



**Full Length Article**

# Describing Phenotypic Variability in Seed Shapes of Weedy Rice Types in Comparison to Cultivated and Wild Rice Types Using Elliptic Fourier Analysis

DENNIS A. APUAN, MARK ANTHONY J. TORRES<sup>†</sup>, MADONNA CASIMERO<sup>‡</sup>, LEOCADIO S. SEBASTIAN<sup>‡</sup> AND CESAR G. DEMAYO<sup>1†</sup>

Department of Agricultural Sciences, Xavier University, Cagayan de Oro City, Philippines

<sup>†</sup>Biological Sciences Department, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Philippines

<sup>‡</sup>Philippine Rice Research Institute, Science City of Munoz, Nueva Ecija, Philippines

<sup>1</sup>Corresponding author's e-mail: [cgdemayo@gmail.com](mailto:cgdemayo@gmail.com)

## ABSTRACT

Weedy rice is a serious threat to food security in a global scale. They invade lowland ricefields by having intermediate phenotypes between rice cultivars and its wild type. They grow sympatrically with the cultivars and compete effectively with the crop that often result to excessive yield loss, but unfortunately this pest is difficult to control due to phenotypic resemblance and close genetic relationships with the rice cultivars. The high variability of weedy rice phenotype in the field is suggestive also that it reflect phenotypic relationships to its wild ancestors. In the current study, we explore the phenotypic affinity of weedy rice in the Philippine archipelago using seed shape. The shape is known to have large genetic bases and so its utility in the study is reliable. Using the Geometric Morphometric (GM) tool specifically elliptic Fourier analysis (EFA) and Multivariate Analysis in statistics, we found that 64% of the weedy rice in the archipelago has phenotypic affinity to 13 wild landraces (AA genome) collected from 15 different locations within West Africa, Caribbean Islands, Latin America, India, Australia, South Asia and Southeast Asia. Ten populations have affinity to *O. meyeriana* (GG genome) in the Philippines and Malaysia. Both weedy populations from Misamis Oriental, Philippines (WRMIS1) and Nueva Ecija, Philippines (WRNE2) have affinity to PsBRc 64 and PSBRc 82, respectively while two populations from Iloilo, Philippines (WRILO1 & WRILO2) have affinity to *O. latifolia* in Costa Rica. Overall results display a complex pattern of phenotypic affinity, thus suggesting multiple origins of weedy rices in the Philippines. © 2011 Friends Science Publishers

**Key Words:** Describing phenotypic variability; Cultivated; Wild rice; Elliptic fourier analysis

## INTRODUCTION

Weedy rice has become a serious problem in the ricefield in a global scale (Ferrero *et al.*, 1999; Mortimer *et al.*, 2000; Cao *et al.*, 2006; Delouche, 2007). In the Philippines, infestation rate is from 1% to 48% (Baltazar & Janiya, 2000). Yield reduction in rice reaches up to 80% (Smith, 1988). This pest is said to be associated to commercial rice varieties planted by direct seeding and broadcasting techniques (Sato, 2000; Delouche *et al.*, 2007). Generally, its form is intermediate between cultivated and the wild types (Groot, 2003; Cao *et al.*, 2006; Vaughan, 2008). In a study that attempted to draw the relationships of weedy rice with the wild types, only the *Oryza rufipogon* and *O. nivara* have so far are used most often as representative wild types to be compared to other wild rice types reported to be weedy in various geographical locations such as *Oryza glaberrima*, *O. barthii*, *O. punctata* and *O.*

*longistaminata* in Africa (Delouche *et al.*, 2007); as well as *O. officinalis* and *O. meyeriana* in China (Sato *et al.*, 2000). Comparison has always been done qualitatively on the gross morphology of the rice plant such as the use of agronomic characters (Federici *et al.*, 2002; Delouche *et al.*, 2007), but not very successful. For example, in Costa Rica, Arrieta (2004) used agronomic characters to infer relationships of weedy rice with the commercial rice varieties and its wild type, but failed to detect small variations especially in shapes of the seeds.

Structural shape of organisms has a large genetic basis (Yoshioka *et al.*, 2004). It is a quantitative character involving many genes, thus variation in shape reflects underlying population genetic structure (Garnier *et al.*, 2005). The utilization of a shape character in determining phenotypic affinity therefore apparently has large basis and high reliability. The shape of seeds of weedy rice are difficult to distinguish and essentially impossible to separate

especially those that mimic or too similar to the cultivated varieties. Qualitative descriptions based on the presence or absence of the awn and colors of the awn, husk, apiculi, sterile lemma and palea were not enough to have a clear discrimination of the species groups. Minimal attention if any has been in quantifying variations in the shape of rice seeds. It is for this reason that understanding how shapes of weedy rice differ from those wild and cultivated types, reliable methods are needed.

With advances in imaging techniques, computer technology, biology and statistics, the descriptions of shapes have become more quantitative in nature. Geometric Morphometrics (GM) is a combination of arrays of methods that has the capability to detect quantitatively variations in shapes. GM technique allows the collection, exploration and quantitative study of morphological shapes of objects (Bookstein, 1991; Marcus *et al.*, 1993; Lele & Richtsmeier, 2001; Zelditch *et al.*, 2004). It differs from traditional morphometrics, which is based on distances, distance ratios, angles etc. since it uses the overall geometry of an object throughout the entire analysis and permits accurate statistical analysis of shapes (McPeck *et al.*, 2008; Mitteroecker & Gunz, 2009; Drake *et al.*, 2010). Most results from the analyses can be visualized as shape changes and interpreted anatomically. This technique has experienced a revolution in the past three decades and is now frequently used to solve questions regarding evolution of complex phenotypes in very diverse organisms (Lawing & Polly, 2009; Mitteroecker & Gunz, 2009; Schaefer & Bookstein, 2009). In plants, GM is successfully used in detecting variations of petal shape of *Primula sielboldii* (Yoshioka *et al.*, 2004), deciphering the fruit type evolution in Bornean *Lithocarpus* (Fagaceae) (Cannon & Manos, 2001), selected wild rice species (Jaranilla *et al.*, 2008) to infer their numerical taxonomy. In the current study, GM analysis was applied to quantitatively describe variations in seed shapes between the weedy rice types and those cultivated and wild species and varieties to answer biological questions involving the phenotypic affinity of weedy rice types with other rice species and varieties. Understanding the phenotypic affinity of weedy rice, when compared to wild and cultivated rice species and varieties will help in the understanding of the possible origin and evolution of this important pest in the rice agroecosystem.

## MATERIALS AND METHODS

A collection of accessions was established for common rice cultivars, wild type rice and the Philippine weedy rice. The wild type rice accessions were obtained from the Germplasm Resource Center (GRC) of International Rice Research Institute (IRRI) (Tables I & II). The weedy rice accessions collected from Luzon and Visayas in the Philippines including the cultivated PSB Rc rice cultivars were obtained from the Philippine Rice Research Institute (PhilRice) (Table III), while those from

Mindanao were obtained through field sampling from the provinces of Zamboanga del Sur, Bukidnon, Misamis Oriental, Agusan del Sur, Agusan del Norte and Surigao del Sur (Table III).

Quantification of weedy rice seed shape was studied using outline analysis based on the elliptic Fourier transform (EFT) method. A total of 7518 seeds from the 99 different accessions were scanned at 2400 dpi using scanjet 2400 scanner. Images of these seeds were digitized by putting a series of 100 equally spaced points along the margin of the curve using the tpsDig ver. 2.05 developed by Rohlf (2005). This procedure generated a total of 1,503,600 Cartesian (x & y) outline coordinates, which was used as data in elliptic Fourier analysis (EFA). The shape of every seed sample was approximated by the first 30 harmonics generated by EFA called Fourier co-efficients, which become the new shape variables. EFA generated a total of 225,540 Fourier co-efficients. Principal component analysis (PCA) of the Fourier co-efficient was done using the Paleontological Statistics software (PAST) ver. 2.03 (Hammer *et al.*, 2001). The eigenvectors and eigenvalues based on variance-covariance matrix of these co-efficients were estimated in PCA and the principal components (PC) contributing large variations were determined using the scree plot (Field, 2005). The Principal Component scores of the samples were used to construct a scatter plot to visualize shape differentiation among accessions.

The non-parametric Kruskal-Wallis test of the PC scores as data sets was done to determine if there are significant differences in shapes among the accessions comprising rice cultivars, wild type and weedy rice. Boxplots were also used to have a graphical visualization of the results.

Phenotypic affinity of weedy rice populations to cultivated or wild types was explored by hierarchical cluster analysis of the PCA scores done by using the un-weighted pair-group moving average (UPGMA) algorithm. The dendrogram generated from the groupings show the degree of phenotypic relatedness of the rice types. Bootstrapping with 1000 resample iterations was used in the analysis.

## RESULTS

Principal Component Analysis (PCA) revealed significant variations and differentiations among 36 weedy rice populations in the Philippines including 2 PSBRc cultivars and 61 landraces (accessions of wild species) collected from 31 countries worldwide (Table I). Kruskal-Wallis test based on principal components scores was highly significant [ $H(98) = 5497, p < 0.01$ ].

Variations as described by PC 1 correspond to the length: width ratio of the seeds. A range of this ratio projected by PC 1 (Fig. 1) revealed that all weedy rice populations from Visayas (WRILO's), except WRILO 3, are relatively stubble in shape. The same observations were found in the weedy rice populations in Luzon, Philippines

except for WRNE 4. The weedy rice populations in Mindanao, Philippines were different because majority of them were slender in shape, except for WRAGM1, WRAGM2, WRSUM1 and WRSUM3. Variations in PC 2, corresponds to the curvature of anterior lemma and ventral curvature of the palea. The reconstructed seed shapes are graphically shown in Fig. 1 and 2 revealed that those seeds having positive PC scores were having less curvature than those seeds with negative PC scores.

The distribution of the 99 landraces/populations in the scatter plot based on the principal component scores (PC 1 & PC 2) of mean shapes generally showed 14 group affinities of weedy rice in the Philippines (Fig. 3). Evidently, each weedy rice type was associated to a single or more landraces (accessions of wild type rice) without apparent geographical pattern. For example, weedy rice populations in Nueva Ecija, Philippines (WRNE's) were associated to landraces whose origins were from different world's geographical region. The same observation was found in weedy rice population in the islands of Visayas and Mindanao.

Results of cluster analysis show that weedy rices in the Philippines were associated to 13 landraces of the following species *O. glaberrima*, *O. spontanea*, *O. rufipogon*, *O. barthii*, *O. nivara*, *O. meyeriana*, *O. latifolia*, *O. glumaepatula* and *O. sativa* (Fig. 4), whose origins are from 14 different geographical locations viz. Burkina Faso, Nepal, Africa, Bangladesh, Cameroon, India, Laos, Philippines, Malaysia, Gambia, Costa Rica, Cuba, Mali, Thailand and Papua New Guinea. One of these landraces (*O. latifolia*) belongs to *O. officinalis* complex with the CCDD genome (Federici *et al.*, 2002), while landraces of *O. meyeriana* (GG genome) belong to *O. granulata* complex (Vaughan *et al.*, 2003)

## DISCUSSION

Shape analysis of seeds of weedy rice types from the Philippines revealed morphological differentiation between them and those cultivated and wild rice species and varieties. Several explanations can be offered to this observed differentiation. One glaring result of this study is the close affinity of weedy rice types with the *Oryza sativa* complex. First, the differentiation can be explained based on the Harlan and de Wet (1971) concept of three levels gene pool, the primary gene pool, which comprised the *O. sativa* species complex readily interbreeds and produces fertile hybrids. The second and third gene pools (e.g., CCDD & GG genomes) have limited to extremely rare genetic exchange. Based on this genetic classification of *Oryza*, weedy rices are expected to have close affinity to common rice cultivars and the wild species belonging to the *Oryza sativa* species complex. Results in the current study are consistent with this assumption. Khush (1997) reported that species in the *O. officinalis* complex have limited genetic exchange with the *Oryza sativa* complex having the AA genome and Morishima (1998) in his study showed that *O.*

**Table I: Landraces and origin of wild type rice species with population ID and accession number at the International Rice Genebank Collection (IRGC)**

Population ID	IRGC Acc. No	Origin	Species
ONV(IN)	80432	India	<i>O. nivara</i> (done)
OOF(MM)	80750	Myanmar	<i>O. officinalis</i> (done)
OLT(FR)	80769	France	<i>O. latifolia</i> (done)
OLT(FR)	80770	France	<i>O. latifolia</i>
OSP(TH)	81970	Thailand	<i>O. spontanea</i>
ORF(AU)	86542	Australia	<i>O. rufipogon</i> (done)
ORF(BD)	88783	Bangladesh	<i>O. rufipogon</i>
ONV(LA)	88814	Lao Peoples Democratic Republic	<i>O. nivara</i>
OMY(PH)	89242	Philippines	<i>O. meyeriana</i>
OMY(PH)	89243	Philippines	<i>O. meyeriana</i>
OMY(PH)	89244	Philippines	<i>O. meyeriana</i>
OLG(MG)	93175	Madagascar	<i>O. longistaminata</i>
OMR(ID)	93265	Indonesia	<i>O. meridionalis</i>
OSP(NP)	93321	Nepal	<i>O. spontanea</i>
WAB(BF)	96848	Upper Volta	WAB 01428
WAB(TD)	96909	Chad	WAB 02158
OPC(TZ)	99576	Tanzania, United Republic of	<i>O. punctata</i>
OLT(CR)	99587	Costa Rica	<i>O. latifolia</i>
OLT(GT)	100165	Guatemala	<i>O. latifolia</i>
OGL(CU)	100184	Cuba	<i>O. glumaepatula</i>
OSP(IN)	100206	India	<i>O. spontanea</i>
OBA(GN)	100223	Guinea	<i>o. barthii</i>
OGB(NG)	100855	Nigeria	C 7608
OGL(BR)	100970	Brazil	<i>O. glumaepatula</i>
OGB(AFRC)	101049	Africa	KONSOVROV
OOF(PH)	101113	Philippines	<i>O. officinalis</i>
OOF(PH)	101137	Philippines	<i>O. officinalis</i>
OMR(AU)	101147	Australia	<i>O. meridionalis</i>
OLG(NG)	101202	Nigeria	<i>O. longistaminata</i>
OLG(BJ)	101205	Benin	<i>O. longistaminata</i>
OLG(CI)	101211	Cote D'Ivoire	<i>O. longistaminata</i>

*meyeriana* will hardly breed and exchange genes with the common rice cultivars.

Results also showed that ten out of 36 field collected weedy rice types (28%) have close affinity to *O. meyeriana* in the Philippines and Malaysia (Figs. 3 & 4). The result is not surprising since landraces of *O. meyeriana* are naturally present in these two adjacent countries (Vaughan, 1994; Qian *et al.*, 2006). Although the natural habitat of the wild landraces of *O. meyeriana* is upland forest, anthropogenic and environmental factors may have brought down these landraces to the lowland through seed dispersal (Gong *et al.*, 2000). The mechanism of genetic drift and natural selection may have produced an ecotype that is well adapted to a disturb lowland. It is also possible that two rarely interbreeding species would grow together sympatrically in lowland agro-ecosystem and the new *O. meyeriana* ecotype may then become weedy rice. It is for this reason that the classification of weedy rice as sub-species of cultivated rice, such as *Oryza sativa* spp. *spontanea* and spp. *fatua* by Vaughan (1989) may be uncertain.

It can be seen also from the results that weedy rice from Misamis Oriental, Philippines (WRMIS1) and Nueva Ecija, Philippines (WRNE2) has affinity to Philippine cultivated rice cultivars PSB Rc64 and PSB Rc82. The result suggests that these weedy populations may have

**Table II: Landraces and origin of wild type rice species with population ID and accession number at the International Rice Genebank Collection (IRGC), The International Rice Research Institute**

Population ID	IRGC Acc. No	Origin	Species
OLG(SL)	101227	Sierra Leon	<i>O. longistaminata</i>
OBA(TD)	101257	Chad	<i>O. barthii</i>
OPC(TZ)	101434	Tanzania, United Republic of	<i>O. punctata</i>
ONV(IN)	101508	India	<i>O. nivara</i>
ONV(IN)	102163	India	<i>O. nivara</i>
OBA(CM)	103591	Cameron	<i>O. barthii</i>
OGI(VE)	103812	Venezuela	<i>O. glumaepatula</i>
OSP(BD)	103826	Bangladesh	<i>O. spontanea</i>
OPC(TZ)	103887	Tanzania, United Republic of	<i>O. punctata</i>
OPC(CM)	104073	Cameron	<i>O. punctata</i>
OMR(AU)	104092	Australia	<i>O. meridionalis</i>
ORF(TH)	104395	Thailand	<i>O. rufipogon</i>
OGI(GM)	104570	Gambia	<i>CG 72 (2)</i>
OMY(MY)	104989	Malaysia	<i>O. meyeriana</i>
OOI(MY)	105086	Malaysia	<i>O. officinalis</i>
OMR(AU)	105305	Australia	<i>O. meridionalis</i>
OLT(SR)	105557	Suriname	<i>O. latifolia</i>
OGI(CO)	105561	Colombia	<i>O. glumaepatula</i>
OMR(ID)	105564	Indonesia	<i>O. meridionalis</i>
OBA(TD)	105606	Chad	<i>O. barthii</i>
OPC(TD)	105607	Chad	<i>O. punctata</i>
OGI(BR)	105670	Brazil	<i>O. glumaepatula</i>
OOI(ID)	105674	Indonesia	<i>O. officinalis</i>
ORF(TH)	105757	Thailand	<i>O. rufipogon</i>
ORF(TH)	105939	Thailand	<i>O. rufipogon</i>
ONV(IN)	106137	India	<i>O. nivara</i>
OSP(ML)	106211	Mali	<i>O. spontanea</i>
OBA(MR)	106291	Mauritania	<i>O. barthii</i>
ORF(KH)	106338	Cambodia	<i>O. rufipogon</i>
OMY(PH)	106474	Philippines	<i>O. meyeriana</i>

evolved in the field through back mutation similar to the observation of Cao *et al.* (2006) in China, where the origin of weedy rice was traced to cultivated rice from Liaoning province. It is also argued that these could be volunteer plants from segregants of degenerating rice cultivars that were continuously planted by the farmers in the rice fields. Zhang *et al.* (2006) has already indicated that segregating populations such as this, usually are highly adaptable to changing environments and thus become weeds. This observation is also commonly observed by rice farmers in the Philippines.

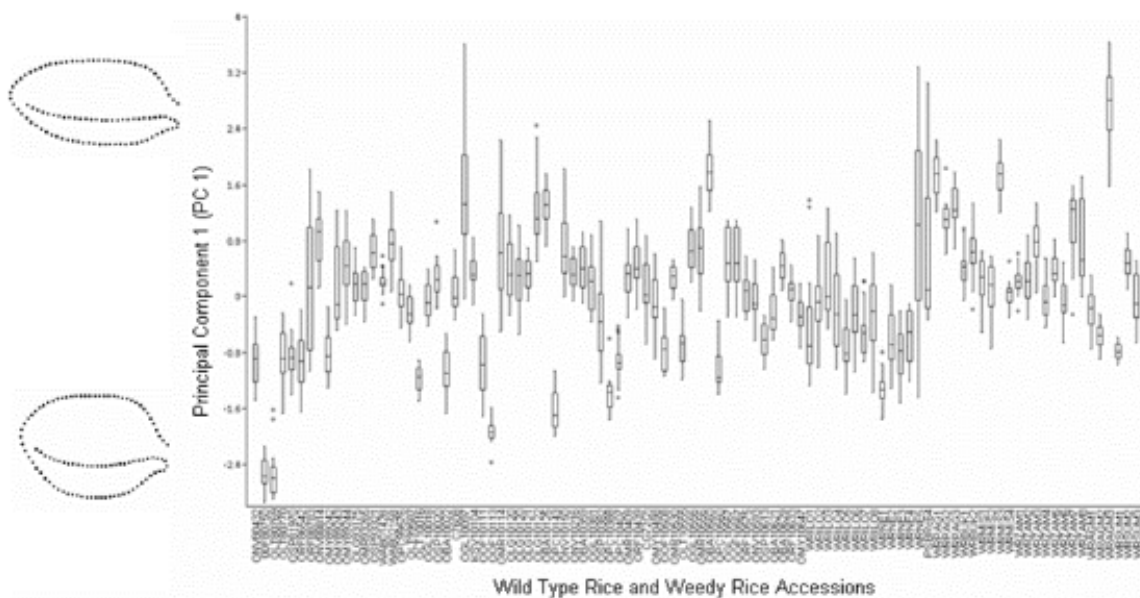
It can be argued also that the weedy rices have originated from rice seeds imported from different rice growing countries. For example, 17% of weedy rice populations in Mindanao, Philippines (Fig. 4, C1) were associated to a breeding line of *O. sativa* cultivar (WAB02158 from Chad) used in developing the cultivated rice NERICA. While, the Philippines had a history of importing seeds, there was no record that it imported seeds of NERICA rice from West Africa for mass production in Mindanao rice fields. David (2007) reported that the country imported the “Bigante” variety from the Bayer Company in India in 2002 and 2003 and a large volume of seeds from China thus strengthening the argument that weedy rices could have been introduced.

**Table III: Accessions of weedy and cultivated rice types**

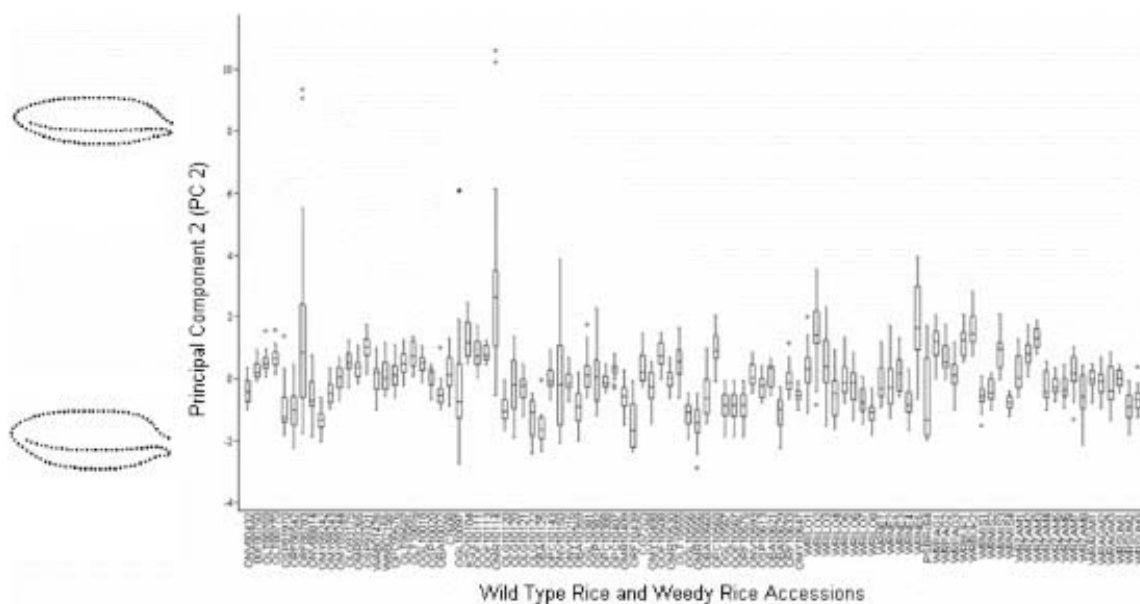
Accessions	Awn (+/-)	Trichome s (+/-)	Awn Color	Husk Color	Apiculi Color	Sterile Lemma/ Palea Color
<b>Weedy Rice</b>						
WRILO1	+	-	straw	straw	ash brown	ivory
WRILO2	-	-	none	straw	straw	ivory
WRILO3	+	+	straw	straw	straw	ivory
WRILO4	+	+	straw	straw	straw	ivory
WRILO5	-	-	none	straw	straw	ivory
WRILO6	+	+	ivory	straw	straw	ivory
WRILO7	-	+	none	straw	straw	ivory
WRILO8	-	-	none	straw	straw	ivory
WRNE1	+	+	ivory	light brown	light brown	ivory
WRNE2	+	+	ivory	straw	straw	ivory
WRNE3	+	-	ash brown	straw	ash brown	ash brown
WRNE4	+	+	ash brown	light brown	ash brown	ivory
WRNE5	+	+	straw	straw	straw	ivory
WRPAG1	-	+	none	straw	ash brown	straw
WRPAG2	-	+	none	straw	ash brown	straw
WRPAG3	-	+	none	straw	ash brown	straw
WRBOK1	-	+	none	straw	ash brown	straw
WRBOK2	-	+	none	straw	ash brown	straw
WRMIS1	+	+	light yellow	straw	straw	light yellow
WRMIS2	-	-	none	straw	straw	light yellow
WRMIS3	-	+	none	straw	straw	light yellow
WRMIS4	+	+	light yellow	straw	straw	light yellow
WRZAM1	-	+	none	straw	straw	straw
WRZAM2	-	+	none	straw	gray	straw
WRZAM3	-	+	none	straw	gray	ivory
WRZAM4	-	+	none	straw	straw	ivory
WRZAM5	-	+	none	straw	gray	ivory
WRZAM6	-	+	none	straw	straw	ivory
WRZAM7	-	+	none	straw	straw	ivory
WRZAM8	-	+	none	straw	ash brown	ivory
WRAGM1	-	+	none	straw	straw	ivory
WRAGM2	-	+	none	straw	straw	ivory
WRAGM3	-	+	none	straw	straw	ivory
WRSUM1	-	+	none	straw	light brown	ivory
WRSUM2	-	+	none	straw	light brown	ivory
WRSUM3	-	+	none	straw	light brown	Ivory
<b>Cultivated Rice</b>						
PSBRc64	-	-	none	straw	straw	straw
PSBRc82	-	-	none	straw	straw	ivory

Results also showed that there are weedy rice types with close phenotypic affinity to more than one landrace (Fig. 4). Weedy rice from Nueva Ecija (WRNE5) for example was found to be of close affinity to *Oryza glaberrima* from Africa and *O. spontanea* from Nepal. The weedy rice type from Agusan Sur (WRAGM1) had affinity to *O. barthii* from Mali, *O. glumaepatula* from Cuba and *O. spontanea* from Bangladesh and many more complex affinity of wild rice types to several wild landraces from several geographical locations. The results could be due to the breeding strategy in producing high yielding varieties (HYV's) of cultivated rice. This strategy of “pyramiding of loci” involves a collection and crossing of several parental ecotypes such that all loci in a quantitative trait like yield are heterozygous or homozygous dominant (Wu, 2009). Thus, newly developed cultivated rice types have genes from different sources including that from wild species (Khush, 1997; Brar & Khush, 2002). The popularly cultivated IR 64

**Fig. 2: Box and whiskers plot showing differences of length to width ratio of the 61 wild rice accessions and 38 weedy rice populations in the Philippines including the 2 PSB Rc rice cultivars**



**Fig. 2: Box and whiskers plot showing differences of curvature at the anterior lemma and ventral palea of the 61 wild rice accessions and 38 weedy rice populations in the Philippines including the 2 PSB Rc rice cultivars**



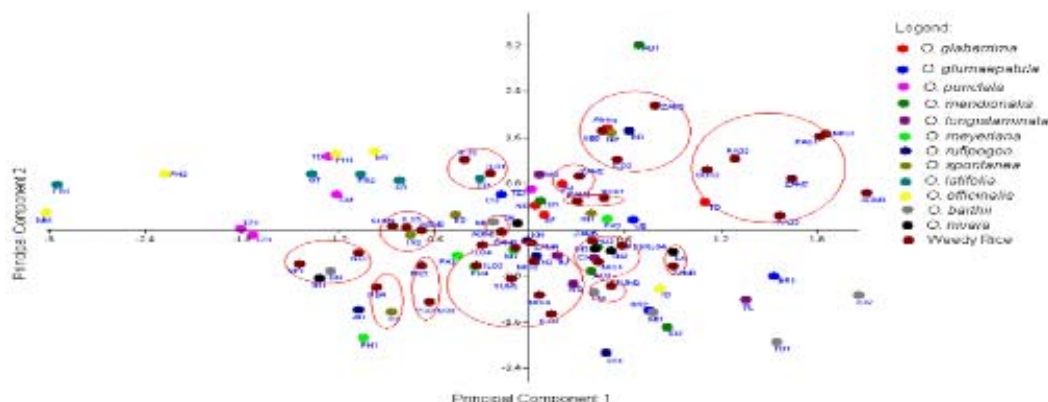
rice for example, has 20 landraces in its ancestry (Khush, 1997). It can therefore be argued that weedy rices evolved from volunteer plants from segregants of degenerating high-yielding varieties (HYV's).

Contamination of seeds from seed exchange or from imported seeds can be sources of weedy rices also. The Philippines is one of the importers of hybrid seeds from countries like China. Impurities during heavy distribution of seeds (David, 2007) are common not only in the Philippines but also in other countries like Vietnam, where 40% of

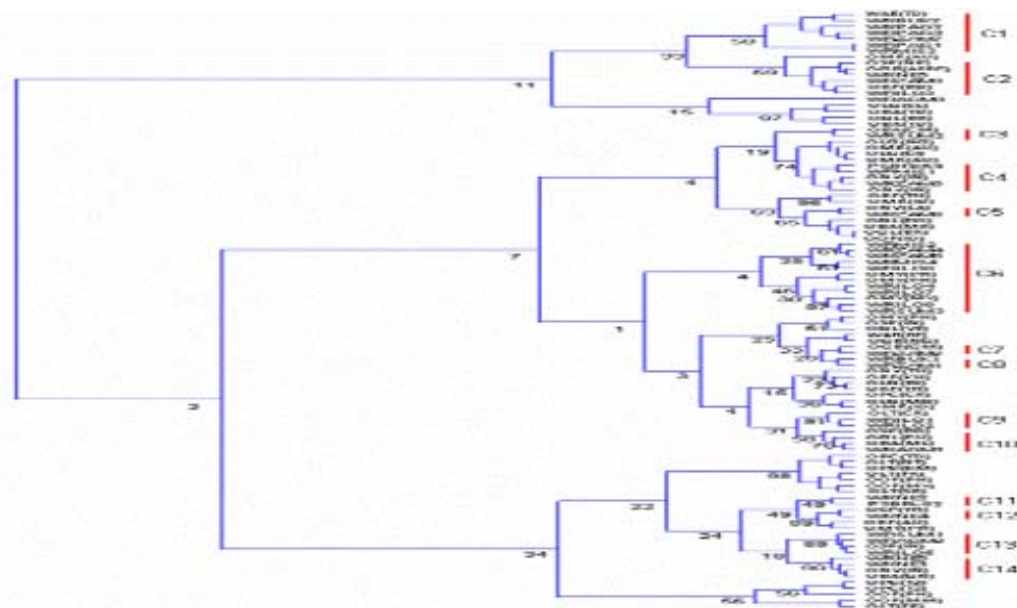
seeds from all the provinces surveyed were contaminated with weedy rice (Mai *et al.*, 2000). The establishment and sympatric growth of contaminants and volunteer segregants in the lowland agro-ecosystem, gave favorable condition for genetic exchange especially in broadcasted and direct seeded system where high density planting results to close proximity among plants allowing high success in gene exchange. This is possible since the reported natural outcrossing rate for rice cultivars ranges from 1.1 to 4% to as much as 8% in wild rice (Delouche, 2007). Niruntrayakul



**Fig. 3:** Distribution of wild and weedy rice based on principal component scores (PC 1 & PC 2) of mean shapes with natural groupings based on Euclidean distance. Labels in international country abbreviations indicate origins: AU-Australia, BD-Bangladesh, BF-Bukina Faso, BJ-Benin, BR-Brazil, CI-Cote D'Ivoire, CM-Cameron, CO-Colombia, CR-Costa Rica, CU-Cuba, FR-France, GM-Gambia, GN-Guinea, GT-Guatemala, ID-Indonesia, IN-India, KH-Cambodia, LA-Laos, MG-Madagascar, ML-Mali, MM-Myanmar, MR-Mauritania, MY-Malaysia, NG-Nigeria, NP-Nepal, PH-Philippines, SL-Sierra Leone, SR-Suriname, TD-Chad, TH-Thailand, TZ-Tanzania, VE-Venezuela. Weedy rice Population in the Philippines origin: NE-Nueva Ecija, ILO-Iloilo, AGM-Agusan del Sur and SUM-Surigao del Sur. Numbers indicate sample/population number



**Fig. 4:** Dendrogram showing phenotypic affinity of weedy rice in the Philippines. WAB and PSBRc- *O. sativa*, ONV- *Oryza nivara*, OOF- *O. officinalis*, OLT- *O. latifolia*, OSP- *O. spontanea*, ORF- *O. rufipogon*, OMY- *O. meyeriana*, OLG- *O. longistaminata*, OMR- *O. meridionalis*, OGB- *O. glaberrima*, OPC- *O. punctata*, OGL- *O. glumaepatula*, OBA- *O. barthii*. Origins are enclosed in parenthesis: AU-Australia, BD-Bangladesh, BF-Bukina Faso, BJ-Benin, BR-Brazil, CI-Cote D'Ivoire, CM-Cameron, CO-Colombia, CR-Costa Rica, CU-Cuba, FR-France, GM-Gambia, GN-Guinea, GT-Guatemala, ID-Indonesia, IN-India, KH-Cambodia, LA-Laos, MG-Madagascar, ML-Mali, MM-Myanmar, MR-Mauritania, MY-Malaysia, NG-Nigeria, NP-Nepal, PH-Philippines, SL-Sierra Leone, SR-Suriname, TD-Chad, TH-Thailand, TZ-Tanzania, VE-Venezuela. Weedy rice Population origin: NE-Nueva Ecija, ILO-Iloilo, AGM-Agusan del Sur and SUM-Surigao del Sur



*et al.* (2009) reported that gene flow between weedy and cultivated rice cultivars reaches up to 52% thus gene flow between rice types with multiple ancestries including segregants or offsprings of seed contaminants may also

explain the complex pattern of phenotypic affinity of weedy rices to cultivated and wild types.

Overall, this study have demonstrated that with limited studies on the shapes of rice seeds shape to allow precise

discrimination between weedy, wild and cultivated types, information generated by the tools of geometric morphometrics specifically the elliptic Fourier method allowed a quantification of the phenotypic diversity of the different rice types and in understanding the phenotypic relationships of this group of rice types to both cultivated and wild relatives. Although there existed other tools such as the application of advanced methods in molecular biology and genetics, the application of GM tools can further strengthen the understanding of the evolution of weedy rice types.

## CONCLUSION

Shape analysis of the seeds of weedy rice types from the Philippines using geometric morphometric analysis reveal a morphological differentiation between them and other cultivated and wild species of rice. Based from the results of this study, weedy rices in the Philippine archipelago may have multiple origins as shown by their phenotypic relationships with other rice types. First, it may have evolved from *O. meyeriana* through drift and selection mechanisms; second, through dispersal of seed contaminants by seed importation or seed exchange; third, volunteer plants from segregants of degenerating high yielding varieties with multiple ancestries and fourth, developed through introgressive hybridization. More studies however, are needed to further test these hypotheses. Breeding experiments, hybridization and genetic evaluation of relationships of the weedy rice types with cultivated, landraces and wild relatives should be undertaken to resolve important issues regarding the evolution of this very invasive pest of the rice agroecosystem.

## REFERENCES

- Apuan, D.A., C.G. Demayo, M.A.J. Torres, M. Casimero and S. Sebastian, 2010. Quantitative evaluation of weedy rice seed shape based on elliptic Fourier descriptor. *In: Proc. Int. Conf. Environ. Computer Sci.*, 4: 391–394
- Arrieta, G., E.L. Sanchez and A.M. Espinoza, 2004. The weedy rice complex in Costa Rica. I. Morphological study of relationships between commercial rice varieties, wild *Oryza* relatives and weedy types. *Genetic Res. Crop Evol.*, 52: 575–587
- Baltazar, A.M. and J.D. Janiya, 2000. Weedy Rice in the Philippines. *In: Baki, B.B., D.V. Chin and M. Mortimer (eds.), Wild and Weedy Rice in Rice Ecosystems in Asia a Review*, pp: 75–76. Limited Proceedings No. 2, Los Banos, Philippines: International Rice Research Institute
- Brar, D.S. and G.S. Khush, 2002. Transferring genes from wild species into rice. *In: Kang, M.S. (ed.), Quantitative Genetics, Genomics and Plant Breeding*, pp: 197–217. Wallingford, CAB International, UK
- Cannon, C.H. and P.S. Manos, 2001. Combining and comparing morphometric shape descriptors with a molecular phylogeny: the case of fruit type evolution in Bornean *Lithocarpus* (Fagaceae). *Syst. Biol.*, 50: 860–880
- Cao, Q., B.R. Lu, H. Xia, J. Rong, F. Sala, A. Spada and F. Grassi, 2006. Genetic diversity and origin of weedy rice (*Oryza sativa* f. *spontanea*) populations found in North-eastern China revealed by simple sequence repeat (SSR) markers. *Annals Bot.*, 98: 1241–1252
- Chase, K., D.R. Carrier, F.R. Adler, T. Jarvik, E.A. Ostrander, T.D. Lorentzen and K.G. Lark, 2002. Genetic basis for systems of skeletal quantitative traits: principal component analysis of the canid skeleton. *Genetics*, 99: 9930–9935
- COGEM Research. *University of Amsterdam and Plant Research International*, pp: 1–55. Amsterdam
- David, C.C., 2007. *The Philippine Hybrid Rice Program: a Case for Redesign and Scaling Down*, pp: 1–60. Philippine Institute for Development Studies (PIDS). Discussion Paper Series, Makati, Philippines
- Delouche, J.C., 2007. *Weedy Rices – Origin, Biology, Ecology and Control*, *FAO Plant Production and Protection Paper 188*, pp: 1–155. Food and Agriculture Organization, Rome, Italy
- Federici, M., A.B. Shcherban, F. Capdevielle, M. Francis and D.A. Vaughan, 2002. Analysis of genetic diversity in the *Oryza officinalis* complex. *Electronic J. Biotechnol.*, 5: 173–181
- Ferrero, A., F. Vidotto, P. Balsari and G. Airoldi, 1999. Mechanical and chemical control of red rice (*Oryza sativa* L. Var. *sylvatica*) in rice (*Oryza sativa* L.) per-planting. *Crop Prot.*, 18: 245–251
- Field, A., 2005. *Discovering Statistics using SPSS*, pp: 632–634. Sage Publication, London
- Garnier, S., P. Alibert, P. Audiot, B. Prieur and J.Y. Rasplus, 2004. Isolation by distance and sharp discontinuities in gene frequencies: implications for the phylogeography of an alpine insect species, *Carabus solieri*. *Mol. Ecol.*, 13: 1883–1897
- Garnier, S., F.M. Jannin, J.Y. Rasplus and P. Alibert, 2005. When morphometry meets genetics: inferring the phylogeography of *Carabus solieri* using Fourier analysis of pronotum and male genitalia. *J. Evol. Biol.*, 18: 269–280
- Gong, Y., T. Borromeo and B.R. Lu, 2000. A biosystematic study of the *Oryza meyeriana* complex (Poaceae). *Plant Sys. Evol.*, 224: 139–151
- Groot, M.H.M., 2003. *Hybridization and Introgression between Crops and Wild Relatives*.
- Hammer, O., D.A.T. Harper and P.D. Ryan, 2001. PAST: Palaeontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4: 9
- Harlan, J.R. and J.M.J. de Wet, 1971. Toward a rational classification of cultivated plants. *Taxon*, 20: 509–517
- Jaranilla, L.G.F., M.A.J. Torres, C.G. Demayo and M.E.B. Naredo, 2008. Outline analysis of rice seeds (*Oryza* spp.) and its potential application in the numerical taxonomy and identification of wild rice varieties. *J. Nature Studies*, 7: 227–240
- Khush, G.S., 1997. Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biol.*, 35: 25–34
- Mai, V., H.V. Chien, A. Vo Van, V. Thi, T. Su Ong and L.V. Thiet, 2000. Rice seed contamination in Vietnam. *In: Baki, B.B., D.V. Chin and M. Mortimer (eds.), Wild and Weedy Rice in Rice Ecosystems in Asia a Review*, pp: 17–19. Limited Proceedings No. 2, Los Banos, Philippines: International Rice Research Institute
- Morishima, H.O., 1998. Genetic difference between wild and cultivated rice. *Agric. Archaeol.*, 49: 30–35
- Mortimer, M., S. Pandey and C. Piggin, 2000. Weedy rice: approaches to ecological appraisal and implications for research priorities. *In: Baki, B.B., D.V. Chin and M. Mortimer (eds.), Wild and Weedy Rice in Rice Ecosystems in Asia a Review*, pp: 97–105. Limited Proceedings No. 2, Los Banos, Philippines: International Rice Research Institute
- Niruntrayakul, S., B. Rerkasem and S. Jamjod, 2009. Crossability between cultivated rice (*Oryza sativa*) and common wild rice (*O. rufipogon*) and characterization of F1 and F2 populations. *Sci. Asia*, 35: 161–169
- Rohlf, F.J., 2005. *Tpsdig, Digitize Landmarks and Outlines, Version 2.05*. Department of Ecology and Evolution, State University of New York at Stony Brook
- Sato, Y.I., 2000. Origin and evolution of wild, weedy and cultivated rice. *In: Baki, B.B., D.V. Chin and M. Mortimer (eds.), Wild and Weedy Rice in Rice Ecosystems in Asia a Review*, p: 9. Limited Proceedings No. 2, Los Banos, Philippines: International Rice Research Institute
- Smith, R.J., Jr., 1988. Weed thresholds in southern U.S. rice (*Oryza sativa*). *Weed Technol.*, 2: 232–241

- Valenzuela, N., D.C. Adams, R.M. Bowden and A.C. Gauger, 2004. Geometric morphometric sex estimation for hatchling turtles: a powerful alternative for detecting subtle sexual shape dimorphism. *Copeia*, 4: 735–742
- Vaughan, D., 1994. *The Wild Relatives of Rice: A Genetic Resources Handbook*, pp: 1–101. International Rice Research Institute, Los Banos, Philippines
- Vaughan, D.A., 1989. *The Genus Oryza L.: Current Status of Taxonomy*. International Rice Research Institute, Manila, Philippines
- Vaughan, D.A., 2008. The evolving story of rice evolution. *Plant Sci.*, 174: 394–308
- Vaughan, D.A., H. Morishima and K. Kadowaki, 2003. Diversity in the *Oryza* genus. *Current Opinion in Plant Biol.*, 6: 139–146
- Weber, K., R. Eisman, L. Morey, A. Patty, J. Sparks, M. Tausek and Z.B. Zeng, 1999. An analysis of polygenes affecting wing shape on chromosome 3 in *Drosophila melanogaster*. *Genetics*, 153: 773–786
- Wu, X., 2009. Prospects of developing hybrid rice with super high yield. *Agron. J.*, 101: 688–695
- Yoshioka, Y., H. Iwata, R. Ohsawa and S. Ninomiya, 2004. Analysis of petal shape variation of *primula seiboldii* by elliptic Fourier descriptors and principal component analysis. *Annals Bot.*, 94: 657–664
- Zeng, Z.B., J. Liu, L.F. Stam, C.H. Kao, J.M. Mercer and C.C. Laurie, 2000. Genetic architecture of morphological shape difference between two *Drosophila* Species. *Genetics*, 154: 299–310
- Zhang, W., S.D. Linscombe, E. Webster, S. Tan and J. Orad, 2006. Risk assessment of the transfer of imazethapyr tolerance from Clearfield rice to red rice (*Oryza sativa*). *Euphytica*, 152: 75–86
- McPeck, M.A., L. Shen, J.Z. Torrey and H. Farid, 2008. The tempo and mode of three-dimensional morphological evolution in male reproductive structure. *American Nat.*, 171: E158–E178
- Drake, A.G. and C.P. Klingenberg, 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. *American Nat.*, 175: 289–301
- Bookstein, F.L., 1991. *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press, Cambridge
- Lawing, A.M. and P.D. Polly, 2009. Geometric morphometrics: recent applications to the study of evolution and development. *J. Zool. (Lond)*, 280: 1–7
- Lele, S.R. and J.T. Richtsmeier, 2001. *An Invariant Approach to Statistical Analysis of Shapes*. Chapman and Hall/CRC, Boca Raton, FL
- Marcus, L.F., E. Bello and A. Garci’a-Valdecasas, 1993. *Contributions to Morphometrics*. Monografías del Museo Nacional de Ciencias Naturales 8. Museo Nacional de Ciencias Naturales (CSIC), Madrid
- Mitteroecker, P. and P. Gunz, 2009. Advances in geometric morphometrics. *Evol. Biol.*, 36: 235–247
- Schaefer, K. and F.L. Bookstein, 2009. Does geometric morphometrics serve the need of plasticity research? *J. Biosci.*, 34: 589–599
- Zelditch, M.L., D.L. Swiderski, H.D. Sheets and W.L. Fink, 2004. *Geometric Morphometrics for Biologists: a Primer*. Academic Press, San Diego, California

(Received 11 February 2011; Accepted 21 May 2011)