Multivariate Analysis of Phenotypic Diversity of Philippine Improved Rice Varieties

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Morphological characterization of modern cultivars provides information regarding preservation of diversity and selection of parents in a breeding program. The study analyzed the diversity for phenotypic traits of 78 improved rice varieties released from 1965 to 1995. Data on morphological and agronomic traits obtained from field evaluation were analyzed by multivariate analysis. Analysis of variance found 8 invariant qualitative traits that provide no contribution in the measure of diversity. A total of 33 traits showed highly significant differences among improved varieties and used in distance analysis. Euclidean distances in pairwise comparison varied from 2.228 to 16.706. Principal component analysis revealed that 6 qualitative characters significantly influenced the variation in released varieties. Cluster analysis permitted separation of improved varieties into 2 major clusters; cluster 1 consisted of 72 (92%) varieties having green-colored base and cluster 2 consisted of 6 (8%) varieties (BP176, PSB Rc34, IR46, UPL R13, UPL R15 and C168) having purple-colored base. Results showed that rice breeders selected for uniform traits as a consequence of design-driven breeding approach, thus reducing phenotypic divergence in improved varieties.

Keywords: Multivariate analysis, morphological diversity, Philippine improved rice varieties

Human-directed crop improvement fixes agronomically important traits as governed by design-driven approach. Selection of breeding lines depends on a given set of criteria found suitable to a particular environment and for a specific application. This process has led to development of homogenous genotypes, making modern varieties morphologically related. Phenotypic similarity poses threats of epidemic of pest and diseases and yield plateau. To avert this problem, characterization is important in breeding program to avoid these inherent danger of phenotypic uniformity. Information on variability in breeding program influences the choice of individuals to cross in hybrid combination towards broadening the genetic base of modern cultivars.

Variability in a group of plants can be estimated at the level of morphology. A range of plant characters are available, but Souza and Sorrells (1989) specifically define cultivar characteristics that can be utilized to determine genetic distance. Several studies used qualitative and quantitative traits to measure relationship in a number of crops: oat (Souza and Sorrells, 1991), corn (Smith et al, 1985a), Triticum aestivum L. (Cox et al, 1985) and Phaseolus Vulgaris L. (Singh et al, 1991). Morphological traits were analyzed using statistical procedures, covariances of allele frequencies summed for all characters and more recently, multivariate statistical methods like cluster analysis and principal component analysis (Crossa et al, 1995).

Evaluation of phenotypic diversity usually reveals important traits of interest to plant breeders. Singh (1989) proposed the existence of 12 gene pools in dry bean on the basis of growth habit, seed morphology and adaptational traits. Shamsuddin (1985) reported that phenotypic distance between spring wheat parents can predict parental specific combining ability in F₁ progeny. Lehman (1984) reported that diversity analysis of barley germplasm identified 11 accessions as being resistant to isolates of leaf rust. Other applications were on evaluation of the structure of germplasm collections, varietal identification in terms of taxonomical classification and phylogenesis analysis.

In rice, development of modern cultivars for the past three decades concentrated towards high yielding plant type: short stature, erect leaves, high tillering, sturdy stems, early maturing and fertilizer responsive.
These high yielding improved varieties have replaced the traditional varieties in the farmers' field. However, improved varieties are not perfect. Several studies showed that rice varieties shared common ancestry which could result from an erosion of genetic diversity. Rice scientists have an obligation to diversify the genetic base of improved varieties, and the first step towards this is to characterize improved rice genotypes at the morphological level.

The objectives of the study were to determine the phenotypic variability of Philippine improved rice varieties released from 1965-1995 and to identify traits that contribute to the total variation in the modern genotypes.

MATERIALS AND METHOD

Materials

A total of 78 rice accessions, comprising of Philippine-bred improved rice cultivars released from 1968 to 1995 were included in the study. Entries represent the breadth of genetic diversity, covering the genetic spectrum of Philippine rice germplasm. The improved rice varieties were released through the National Seed Industry Council, formerly Philippine Seed Board, encompassing all rice agro-ecological systems such as irrigated lowland, upland, rainfed-lowland, cold and saline areas. Among the improved varieties, seven are traditional cultivars developed through pureline selection, while the remaining entries are products of conventional breeding. Conventionally bred varieties were developed by different breeding institutions like International Rice Research Institute (IRRI), Philippine Rice Research institute (PhilRice), Bureau of Plant Industry (BPI) and College of Agriculture-University of the Philippines at Los Baños (CA-UPLB). Seeds of the modern varieties were obtained from Philippine Rice Research Institute at Maligaya, Muñoz, Nueva Ecija.

Experimental Design

The experiment was conducted in irrigated lowland condition during the 1996 dry season at the Central Experiment Station of the Philippine Rice Research Institute in Muñoz, Maligaya, Nueva Ecija. Cultivars were laid out in randomized complete block design with three replications. Plot size was 3.5 m long and 1 m wide. Hill and row spacing was 20 cm.

Cultural Management

Recommended cultural practices were followed for the region. Fertilizer rate was 120-60-60 kg NPK ha⁻¹. The fertilizer was applied basally a month after transplanting and top dressed at panicle initiation stage. Herbicide (Machete) was applied to minimize infestation of weeds. Plots were fully protected from insect pests and diseases. Rat and bird damages were also controlled.

Data Collection

Data were collected for 14 quantitative traits and 27 qualitative traits following the Descriptor for Rice Oryza sativa L. (IRRI, 1980).

MORPHO-AGRONOMIC TRAITS

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Culm length                  Blade pubescence
Culm number                  Blade color
Culm diameter                Basal leaf sheath color
Effective tiller number      Leaf angle
Panicle length               Flag leaf angle
100-grain weight             Culm angle
100 grain weight             Culm strength
Plant height                 Panicle type
Days to heading              Secondary branching
Days to maturity             Panicle exsertion
Leaf length                  Panicle shattering
Leaf width                   Awning
Sterile Lemma Length         Awn color
                         Lemma and Palea color
Spikelet fertility           Endosperm type
Stigma color                Sterile Lemma Color
Seed coat                   Ligule color
Data Analysis

The data were analyzed on a plot mean basis. Initial analysis of variance was performed to verify genetic variation in the traits measured. The few traits with little genetic variation, based on F-test, provided no contribution for further analyses. Correlations between traits were calculated based on the means of each genotype. The traits left for study were standardized to unit variance using NTXSYS (Numerical Taxonomy and Multivariate Analysis System) programs (Rohlf, 1990). Standardizing the data matrix is a common procedure to eliminate scale differences in the morphometric traits, which has the effect of making all attributes equally important to the analyses. Numerical measures of likeness between each pair of accessions were conducted to produce a symmetrical square matrix which are necessary for classification techniques. Distance matrix was constructed by means of Euclidean Distance Coefficients, \( e_{ij} \). Cluster analysis was carried out for morphology-based genetic distance matrix of the rices evaluated, applying the widely used UPGMA (Unweighted Pair-Group Method of Arithmetic Mean) clustering method in the NTYSN. Principal component analysis was done using NTYSN and SAS programs (SAS Institute 1983).

RESULTS AND DISCUSSION

Analysis of Character Means for Both Qualitative and Quantitative Traits

Little variation was observed among Philippine improved rice varieties released from 1965 to 1995 based on 41 morphological and agronomic characters evaluated. From the total attributes examined, only 33 traits exhibited highly significant differences for F test \((P<0.01)\) among improved varieties. Released cultivars were found homogenous for seed coat color, sterile lemma color, secondary branching, panicle axis, auricle color, ligule shape, blade angle at heading and culm strength. It appears that straw lemma color, translucent grains, drooping panicles with the presence of secondary branching, green auricle color, 2-cleft ligule, erect blade angle at heading and non lodging susceptible culms are some of the traits equated to a modern plant type regardless of ecosystem (irrigated, rainfed, upland, saline and cool elevated areas). Accumulation of similar traits among improved cultivars demonstrated that breeders shared common breeding objectives in developing high yielding plant type. Finding lack of variability in a particular trait in two individuals being compared would have an insignificant effect on the measure of relationship between the individuals (Romesburg, 1984).

In the analysis of quantitative traits, the coefficients of variability varied from 2.5\% (maturity) to 15.5\% (productive tillers). Modern varieties undergo continuous selection and purification with emphasis on visible traits. After several seasons of purification, as indicated by low coefficient of variability, released varieties become homogeneous in some traits like maturity. Observation on the distribution of the quantitative traits showed that all measurements were not too far from normal random distribution (Figure 1). Bimodal phenotypic distribution was exhibited by their plant heights and culm lengths showing two peaks for 95 cm / 130 cm and 65 cm / 110 cm, respectively. Other traits like maturity, grain length, grain weight and heading showed a unimodal distribution with most varieties highly skewed to the right. High variability was found in maturity, plant height and culm length with standard deviation values of \( s=8.59 \), \( s=15.84 \), and \( s=15.39 \), respectively. Low variability was found in leaf width \(( s=0.08 \) ), ligule color \(( s=0.11 \) ) and grain width \(( s=0.16 \) ).

Reduced plant stature is the most obvious trait of improved rice cultivars with an average height of 99 cm. Almost 80\% of the released varieties had semi-dwarfing (sd-1) gene, which came from Dee-gee-woogen (Hargrove, 1979). This gene governing short statureness contributed most of the production gains of the green revolution due to associated improvements in harvest index and non-lodging susceptible under heavy dosage of fertilizer. Improved varieties were mostly medium maturing with 121-147 days. Longer maturity allowed plants to have more time to photosynthesize thus more photosynthates produced which are needed at grain filling stage (Khush, 1991). Still very few varieties had 110 days growth duration. IR28, IR30, IR36, PSBRc 10 and PSBRc 12 were among the early maturing varieties. Traditional varieties released by National Seed Industry Council formerly Philippine Seed Board include PSB Rc 16 (Enanno), PSB Rc 36 (Enanno II), PSB Rc 38 (Rinara) and PSB Rc 40 (Chayong). These varieties had medium maturity, intermediate height and low tillering. IR64, C-63G, BPI Ri10, PSB Rc 18, PSB Rc 20 and PSB Rc 22 known for good grain quality, had green internode, green apiculus and yellow stigma. Magat, the country's first hybrid
Correlation Among Morphological and Agronomic Traits

Correlation coefficients between traits measured in improved varieties are shown in Table 1. Maturity was found poorly correlated with other morpho-agronomic traits. It exhibited negative correlation with 14 traits and found slightly correlated with grain width (r=0.43) and plant height (r=0.42). This indicates that some medium maturing released varieties had wider grain width and taller plant height compared to some early maturing released varieties. The participation of non-laminar photosynthesis in grain filling is quite evident in the correlation between grain weight and sterile lemma length. Sterile lemmas contribute to the total photosynthates stored in the grains. Plant height was significantly correlated (r=0.61) with leaf length showing the principle of morphogenetic compatibility in the architectural plant type in rice. Significant correlation was also found between plant height and apiculus color. Varieties showing purple or red apiculus were tall, while cultivars with green apiculus were short. Other traits that were strongly correlated with plant height and apiculus color were blade color, culm angle, culm color, culm height and basal leaf sheath color.

Based on the correlation analysis, a number of traits were directly associated with other traits regardless of plant type or architectural configuration of the whole plant.

Euclidean Distances Among Improved Varieties

Estimates of phenotypic or genotypic distance were obtained from qualitative and quantitative data measured from the total samples. This assumes that the differences between characters reflect the genetic divergence of the genotypes being compared. Distance coefficients between pairs of improved varieties using
Euclidean geometry are shown in Figure 2. The mean phenotypic distance of improved varieties was \( e_{\mu} = 7.55 \) suggesting a low phenotypic diversity in released cultivars.

In improved varieties, Euclidean distances in pairwise comparison ranged from 2.23 to 16.71 from a total of 3081 pairs. The lowest distance coefficient was exhibited by IR24 and IR45. These two varieties showed similarity in almost all qualitative traits measured. The highest degree of phenotypic resemblance was detected as one served as a parent to another. IR24 is part of the ancestry of IR45. Not only that they shared similar traits with each other, but with all released varieties as well. This is quite evident in the mean phenotypic distance exhibited by these two varieties being the lowest; IR24 (5.90) and IR45 (5.73). On the other hand, the highest phenotypic distance was exhibited by UPL Ri3 and BPI Ri10. The immediate parents of BPI Ri10 are mutants of BE-3-37-5 and BPI-121-40. It is one of the released varieties that has no semi-dwarfing gene. Unlike UPL Ri3, it has the sd-1 gene of Dee-gee-woo-gen through its IR20 parent. Malagkit sungsong, as the maternal parent, contributed most to the unique characteristics of UPL Ri3 being an upland released variety. Traits that were inherited from the maternal parents were purple apiculus, purple auricle, purple internode and purple basal leaf sheath. Because of these unique characteristics, it exhibited the highest mean

![Figure 2. Summary of Euclidean distances in pairwise comparison of 78 modern rice varieties.](image)

The genetic distance of \( e_{\mu} = 13.68 \) across all released varieties while BPI Ri10 had the third to the highest mean phenotypic distance across all varieties with \( e_{\mu} = 10.78 \).

The contribution of IR8 seems to be diluted in most of its derivatives. The phenotypic distance in comparison with other varieties varied from 8.40 to 13.25 with an average distance coefficient of 9.24. IR8 derivatives (mainly IR24) were involved in high degree of crossing in variety development (De Leon, 1995). Thus IR8 gene complexes were diluted after extensive hybridization in most recently released varieties. Highest dissimilarity of IR8 was observed in comparison with BPI Ri10. IR8 can be traced to its initial parents Cina, Latisail and Dee-geo-woo-gen while BPI

RI10 had Pa Chiam and Seraup Besar in its ancestry.

Most IR varieties extensively shared similar traits with other IR varieties with Euclidean distance coefficients of less than 6.00. IR varieties were patterned to the prototype of IR8. It is expected that the resulting derivatives differ in just few traits. IR8, IR48, IR46, IR60, IR62, and PSB Rc1 were the only varieties bred by IRRI with a considerable amount of unique characters from other IRRI-bred varieties with genetic distance higher than 8.0. PSB Rc1 possessed a different plant type because this was bred for upland condition. IR64, another IRRI-bred variety, showed high degree of phenotypic differences from upland varieties such as C168 and UPLRi5, and from irrigated varieties such as BPI Ri10 and PSB Rc34. PSB Rc26H, the first IRRI-bred released hybrid, had an average Euclidean distance of 7.06. Hybrid characteristics that differ from other improved varieties were mostly agronomic in nature.

PSB Rc14, a rainfed variety bred at UPLB, was one of the newly released varieties with highest degree of phenotypic resemblance with other varieties with a mean distance of \( e_{\mu} = 5.89 \). It had a combination of desirable features such as profused tilling, dark green and erect leaves and sturdy stems. Similarity in traits with other irrigated varieties could be one reason why it is popular among irrigated farmers. C4-63G, one of the most popular varieties, shared similar morphology with other released varieties. It showed high degree of similarity with PSB Rc16, a traditional variety with modern features released for rainfed dry-seeded and with its sister line C4-137 with Euclidean distance value of less than 4.00. Newly released varieties that have low mean phenotypic distances are as follows: PSB Rc4 (6.92), PSB Rc6 (6.32), PSB Rc18 (5.97), PSB Rc20 (6.09), PSB Rc28 (6.28) and PSB Rc32 (6.42). Only PSB Rc34 (Burdagol) showed a different plant type in newly released varieties with a genetic distance of 10.27.

Ecosystem-based distance analysis showed that upland varieties were the most diverse in terms of phenotype compared to the other ecosystem groups. Upland rices consisting of 6 varieties had a mean distance of 8.90 from pair comparisons among them, followed by rainfed lowland rices of 10 varieties with mean distance of 7.55. Irrigated lowland rices of 62 varieties had the lowest mean distance of 7.45 in comparison with each other.

**Cluster Analysis Using Phenogram of Improved Cultivars**

Associations among 78 Philippine improved rice cultivars revealed by UPGMA cluster analysis are presented in Figure 3. Genetic distance estimates based on morphological markers were used for cluster analyses in order to present the genetic relationships as phenogram.
The phenogram revealed that modern varieties were classified into two main clusters. The larger cluster, which was assigned as cluster 1, consisted of 72 varieties accounting for 83\% of the improved varieties. Most of the IR8 and Dee-gee-woo-gen derivatives belong to this group. The smaller cluster, which was assigned as cluster 2, consisted of 6 varieties namely; UPL Ri3, IR46, UPL Ri5, C168, PSB Rc 34 (Burdagol) and BPI 76. Three of the varieties in cluster 2 do not have the semi-dwarfing gene (sd-1). Traits that were distinct in the formation of the 2 groups were apiculus color, stigma color, basal leaf sheath color, blade color, plant height, culm length, collar color and internode color. Cluster 1 had varieties with green apiculus, yellow to white stigma, green basal leaf sheath color, green blade color, green collar color, green internode color and short to intermediate height. Varieties in cluster 2 were characterized by purple apiculus, purple stigma, purple basal leaf sheath, purple line internode, purple leaf margin, purple collar and intermediate height.

The composition of the subgroups in cluster 1 is highly heterogeneous but some distinct clusters with established classification can be discerned. BPI R10, IR30, IR8 and PSB Rc1 formed single cluster each separating from the rest of the varieties in cluster 1. It means that aside from having green internode, green apiculus, green basal leaf sheath and yellow stigma these varieties exhibited other unique characters making them phenotypically divergent. BPI R10 had short stature, early maturity, high tillering ability and short, dark green, broad leaves. IR8 differ in other improved varieties by exhibiting medium maturity, short height, high tillering ability and short, light green narrow leaves. IR30, a derivative of IR8, had early maturity, short height and low tillering ability. PSB Rc 1, an upland cultivar, diverged from the rest of improved varieties by having brown furrow and small density seeds, low tillering and intermediate height. Traditional rainfed lowland rice varieties (PSB RC16, PSB RC38, PSB RC40 and PSB RC36) formed one sub-cluster along with improved varieties (C4-63G, C4137, IR5, IR22, PSB RC24 and PSB RC42) for having intermediate stature, medium maturity and low tillering ability. These varieties had 50 percent of their genes from a traditional or landrace parent. IR58 was closely related morphologically to its parent IR28 by forming another sub-cluster. Sister lines BPI Ri1 and BPI Ri3 with UPL R11 formed another subgroup as they shared the same immediate parent in their pedigree which is Panpet 63. Previously mentioned IR24 and IR45, formed another group as one served as a parent of another.

In-depth Analysis of Genetic Diversity of Improved Varieties - Evaluation of Design Breeding Approach

Results of the morphology-based genetic diversity analysis showed that improved varieties shared a high degree of resemblance in a number of characters evaluated. It has been observed that varieties were found almost similar in some traits that are directly involved in achieving high yield potential and differ in most traits which are indirectly involved or insignificant in achieving higher yield. In the present study, traits that contributed to genetic divergence which resulted to formation of two groups were apiculus color, blade color, internode color, stigma color and auricle color. Low variability in some traits could be attributed to designed-driven breeding approach followed by the different breeding institutions, BPI/PhilRice, UPLB, and IRRI, in developing modern rice for each particular environment. In design-driven approach, few traits were identified believed worthy of attention in a breeding program. These identified traits were used as a basis of selection in segregating generations. Fixation of these traits resulted in the formation of homogeneous phenotypes, which is quite evident in our existing varieties.
Ideotype-breeding was popularized in search for characteristics governing high dry matter production and its transformation to economic yield (Hunt, 1993). Borlaug developed a semi-dwarf high yielding architectural plant type for wheat which became the model in improving cereal-grain crops. IRRI in early 1960’s selected and released a semi-dwarf variety from the cross of Peta and Dee-geo-woo-gen. This semi-dwarf variety, IR8, with high yield potential, displaced many traditional varieties in the farmers’ field and became the prototype model for succeeding rice varieties.

The main thrust of ideotype breeding over the past 35 years has been morphological. This guided the breeders in the process of selecting parents to be hybridized and breeding lines in segregating populations to be advanced. A number of disadvantages were found with the use of this design driven breeding approach (Hunt, 1993). The first major disadvantage is that the concept implies that there is a single superior type for a given climatic or agricultural region. The superiority of single plant type per se is not a problem, the problem comes in when there is a low genetic variability in the traits considered. Frequent utilization of the gene with limited source, as driven by ideotype breeding, resulted in most varieties genetically uniform in that particular locus. A good example is the utilization of semi-dwarfing gene in many breeding programs. Semi-dwarfing gene governing short statureness was found to contribute greatly in producing high yield. Since the identified source of the semi-dwarfing gene came only from Dee-geo-woo-gen, most released varieties from the Philippines had the same copy of the gene via IR8. The second major problem is that limited genetic improvement in most improved varieties has resulted in ideotype breeding. Most selections are patterned after one single plant type, thus varieties to be released are of the same appearance which could only differ in one or few traits. Problem associated to phenotypic resemblance is the plateauing of yield in modern varieties, wherein a narrow genetic base is often suspected.

These problems of yield plateau and genetic uniformity have influenced the breeders to revolutionize the trend in rice breeding by establishing new prototype for higher yield, augmenting the flow of distantly related parents into the breeding and finding new source of important genes with the aid of biotechnology. IRRI is now developing a new plant type for rice best-suited for direct seeding. This new ideotype has low tillering, intermediate stature, dark green leaves, large and long panicles and with high density grains. Breeders of IRRI were able to develop breeding lines for this prototype but which cannot be released for farmer use until year 2000 because they are still in the process of incorporating resistant genes in abiotic and biotic stresses (Khush, 1991). Philippine Rice Research Institute (PhilRice) is also trying to develop a new prototype but this is still in the early phase of breeding process (PhilRice, 1995). In conclusion, the problems of genetic uniformity had been felt by the breeders and they are now exerting effort to increase the diversity in the current and future cultivars.
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LITERATURE CITED


