Iwata *et al.*, 2010). Then, the more number, long and heavy grains will eventually lead to high yield. Grain related traits however are easily affected by the environment as stated by Cantila *et al.* (2017) on their research on evaluating rice types outside its natural environment in the Philippines. That is why rigid evaluation should be done on these traits to determine positive yield outcome. However, the full adoption of high-yielding varieties of cereal has resulted in a dramatic reduction in food diversity and micronutrient intake (Cakmak *et al.*, 2010). Also, achieving high yields in research overlooked micronutrient concentration on crops'

edible parts, leading to a little information on this aspect (Nube and Voortman, 2006). More than half of the world's population were affected by micronutrient deficiency dubbed as the "hidden hunger" (Khush *et al.*, 2012). Micronutrients played a vital role in cellular and hormonal responses, signaling and functions (Guerrant *et al.*, 2000; Kapil and Bhavna, 2008). The micronutrients accumulated in grains are also the internal traits that have importance to grain quality (Waters and Sankaran, 2011); examples of micronutrients are copper, iron, manganese and zinc. The study aimed to analyze genetic variation (GV) in eight traits, determine correlation in traits, and identify clusters in the 20 traditional rice varieties (TRVs).

Materials and methods

The Genetic Resource Division of the Philippine Rice Research Institute (PhilRice), Central Experimental Station, Nueva Ecija provided the 20TRVs such as Awot, Azucena, Binatang, Camuros, Chumi-i-tinawon, C2, C22, Dinorado1, Dinorado2, Hinomay, Hinumay, Kalinayin, Kasagpi, Kutibos, Malan, Milbuen3, Milpal18, Palawan, Pututan, and Wag-wag as the experimental units in the study.

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The experimental units were laid out triplicates in randomized complete block design at PhilRice, Midsayap Experimental Station from May 4 to August 29, 2017. The cultural management including weeding, fertilizer application, and chemical application protected and developed the plants. After harvesting, threshing, winnowing and cleaning; the grains were gathered data. For each experimental unit, grain length (Gl) and width (Gwd) was measured by averaging 10 random seeds using Vernier caliper. Similarly, grain weight (Gwt) was measured by weighing 1000 grains while grain yield (Gy) was determined by using the standard evaluation for rice (IRRI, 2014). For micronutrient content analysis, 20 g of each experimental unit was analysed using atomic absorption spectrophotometer and its concentration [copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn)] was determined using the methods described in previous report by Xu *et al.* (2015) at the University of Southern Mindanao Agricultural Research Center. STAR 2.01 (IRRI, 2014) provided the descriptive statistics and analysis of variance (ANOVA). XLStat. The correlation was computed following Pearson's coefficient, and cluster analysis following the agglomerative hierarchical on unweighted pair group method arithmetic average or UPGMA and Euclidean distance' coefficient.

Results and discussion

Importance of genetic variation

The pace of varietal development depends on the amount of GV identified in the germplasm (Kumbhar *et al.*, 2015). The GV in eight traits was found highly significant in 20 TRVs with samples shown in Figure 1. The mean for Gl, Gwd, Gwt and Gy was 8.56mm, 2.8mm, 20.01g and 2.13t/ha, respectively (Table I). These values were within the range of other rice research like Nurhasanah (2017) in Gl, Nirmaladevi *et al.* (2015) in Gwd, Cantila *et al.* (2017) and Ortuoste and Ortuoste (2014) in Gy. Gl, Gwd and Gwt are very important to rice since they have the direct relationship to grain's quality and quantity (Maji and Shaibu, 2012). Cu, Fe, Mn and Zn contents, on the other hand, had a mean of 8.64ppm, 14.56ppm, 20.79ppm and 30.26ppm, respectively (Table I). The micronutrients in TRVs followed an order as Zn > Mn > Fe > Cu. The values of different micronutrients were in parallel to the values found by different research like Genc *et al.* (2001) and Depar *et al.* (2011) in Cu, Frossard *et al.* (2000) and Gregorio *et al.* (2000) in Fe, Heinemann *et al.* (2005) in Mn, and Genc *et al.* (2001), Graham *et al.* (1999) and Gregorio *et al.* (2000) in Zn. In plants, the leaves act as sink tissues where micronutrients are vital to photosynthesis

Table I. Eight traits' means, mean squares and coefficient of variation showing the significance of genetic variation

Traits	Mean±SE	Mean Squares			CV (%)
		Replication	Genotype	Error	
Gl (mm)	8.56 ± 0.25	0.13	1.646**	0.09	3.51
Gwd (mm)	2.8 ± 0.18	0.06	0.2994**	0.05	8.01
Gwt (g)	20.01 ± 1.6	7.82	19.9342**	3.84	9.8
Gy (t/ha)	2.13 ± 0.54	0.57	1.9474**	0.44	31.17
Cu (ppm)	8.64 ± 1.82	30.42	54.29**	4.98	4.3
Fe (ppm)	14.56 ± 2.05	5.13	28.25**	6.31	5.75
Mn (ppm)	20.79 ± 1.77	35.18	74.8864**	4.73	10.46
Zn (ppm)	30.26 ± 1.97	14.20	72.0172**	5.83	7.98

Footnote: SE = standard error, ** significant at p< 0.001 level, CV = coefficient of variation, Gl=grain length, Gwd=grain width, Gwt=grain weight, Gy=grain yield, Cu=copper, Fe=iron, Mn=manganese, and Zn=zinc.

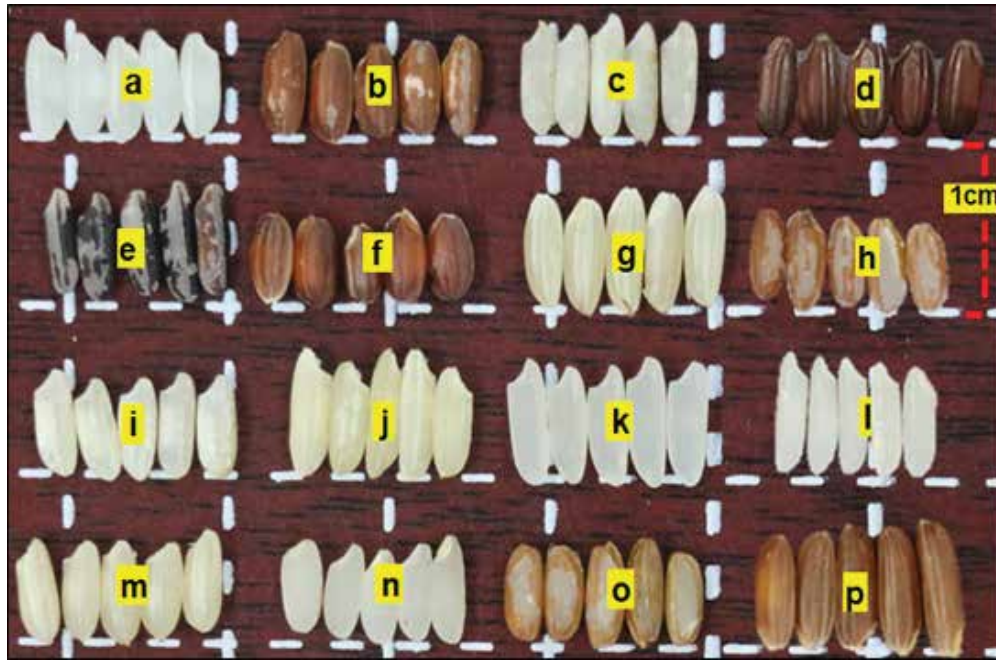


Fig. 1. Grain samples showing genetic variation

Legend: a=Camuros, b=Binatang, c=Chumi-i-tinawon, d=Palawan, e=Kasagpi f=Pututan, g=Hinomay, h=Kasagpi, I=Hinumay, j=Milbuen3, k=Azucena, l=Awot, m=Kalinayin, n=Dinorado1, o=Dinorado2 and p=Kutibos.

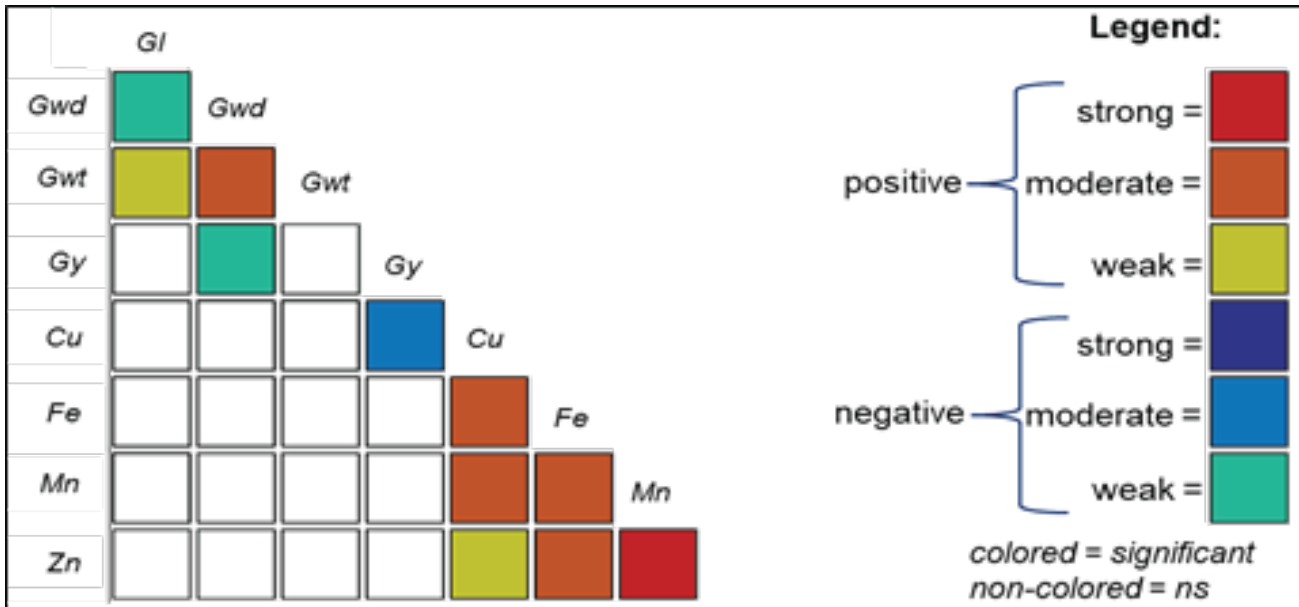


Fig. 2. Correlation analysis showed the relationship of the eight traits

Legend: Gl=grain length, Gwd=grain width, Gwt=grain weight, Gy=grain yield, Cu=copper, Fe=iron, Mn=manganese, and Zn=zinc.

and other metabolic mechanism (Gupta *et al.*, 2015). Plants transport micronutrients from the source tissues to edible seeds during the grain filling stage (Waters and Sankaran, 2011). The accumulation lies on its soil ion availability, root uptake efficiency, shoot translocation, and seeds' storage (Grusak 1999; Olsen and Palmgren, 2014). Micronutrients in the grains is important to humans since Cu deficiency leads to skeletal defects, Fe deficiency leads to anemia, Mn deficiency leads to tremors and stiff muscles and Zn deficiency leads to dwarfness and infertility (Sebastian and Prasad, 2015). GV based on grains, therefore, is vital for rice research.

Correlation in traits

Pearson correlation analysis identified the relationship between eight traits. Taylor (1990) identified correlation as follows: weak if $r = 0.01-0.35$, moderate if $r=0.36-0.67$ and strong if $r=0.68-0.99$. Cantila and Quitel (2017) applied this implication in their rice research on foreign genotypes Only

11 significant pairs, however, were obtained with 8 pairs in positive and 3 pairs in negative correlation (Figure 2). Positive and strong correlation was found between Mn and Zn contents, positive and moderate between Gwd and Gwt, Fe and Zn contents, Fe and Cu contents, Fe and Mn contents and, Cu and Mn contents, and negative and moderate between Gy and Cu content (Figure 2). Among the trait pairs based on grain morphology, only Gl and Gwt was positive and significantly correlated while the rest was in weak or not significantly correlated. Not enough information is available about micronutrient accumulation in relation to an increased yield or biomass (Xue *et al.* 2014). The negative relationship of yield and Cu content has been pointed out by Garvin *et al.* (2006) and Murphy *et al.* (2008) that high grain yields may lead to a decrease of micronutrients in the grains. On the other hand, it was noted that all micronutrients were positively correlated to each other. Roberts *et al.* (2004) reported that phytosiderophores, an amino acid synthesized and released

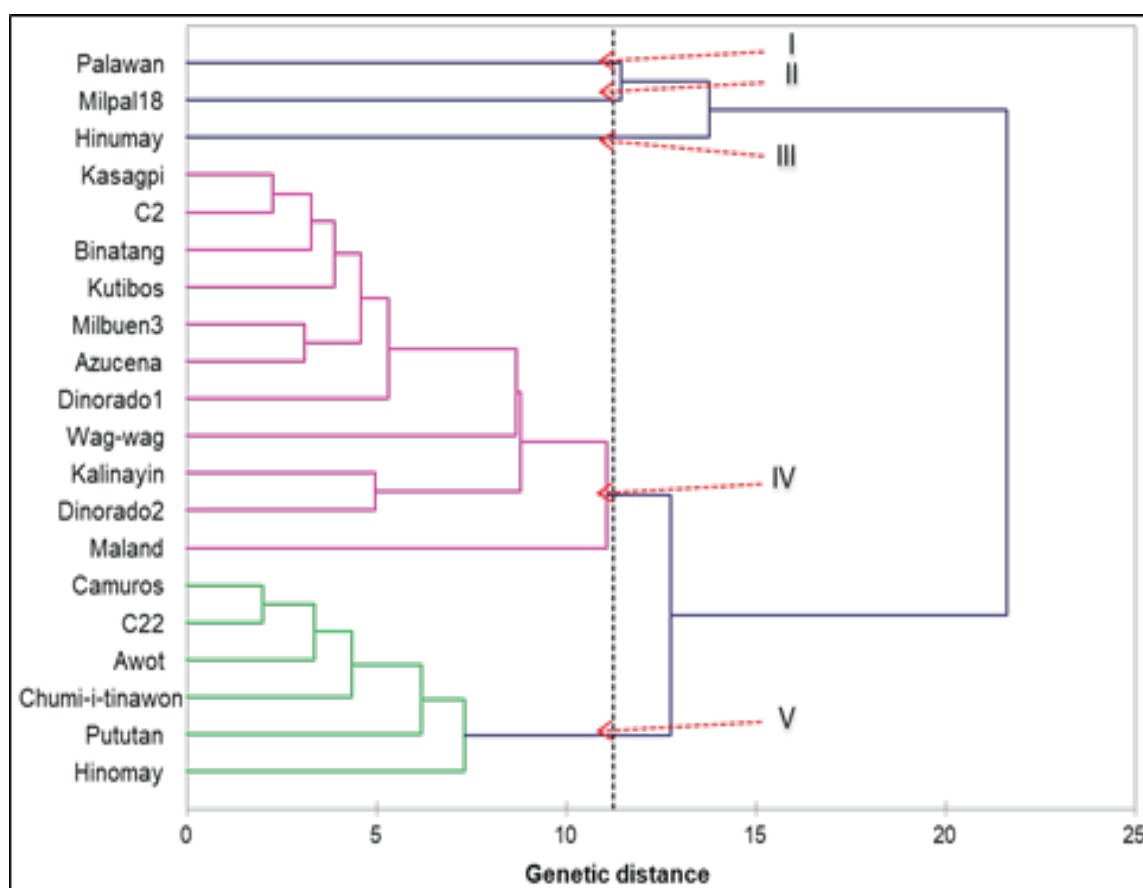


Fig. 3. Clustering of 20 TRVs based on the eight traits

Table II. Mean values of TRVs in eight traits

Clusters	TRVs	Gl	Gwd	Gwt	Gy	Cu	Fe	Mn	Zn
I	Palawan	9.43	3.03	21.90	1.22	14.69	32.25	28.13	34.73
II	Milpal18	9.78	2.49	23.50	2.05	8.03	30.38	19.85	31.43
III	Hinumay	8.80	2.50	17.07	3.09	8.22	25.29	32.69	35.22
	Azucena, Binatang, C2, Dinorado1, Dinorado2,								
IV	Kalinayin, Kasagpi, Kutibos, Malan, Milbuen3 and Wag- wag Awot, C22, Camuros, Chumi-i-	8.32	2.85	19.97	2.14	8.65	12.94	22.16	32.71
V	tinawon, Hinomay and Pututan	8.61	2.78	19.68	2.11	7.79	10.17	15.22	24.01

Footnote: Gl=grain length, Gwd=grain width, Gwt=grain weight, Gy=grain yield, Cu=copper, Fe=iron, Mn=manganese, and Zn=zinc.

by gramene monocots, chelate Fe and forms a stable Fe. This interaction then leads to the translocation of Zn, Cu and Mn across the plant cell (Ueno *et al.*, 2009). The relationship result especially between micronutrients suggests that increasing all micronutrients in a grain can be done in one variety.

Clusters in TRVs

The genetic distance, Euclidean values, of the 20 TRVs was computed using eight traits. Euclidean values were

ranged from 2.0 to 30.5 among 190 pairs of TRVs (Figure 3). Among the TRVs, Camuros and C22 are the closest with 2.0, followed by C2 and Kasagpi with 2.3 and Awot and Camuros with 2.5 (Figure 3). In contrast, the farthest pairs were in C2 and Palawan with 30.5, Camuros and Palawan with 29.3 and Awot and Palawan with 29.0 (Figure 3) indicating high genetic difference between paired TRVs. Breeding Palawan to C2, Camuros or Awot may give offsprings with high genetic variation. However, analysis showed five clusters. Cluster IV had 11 TRVs while cluster V had 6 TRVs (Figure 3; Table II). The means

in Gl (8.32mm), Gwd (2.85mm), Gwt (19.97g) and Gy (2.14t/ha) of cluster IV members were much nearer to the overall means (Table I). On the other hand, the mean in Cu, Fe, Mn and Zn contents of cluster V members were the lowest. Another, clusters I, II and III had Palawan, Milpal18 and Hinumay, respectively. Unique membership can be due to its highest values observed in a TRV. Palawan in cluster I had the highest values in Gwd with 3.03mm, Cu content with 14.69ppm and Fe content with 32.25ppm (Table II). Milpal18 in cluster II had the highest values in Gl with 9.078mm and Gwt with 23.5g (Table II). Hinumay in cluster III had the highest values in Gy, Mn and Zn with 3.09t/ha, 32.69ppm and 35.22ppm, respectively (Table II). Two out of three clusters (I and III) with unique membership and Cluster V had their grouping as a result of varying grain nutrient contents. Micronutrients are indeed variable in rice, indicative of better opportunity for breeders to select genotypes as parents for increasing micronutrients in rice grains.

Conclusion

High significant variation in 20 TRVs was obtained throughout the eight traits while 11 significant correlations were found in 28 pairs among traits. It was noted that micronutrients' relationship was significantly positive, result imply that increasing micronutrients in a grain is achievable.

Acknowledgment

The authors would like to express their gratitude to Mr. Elmer F. Bedia, field laborer of the study and to the staff and management of PhilRice, Midsayap Experiment Station, Bual Norte, Midsayap, Cotabato.

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