

The Study of Quantitative Traits with Different Statistical Parameters in Registered Inbred Rice (*Oryza sativa* L.)

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The primary quantitative trait grain yield (GY) and secondary traits *viz.*, days to maturity (DM), number of productive tillers (NPT), plant height (PH), panicle weight (PW), spikelet fertility (SF), spikelet number per panicle (SNP), and thousand seed weight (TSW) of 18 Philippine registered inbred rice were studied using different statistical parameters *viz.*, correlation analysis, genotypic and phenotypic coefficient of variability (GCV and PCV), broad sense heritability (H^2b), and genetic advance (GA). There was a significant, positive, and strong correlation between DM and PH, PW and SNP, PW and GY, and SNP and GY. GCV showed moderate variability in PW with 11.94% and NPT with 10.55%. PCV also showed moderate variability in NPT with 17.23%, GY with 14.3%, PW with 13.89% and SNP with 12.67%. All traits except for PW and SNP in GCV and traits except for NPT, GY, PW, and SNP in PCV showed low variability. H^2b too had PH with 79.26%, PW with 73.91%, and SNP with 60.39% as high heritability while GA expressed to the mean (GAM) had PW with 21.14% as high genetic gain. The study found out that PW and SNP had positive and strong association to GY, but only PW had consistent and considerable amount of genotypic and phenotypic variations. Furthermore, high H^2b along with high GAM was only obtained in PW. Therefore, the different statistical parameters were in congruent with the implication that higher grain yield can be achieved by attaining genotypic selection in PW.

Key words: genetic advance, heritability, quantitative, secondary traits, yield

INTRODUCTION

Rice is undeniably the most prominent crop in the Philippines and throughout Southeast Asia. Philippine rice production had been increased due to the adoption of registered rice varieties (Sombilla & Quilloy 2014). Registered rice varieties aside from possessing genes for biotic and abiotic resistance were developed to be high yielding. In the Philippines, Cantila and co-workers (2016) found out that the days to 50% flowering, days to maturity, number of filled grains per panicle, number of tillers, one thousand grain weight, plant height, panicle length, panicle weight, spikelet fertility, and spikelet number per panicle have positive correlation with grain

yield based on four hybrids, four special type of rice, and 21 inbred rice. Yield is the result of contributory effects of multiple traits, especially the secondary traits associated to yield (Yoshida 1983). According to Yano and Sasaki (1997), these traits have higher heritability and are less affected by the environmental than yield itself. Studying these traits therefore provides breeders what trait or traits to focus in the genotypic selection that leads yield improvement (Akhtar et al. 2011). In addition, secondary traits are morphologically-based, data are easy to extract (Fufa et al. 2005) and its expression is a result of genetic (G), environment (E), and GxE interaction (Johannsen 1911). Several efforts have been made on studying phenotypic divergence using quantitative traits of rice with experimental materials such as inbred

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(traditional, line segregants, etc.), hybrid, and combination of hybrid and inbred (Cantila et al. 2016, Nirmaladevi et al. 2015, Lakshmi et al. 2014, Augustina et al. 2013, Sanni et al. 2012). Given the foregoing, the study utilized different statistical parameters to determine the level of association between traits, measure the genotypic and phenotypic variability, and estimate the heritability and genetic advance of grain yield and secondary traits of the registered inbred rice. The information that will be obtained shall be useful in the development and improvement of rice breeding program strategies focusing on yield improvement.

MATERIALS AND METHODS

Experimental set-up and management

Eighteen inbred rice registered on the National Seed Industry Council (NSIC), Philippines (Table 1) were laid out in Randomized Complete Block Design (RCBD) replicated three times at General Santos City, Philippines. Temperature ranges between 29.8° C and 34.52° C with a mean of 32.46° C while relative humidity ranges between 39.64% and 61.2% with a mean of 47.65% during eight months in two seasons. The experimental materials were

prepared and sown in dry seeding method. Two seedlings per hill were transplanted after 21 days at 20 cm x 20 cm in 10 m² plot size. Fertilizers were applied as follows: 30 kg/ha of 14-14-14 as basal; 450 kg/ha of 14-14-14, 50 kg/ha of 16-20-0 and 450 kg/ha of 21-0-0 at tillering stage; and 257 kg/ha of 16-20-0 at panicle initiation stage. Chemical spraying, herbicides spraying, spot weeding, and water leveling protected the experimental plants. Plants were harvested at 80-85% maturity of grains.

Data management and analyses

Data onto primary trait grain yield (GY) and secondary traits such as days to maturity (DM), number of productive tillers (NPT), plant height (PH), panicle weight (PW), spikelet fertility (SF), spikelet number per panicle (SNP), and thousand seed weight (TSW) in rice were gathered for two seasons: from 2015's wet season to 2016's dry season. DM was done by counting the days from sowing until the grains reach 80-85% maturity and GY in tons per hectare (t/ha) was computed using this formula: [Panicle number per m² x spikelet number per panicle x filled spikelet % x 1000 seed weight (g) x 10⁻²] ÷ 1000. The mean for 10 randomly selected plants per plot was used for traits: NPT by counting the number of productive tillers per plant, PH in centimeters (cm) by measuring the base up to the tip of the panicle one week before harvesting, PW in grams (g) by weighing the panicle, SF in percentage (%) by counting all filled spikelets divided by the total number of spikelet per panicle multiplied by 100, and SNP by counting all the spikelet in a panicle. However, thousand seed weight (TSW) in grams was done by counting and weighing the 1000 seeds from random panicles within the entry.

The combined means of each trait in two seasons were then tested using Kolmogorov-Smirnov (Smirnov 1939, Kolmogorov 1941), Lilliefors (Lilliefors 1967), and Shapiro-Wilk (Shapiro & Wilk 1965), as well as outliers' tests like Dixon (two-tailed test) (Dixon 1980) and Mandel's *h* statistics (upper-tailed test) (Mandel 1985) using XLStat software by Addinsoft (2010). Data onto each inbred genotype in combined means (two seasons) were subjected to analysis of variance using RCBD through Statistix 9.0 by Analytical software (2009). Mean squares (MS) were used to estimate variance components in Microsoft Excel. Formulas were employed to obtain the following: Wricke & Weber (1986) for the genotypic variance (GV) = (MS_G - MS_E) ÷ replication, and phenotypic variance (PV) = GV + MS_E; Singh & Chaudhary (1985) for the genotypic coefficient of variability (GCV, %) = [(GV^{1/2}) ÷ mean of the trait] × 100, and phenotypic coefficient of variability (PCV, %) = [(PV^{1/2}) ÷ mean of the trait] × 100; Allard (1960) for the broad sense heritability (H²b) = (GV ÷ PV) × 100; and Shulka and

Table 1. Eighteen Philippine registered inbred rice with their corresponding average yield (t/ha), year registered and breeding institution based on NSIC, Philippines.

NSIC registration number	Average yield (t/ha)	Year registered	Breeding institution
NSIC Rc158	6.89	2006	International Rice Research Institute (IRRI)
NSIC Rc212	5.92	2009	
NSIC Rc214	6.22	2009	
NSIC Rc222	5.83	2009	
NSIC Rc238	6.15	2011	
NSIC Rc302	5.23	2012	
NSIC Rc352	5.14	2014	
NSIC Rc356	5.02	2014	
NSIC Rc150	6.45	2006	
NSIC Rc154	6.97	2006	
NSIC Rc160	6.29	2006	
NSIC Rc216	4.92	2009	
NSIC Rc224	5.39	2010	
NSIC Rc226	6.17	2010	
NSIC Rc298	5.52	2012	
NSIC Rc300	5.36	2012	
NSIC Rc308	6.61	2013	
NSIC Rc358	5.6	2014	

co-workers (2006) for the genetic advance (GA) = $k \times PV^{1/2} \times H^2b$, where k = selection differential at 5%, and genetic advance expressed to the mean (GAM) = $(GA \div \text{mean of the trait}) \times 100$.

RESULTS AND DISCUSSION

Validity of data

The scope of variation was expressed in range coupled with mean and standard error for the eight traits (Table 2). ANOVA revealed high significant differences ($p < 0.001$) for traits PH, PW, and SNP; significant differences ($p < 0.01$) to GY, NPT, SF, and TSW; and insignificant differences to DM among 18 registered inbred rice (Table 2). It can be noted that percentage towards total mean squares (MS) of $\leq 32\%$ in MS for genotype (MS_G) will lead to insignificance while $\leq 5\%$ in MS for error (MS_E) will lead to high significance (Table 2). Coefficient of variation (CV) was between 2.58% (PH) and 13.62% (NPT) (Table

2), and Maphumulo and co-workers (2015) showed that CV with $< 20\%$ indicates low experimental error. Variation was also tested for normality using Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilk. Kolmogorov-Smirnov test is an empirical distribution function that is very sensitive to extreme values. Lilliefors is a modified Kolmogorov-Smirnov and less conservative than the latter (Razali & Wah 2011). On the other hand, Shapiro-Wilk test is best suited to samples < 5000 observations (Shapiro & Wilk 1965). Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilk p -values were in a range of 0.131-0.996, indicating greater values than alpha of 0.05 (Table 3). Distribution of traits shown in Figure 1 had followed a normal distribution. Dixon's test as two-tailed and Mandel's h statistics as upper-tailed tests (Table 3) was further used to identify outliers in the data. Outliers are extreme data that lead to non-normality in the distribution (Wilrich 2013). While it is true that a perfect normal distribution is considered a myth (Elliot & Woodward 2007), different tests in normal distribution were done to satisfy the statistical requirement.

Table 2. Statistical parameters used to determine significance among the eight quantitative traits in Philippine registered inbred rice.

Traits	Range	Mean \pm SE	Mean squares (MS)			CV (%)
			Block (%)	Genotype (%)	Error (%)	
Days to maturity (DM)	102.5-117	110.66 \pm 0.48	30.37(53)	18.44 ^{ns} (32)	8.77(15)	2.68
Grain yield (GY in t/ha)	3.69-7.1	5.40 \pm 0.10	0.38(21)	1.09*(60)	0.35(19)	11
Number of productive tillers (NPT)	7-15.7	11.09 \pm 0.26	5(37)	6.39*(47)	2.28(17)	13.62
Plant height (PH in cm)	74-97	87.88 \pm 0.69	44.5(39)	64.31**(56)	5.16(5)	2.58
Panicle weight (PW in g)	2.11-3.93	2.82 \pm 0.06	0.62(60)	0.38**(37)	0.04(4)	6.81
Spikelet fertility (SF in %)	67-89.6	79.46 \pm 0.7	27.75(30)	48.7*(53)	14.97(16)	4.87
Spikelet number per panicle (SNP)	89-147.2	117.29 \pm 2.01	127.47(18)	487.77**(69)	87.52(12)	7.98
Thousand seed weight (TSW in g)	20-28.2	23.78 \pm 0.24	0.71(8)	6.11*(71)	1.75(20)	5.57

SE=standard error, **significant at $p < 0.001$ level, *significant at $p < 0.01$ level, ^{ns} not significant, enclosed values are percentage contribution relative to the total mean squares, CV=coefficient of variation.

Table 3. Normality tests and outlier's tests used to evaluate the normal distribution and extreme values in the eight quantitative traits in Philippine registered inbred rice, respectively.

Traits	Normality tests				Outlier's tests
	Kolmogorov Smirnov p-value	Lilliefors' p-value	Shapiro-Wilk p-value	Dixon's p-value	Mandel's h statistics (h within=-1.749 to 1.749)
Days to maturity (DM)	0.79**	0.391**	0.275**	0.589**	1.142
Grain yield (GY in t/ha)	0.962**	0.785**	0.814**	0.65**	-1.01
Number of productive tillers (NPT)	0.455**	0.075**	0.137**	0.72**	-0.894
Plant height (PH in cm)	0.996**	0.95**	0.131**	0.658**	0.676
Panicle weight (PW in g)	0.63**	0.196**	0.175**	0.868**	-1.063
Spikelet fertility (SF in %)	0.764**	0.352**	0.261**	0.84**	0.504
Spikelet number per panicle (SNP)	0.861**	0.518**	0.335**	0.807**	1.278
Thousand seed weight (TSW)	0.876**	0.548**	0.569**	0.331**	-0.634

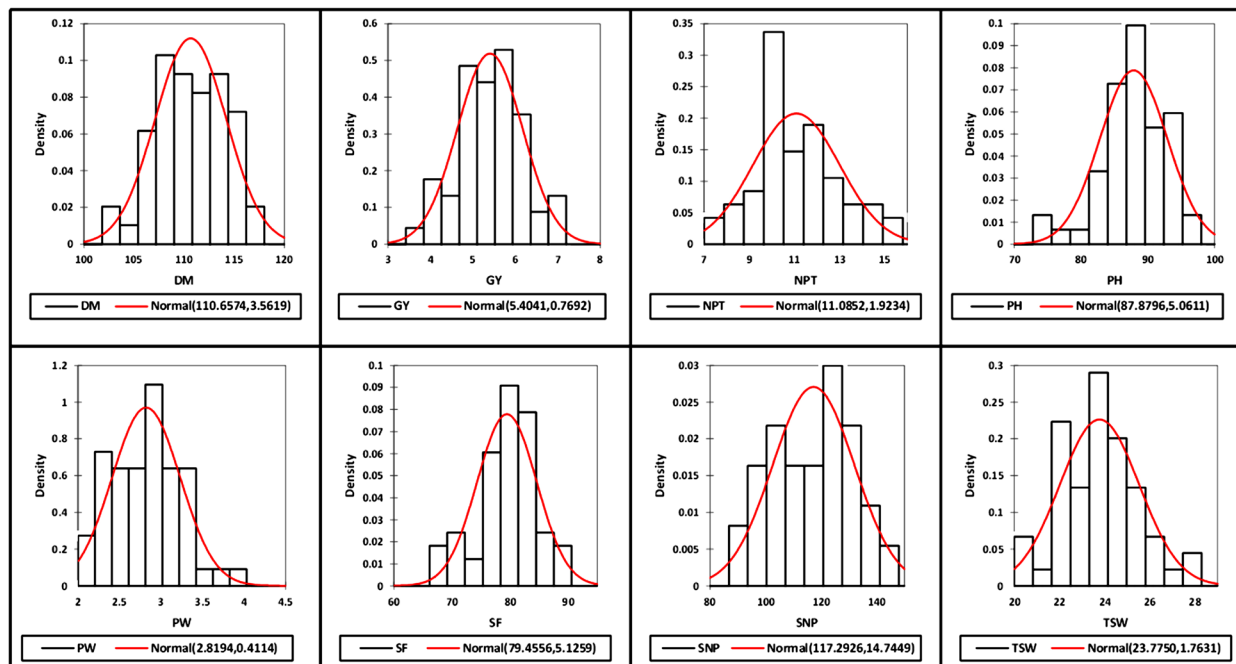


Figure 1. Histograms of eight quantitative traits based on Philippine registered inbred rice.

Association between traits

Yield is a result of the complex traits directly or indirectly affecting each other (Yoshida 1983). That is why information on association between quantitative traits should be done. Pearson's correlation analysis was used to determine this relationship. Taylor (1990) used $r = 0.01-0.35$ as weak, $r = 0.36-0.67$ as moderate, and $r = 0.68-0.99$ as strong association. Only 13 significant associations with 11 positive and two negative were found in the 28 combination between traits. DM and PH, PW and SNP, GY and PW, and GY and SNP had positive and strong associations (Figure 2). Similarly, Ashfaq and co-workers (2012) found significant and positive association between DM and PH, PW and SNP, GY and PW, and GY and SNP when studying rice genetic divergence. It was found out that short varieties were early maturing while taller varieties were also late-maturing while the association between GY, PW, and SNP had been previously reported by Yoshida (1983). PhilRice (2007) also use weight and spikelet number attributes in deriving data for grain yield, indicating closer relationship between these traits. DM and GY, DM and PW, DM and SNP, PH and GY, PH and PW, PH and SNP, and PH and TSW on the other hand had positive and moderate associations. However, rice breeders usually consider strong than moderate association for traits. In different manner, negative and moderate associations were found in traits between NPT and PW, NPT and SNP, NPT and TSW, and PH and SF. Adams & Grafius (1971) reported that negative association is due to the competition within the plant like nutrient allocation to different plant organs (Lakshmi et al. 2014).

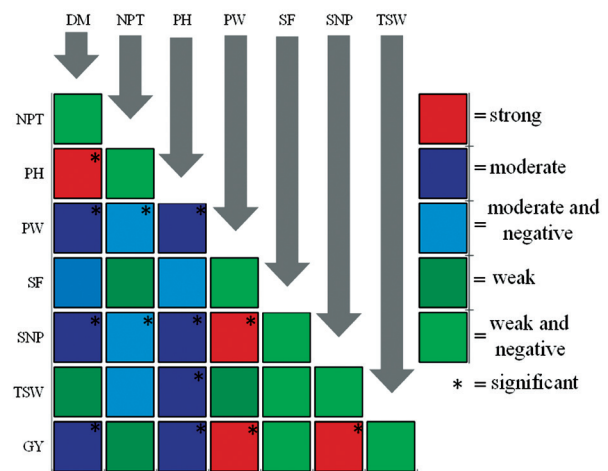


Figure 2. Association between quantitative traits based on Philippine registered inbred rice.

It can be implied that, as the number of productive tillers increases, panicle weight, spikelets' number and grain yield decreases. Also, as the varieties grew taller, few spikelets were filled.

Variability, heritability, and genetic advance

Variance components *viz.*, genotypic variance (GV), phenotypic variance (PV), genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) were also used. GV was between 0.11 (PW) and 133.42 (SNP); PV from 0.15 (PW) to 220.94 (SNP); GCV

Table 4. Variability, heritability and genetic advance of the eight quantitative traits based on Philippine registered inbred rice.

Traits	GV	PV	GCV (%)	PCV (%)	H ² b (%)	GA	GAM (%)
Days to maturity (DM)	3.22	11.99	1.62	3.13	26.88	1.92 (days)	1.73
Grain yield (GY in t/ha)	0.25	0.6	9.2	14.3	41.34	0.66 (t/ha)	12.18
Number of productive tillers (NPT)	1.37	3.65	10.55	17.23	37.53	1.48 (tillers)	13.32
Plant height (PH in cm)	19.72	24.88	5.05	5.68	79.26	8.14 (cm)	9.27
Panicle weight (PW in g)	0.11	0.15	11.94	13.89	73.91	0.6 (g)	21.14
Spikelet fertility (SF in %)	11.24	26.21	4.22	6.44	42.89	4.52 (%)	5.69
Spikelet number per panicle (SNP)	133.42	220.94	9.85	12.67	60.39	18.49 (spikelets)	15.76
Thousand seed weight (TSW)	1.45	3.2	5.07	7.53	45.37	1.67 (g)	7.03

GV=genotypic variance, PV=phenotypic variance, GCV=genotypic coefficient of variation (%), PCV=phenotypic coefficient of variation (%), H²b=broad sense heritability (%), GA=genetic advance, GAM=genetic advance expressed to the mean (%).

from 1.62% (DM) to 11.94% (PW); and PCV from 3.13% (DM) to 17.23% (NT) (Table 4). In terms of variability, Deshmukh and co-workers (1986) reported that GCV and PCV values of >20% are high, 10-20% are moderate, and <10% are low. GCV revealed that DM, GY, PH, SF, SNP, and TSW have low variability while NPT and PW have moderate variability. In the same way, PCV revealed that DM, PH, SF, and TSW have low variability but GY, NPT, PW, and SNP have moderate variability. Levels in genotypic and phenotypic variations were determined, but not the heredity of the trait. Heritability extracts information on the heredity while separating the environmental effect in the total variation (Allard 1960). To be specific, heritability in broad sense (H²b) with additive, dominant, and epistatic variances was used (Nirmaladevi et al. 2015, Allard 1960, Hanson et al. 1956). Robinson and co-workers (1949) reported high, moderate, and low heritability if H²b>50%, H²b =50%, and H²b<50%, respectively. High H²b was found out in PH with 79.26%, PW with 73.91%, and SNP with 60.39% (Table 4), showing transmissibility to the next generation. However, H²b does not give values of genetic gain. Larik and co-workers (2000), Johnson and co-workers (1955) and Burton & De Vane (1953) pointed out that high H²b should be accompanied by high genetic advance (GA) for selection to be effective. GA measures genetic gain for a trait under selection pressure (Wolie et al. 2013). Nirmaladevi and co-workers (2015) used H²b with >60% and GA expressed to the mean as GAM with >20% as the statistical parameter in rice. The study found out that only PW had high GA with 0.6 g equivalent of GAM with 21.14% (Table 4). This means that in 5% selected best progenies in PW, the succeeding generation will gain an estimated 0.6 g. The estimated GA, 0.6 g, will be added to the overall PW of 2.82 g and makes the next generation having an estimated 3.42 g PW.

CONCLUSION

Statistical parameters suggest that there is an adequate and considerable variability present in the quantitative traits based on the registered inbred rice. Yield has been the primary concern in any Philippine rice varietal breeding and development. Among the secondary traits, only SNP and PW had positive and strong association with yield but only PW was consistently detected as moderately variable. On the other hand, high heritability was recorded in PH, PW, and SNP with 79.26%, 73.91%, and 60.39%, respectively. These traits could be effectively passed to the next generation. However, PW was the lone trait with high GA (0.6 g) and GAM (21.14%). Therefore, genotypic selection in PW should be maximized as this trait was more effective and close in attaining higher grain yield in inbred rice.

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