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Intra-panicle Variation in Seed Shapes of Weedy Rice

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Abstract: Weedy rice as one of the serious pest in the ricefield evolved as an intermediate form between the common rice cultivars and wild type rice by possessing back its feral characters such as early shattering of grains and dormancy. As member of the genus *Oryza*, it shared many common characteristics. One of these is the canalized pattern of seed differences along the length of the panicle, especially seed size which was reported to be the result of asymmetric resource allocation brought about by hormone polarity that dictates gradient of sink strength. Seed size was known to be correlated to seed shape, however information regarding seed shape differences in the panicle is lacking. In the current study, we hypothesized that intra-panicle variation of seed shape is present. Using Geometric Morphometric (GM) tool and multivariate statistics, we found significant shape differences between seeds at the distal end of the panicle and the combined seeds of the mid-panicle and seeds at the proximal end. This result was consistent and parallel to previous studies on seed size differences along the panicle length. Results were discussed in an evolutionary context, highlighting the observations as a canalized trait advantageous for weedy rice to enhance reproductive success and optimize fitness. Such trait is argued to facilitate its colonization, establishment and spread in the ricefield.

Key words: weedy rice, Oryza, geometric morphometrics

INTRODUCTION

The cryptic invasion and competitive power of weedy rice in the field remain enigmatic. Similarity in morphology of the weedy rice to the cultivated rice varieties could serve to conceal itself during invasion, particularly using the unit of dispersal – the seeds. The shape, size and weight of the seeds are important characters in rice grains that relate to quality and yield (Fitzgerald *et al*., 2008). Seed size and weight are highly correlated characters related to yield, but these two variables exhibit variations within the panicle due to differential grain filling between superior and inferior spikelets (Rajala and Sainio, 2004; Yang *et al*., 2006). Seed size and shape on the other hand are traits related to rice quality (Bai *et al*., 2010; Fitzgerald *et al*., 2008, Khan *et al*., 2009 and Ferrero, 2004). Since these two traits are correlated (Berge and Penin, 2004), question immediately arises whether there could be variation of seed shape along gradient of early flowering spikelets and late flowering spikelets from the distal to proximal end of the panicle. Secondly, how such variation is viewed in evolutionary context in relation to fitness enhancement and competitive ability?

The variation of seed size and weight within panicle was elaborated to some detail in physiological terms by a number of workers (Ahmed *et al*., 2008; Murchie *et al.,* 2002; Sadras and Egli, 2008; Wu *et al*., 2008; Yang *et al*., 2006). Superior spikelets at the distal part of the panicle, which flower early during development often develop large and heavy grains compared to inferior spikelets at the proximal end of the panicle. Inferior spikelets usually produce small and light weight grains and sometimes having unfilled grains. Networks of hormones and their interactions are reported to be responsible for the differential grain filling and variations in size (Wu *et al*., 2008; Yang *et al*., 2006). Sadras and Denison (2009) invoked the concept of sink-sink competition to explain such variation, and Wu *et al*. (2008) reported that levels of hormones in different locations of the panicle are responsible for local mobilization of carbohydrates. Given these physiological dynamics which explain variation in size within the panicle, we hypothesize that shape as well must vary as

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affected by these same physiological interactions considering that size is known to correlate with shape. Furthermore, information regarding seed shape variation within the panicle is lacking.

While there are strong physiological supports to explain intra-panicle variation of seeds size, there is also paucity of information relative to seed shape particularly the implication of shape variation to fitness and invasion of weedy rice into the field. One of the enigmatic aspects of weedy rice invasion is how they optimize fitness and gain competitive power over the common cultivars of *Oryza sativa*.

In the current study, we explore such variation by combining geometric outline of the seeds with multivariate statistics and attempt to interpret the results in evolutionary context especially related to the reproductive success of weedy rice. The ability to produce more offspring and gain foothold in the field is in itself a competitive advantage of the weeds. Although at the cursory inspection on seed shape of the *Oryza* group, seeds may not be distinguished due to seemingly slight differences but its variation can be detected and measured using geometric morphometric tool (Klingenberg and Monteiro, 2005; Landlade et al., 2005).

Here, we show that intra-panicle variation in seed shapes of weedy rice exist, and much of these variations are attributed to the length:width ratio. Seeds in the upper portion of the panicle have different shapes compared to the middle and lower portions. This pattern of variations seems to correspond to the reported variation of seed size in rice, in which seed size in the superior spikelets is significantly different from that in the inferior spikelets within the panicle. And such pattern of variation may be used effectively as a mode of invasion.

MATERIALS AND METHODS

Weedy rice samples were collected from four different locations in the Caraga region in Mindanao, Philippines: 2 municipal locations in Agusan del Sur and 2 municipal locations in Surigao del Sur provinces. A total of 3513 seed samples were collected, air dried and scanned at 2400 dpi resolution using hp scanjet scanner (Fig. 1). Each of these seeds was then digitized by locating a series of 100 points along the margin of the curve using the tpsDig software ver. 2.05 of Rohlf (2002, 2006). The Outline data obtained in the form of 100 consecutive x and y cartesian coordinates were then exported to the matrix of PAST (Paleontological Statistics) software ver.2.03 (Hammer *et al.* 2001). Using this software, Elliptic Fourier Analysis (EFA) was done to transform Cartesian coordinates into new shape descriptors.

The new shape descriptors were then summarized by Principal Component Analysis (PCA) based on the variance-covariance matrix. Amount of variation in seed shapes was determined through PCA, and identification of the new variable contributing large variation was through the use of scree plot as described by Field (2005). Kruskall-Wallis test was implemented to determine significant differences in shapes using SPSS ver. 17. The scores of Principal Component 1 were used to construct box and whisker's plot to visualize differences in shapes.

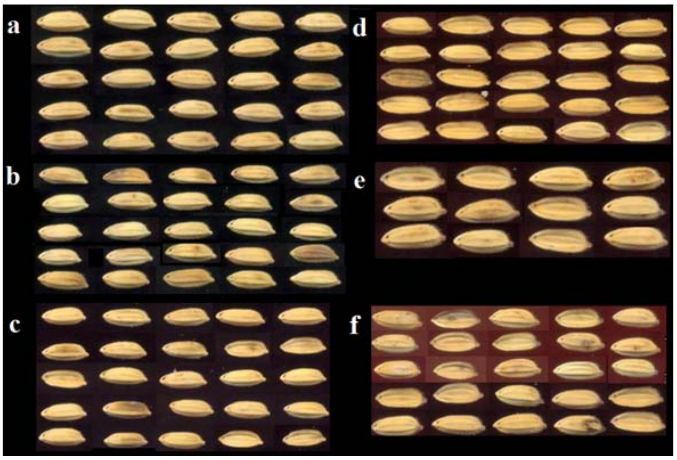

Fig. 1: Representative seed samples of weedy rice collected from Agusan del Sur and Surigao del Sur, Philippines. (a) AGD, (b) AGP, (c) AGM (d) SUP, (e) SUD, (f) SUM. (*Note: The first two letters of the sample ID such as AG and SU refers to Agusan Sur and Surigao Sur samples respectively. The last letter refers to seed location in the panicle e.g. P-proximal seeds, M-mid panicle seeds and D-distal seeds).

RESULTS AND DISCUSSION

Elliptic Fourier Analysis (EFA) successfully captured the intra-panicle variation in seed shapes of 3513 seed samples of weedy rice collected from the provinces of Agusan del Sur and Surigao del Sur, Philippines. A total of 351,300 cartesian coordinates of the seed outline were transformed effectively by EFA into new quantitative shape descriptors called elliptic Fourier descriptors. Below is the shortened quantitative Fourier descriptors into which further analysis was based (table 1).

Table 1: The Elliptic Fourier Descriptors as a new quantitative shape descriptors derived based on transformation algorithm of EFA.

Sample ID	size	cosx1	sinx1	cosy1	siny1…...	..…cosy29	siny29	cosx30	sinx30	cosy30	siny30
AGP1	94.694	0.253	-0.645	0.906	0.420	-0.002	0.001	0.001	-0.003	0.000	-0.001
AGP1	98.906	0.280	-0.795	0.866	0.451	-0.001	-0.001	0.007	-0.005	0.000	0.001
AGP1	97.940	0.273	-0.722	0.882	0.451	0.000	0.002	0.003	-0.004	0.000	-0.001
AGP1	103.783	0.174	-0.815	0.846	0.411	0.000	-0.001	0.003	-0.003	0.001	0.002
AGM4	94.408	0.280	-0.628	0.799	0.572	-0.002	-0.002	0.002	0.000	0.000	0.005
AGM4	92.831	0.154	-0.795	0.882	0.378	0.002	0.001	0.003	-0.003	-0.001	0.001
AGM4	101.793	0.239	-0.822	0.788	0.475	-0.001	0.003	0.004	-0.003	-0.001	0.000
AGD1	80.899	0.446	-0.707	0.875	0.474	-0.001	0.005	-0.001	-0.003	-0.002	-0.001
AGD1	100.967	0.310	-0.838	0.821	0.475	-0.001	-0.002	0.007	-0.003	-0.001	0.004
AGD1	100.391	0.438	-0.703	0.798	0.591	-0.003	-0.002	0.007	0.000	-0.003	0.003
AGD1	93.373	0.266	-0.615	0.845	0.520	0.002	0.002	0.001	0.001	-0.003	0.003
SUP6	103.680	0.117	-0.680	0.846	0.450	-0.001	0.000	0.003	-0.003	0.000	-0.001
SUP6	102.475	0.090	-0.784	0.834	0.396	-0.002	0.001	0.003	-0.004	-0.001	-0.001
SUP6	103.905	0.242	-0.779	0.776	0.513	-0.001	0.001	0.006	-0.004	0.002	-0.005
SUP6	103.988	0.067	-0.836	0.806	0.367	-0.002	0.001	0.008	-0.002	0.005	-0.001
SUM7	98.827	0.263	-0.631	0.822	0.543	-0.001	0.000	0.003	-0.003	0.000	0.001
SUM7	104.911	0.193	-0.805	0.769	0.479	0.000	0.001	0.008	0.000	0.000	0.002
SUM7	99.875	0.215	-0.756	0.795	0.503	-0.002	0.002	0.004	-0.002	-0.001	-0.001
SUM7	102.993	0.171	-0.770	0.834	0.446	-0.002	0.002	0.005	-0.003	0.002	-0.002
SUD1	112.140	0.142	-0.692	0.802	0.499	-0.001	-0.001	0.007	0.001	0.000	0.002
SUD1	100.624	0.094	-0.764	0.855	0.390	-0.001	0.001	0.007	-0.002	0.003	-0.002
SUD1	98.450	0.168	-0.762	0.866	0.417	0.002	0.002	0.006	-0.005	-0.002	-0.001
SUD1	93.752	0.204	-0.827	0.844	0.420	-0.001	0.001	0.008	-0.002	0.001	-0.001
SUD1	100.643	0.230	-0.789	0.842	0.455	-0.002	0.001	0.007	-0.002	0.002	0.000

The first two letters of the sample ID such as AG and SU refers to Agusan Sur and Surigao Sur samples respectively. The last letter refers to seed location in the panicle e.g. P-proximal seeds, M-mid panicle seeds and D-distal seeds.

Principal Component Analysis (PCA) of these Elliptic Fourier Descriptors revealed that 97.8% of the total variance was accounted for by the first two principal components (PC 1 and PC 2 in table 2). Eighty five percent (85%) of the variation was due to length:width ratio of the seeds represented by PC1. The second largest variation in seeds (12%) was accounted for by PC 2, and can be attributed to the ventral curvature of the palea and anterior curvature of the lemma. This was deduced based on cartesian coordinates with high component loading on PC2. The y coordinates from these two regions of the seeds (anterior curvature of the lemma and ventral curvature of the palea), were found to have high positive loading on PC2 ranging from 0.080 to 0.081.

Auxin hormone may be responsible for endosperm cell extensibility on these seed regions with high loading, and genes coding for transport molecules mediating auxin efflux, like PIN proteins in the Arabidopsis model (Petrasek *et al*., 2006 and Sauer *et al*. 2006), may be acting on these curvature regions affected by PC 2. Auxin hormone itself as suggested by Sauer *et al*. (2006) acts as polarizing cue via AUX/IAA-ARF (Indole-3-acetic acid-auxin response factor) pathway, linking individual cell polarity with tissue and organ polarity through control of PIN polar targeting- a kind of feedback regulation. Sterol composition of cells has also been indicated by Willemsen *et al*. (2003) to be required for cell growth polarity.

The scattering of seed samples along the axis of principal component 1 (PC 1) shows a dense cluster at the negative side, near and below the zero mean (fig. 1). These are the samples with low length to width ratio, and thus seeds are stubby in shape. Seeds with high length to width ratio are in the positive side of the PC1, thus seeds are slender with less curvature at the anterior lemma.

The amount of variation that PC 1 explained was exceedingly large compared to the other principal components, thus worthy of further examination. We used principal component scores of PC 1 to construct the box and whisker's plot (fig. 2), and it showed that only seeds from the distal end of panicle from samples obtained from Surigao Sur have the highest median PC score value - the median score was above the zero mean. Shape reconstruction of these distal seeds by logging the Y coordinates revealed a slender seed shape.

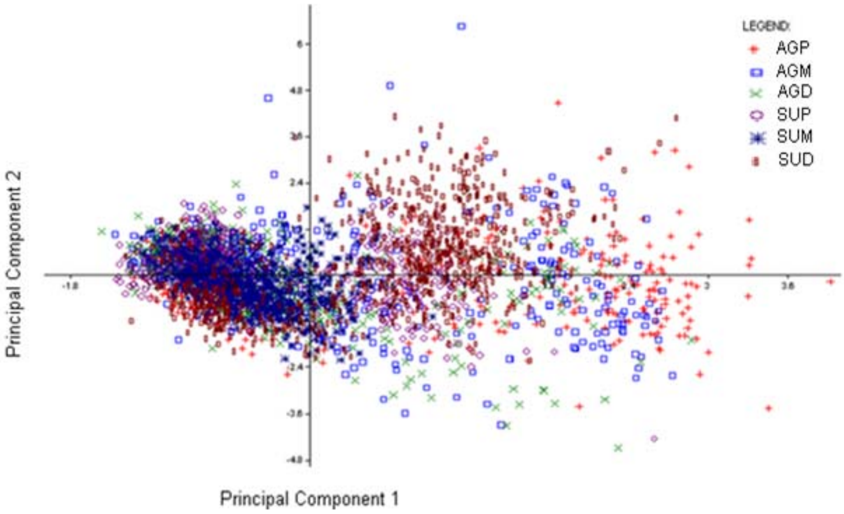

Fig. 1: Scatter diagram of 3513 seed samples taken from the following locations of the panicle: P-proximal, M-mid panicle and D-distal. AG and SU are sources of panicles namely Agusan Sur and Surigao Sur respectively.

The rest of the seed groups with PC score below zero mean, revealed a stubby shape (fig. 2). Notably, all seeds from Agusan def Sur were stubby (with median score below zero mean), but seeds differ in degree of stubbiness from distal to proximal end as exhibited by their mean ranks (table 3).

Unlike PC 1 in which large mean rank was associated to large length:width ratio resulting to slender seeds, the large mean rank in PC 2 on the other hand was associated to less curvature at the anterior lemma and ventral palea. This would mean that large PC 2 score produce small angular projection during growth and development of seeds, thus resulting to a more flattened lemma and palea. Reconciling and summarizing these information contained in PC 1 and PC 2 revealed a general pattern consistent with the one observed by Yang *et al.* (2006), *i.e.* superior spikelets (distal end of panicle) developed into large seeds compared to inferior spikelets (lower end of the panicle).

Kuskall-Wallis test found significant shape differences between distal seeds and mid-panicle and proximal seeds ($P< 0.01$). Mid panicle seeds and proximal seeds however were not significantly different from each other ($P>0.05$). Discriminant function analysis showed that 61.2% of 3513 seed samples were correctly classified ($P< 0.01$), and Cluster analysis corroborates this observation showing two groups: the group of distal seeds and the group of mid panicle and proximal seeds combined. This was supported highly by bootstrap value of 100% based on 1000 iterations (dendogram not shown).

This difference in seed shape from distal to proximal end of the panicle could be due to asymmetric distribution of photosynthates, and this in turn was ascribed to developmental or architectural constraints (Wolfe and Denton, 2001) and hormone polarity (Sauer *et al.*, 2006 and Wu *et al.*, 2008). The differential grain filling and grain filling rate, were documented by a number of workers. Sadras and Egli (2008) have already indicated that grain filling is responsible for the 73% variation in seed size (which is correlated to shape through its allometric effect). Rajala and Sainio (2004) also suggested in their report that grain filing rate differs depending on the position of the grains in the panicle. They found that the uppermost primary floret that was pollinated first also had the highest cell number, and also the ones that tend to produce the largest and heaviest grains. Difference in hormone concentration between superior and inferior grains (or florets) was identified as having direct involvement (Wang *et al.*, 2006 and Yang, *et al.*, 2001).

Yang *et al.* (2006) attributed the differential grain filling between superior and inferior grains to differences in Abscissic acid (ABA) and ethylene ratio, highlighting that high ABA: ethylene ratio at the superior grain promote endosperm cell division. Moreover, brassinosteroids stimulate the flow of assimilates to the dividing and enlarging cells (Wu *et al.*, 2008) at the superior florets and less assimilate flow to the inferior florets having low brassinosteroid concentration, while the cytokinin levels was correlated to grain filling rate (Zhang *et al.*, 2010).

Table 2: Results of principal component analysis showing the first two factors (PC's) contributing large eigenvalue and variance.

Principal Components (PC)	Eigenvalue	% Variance	Cumulative Variance (%)
1	2073710	85.148	85.148

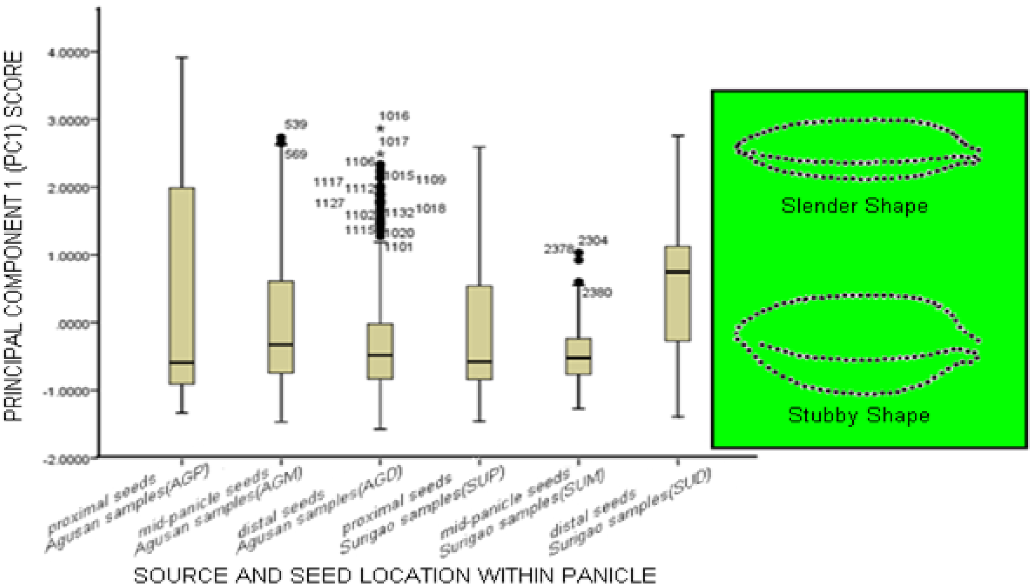

Fig. 2: Box and whiskers plot based on PC 1 scores of seeds from distal to proximal end of panicle collected from Agusan Sur and Surigao Sur Provinces. Seed shapes corresponding to positive and negative PC scores included at the left flank.

Table 3: Mean rank of seed samples identified by sample ID in PC 1 and PC 2 with correspon-ding sample size.

Seed Source	Location within Panicle	Sample ID	Sample Size	Mean Rank PC 1	Mean Rank PC 2
Agusan Sur	Proximal	AGP	438	1808.15	1726.34
	Middle	AGM	533	1805.53	1794.61
	Distal	AGD	531	1506.91	1670.40
Surigao Sur	Proximal	SUP	537	1512.79	1924.41
	Middle	SUM	677	1406.41	1475.41
	Distal	SUD	797	2325.40	1932.79
TOTAL			3513		

This asymmetric distribution of hormones in the panicle, with auxin used as model, was viewed by a number of workers as a canalized pattern (Sauer *et al*., 2006; Lagan *et al*., 2004; Mitchison, 1981 and Sachs, 1981). While canalization in this respect was defined differently, in that it refers to polarity of hormone diffusion (either facilitated diffusion or by polar transport) and such pattern was found to be robust by Lagan *et al*. (2004). This means that it has solid genetic and epigenetic basis that restricts and limits phenotype variation amidst environmental perturbations. This view was still consistent with the evolutionary view of canalization (Siegal and Bergman, 2002).

This canalized pattern of seed differences often accompanies shape changes as a consequence of size differences, here emphasizing our results that distal seeds differ in shape from that of mid panicle and proximal seeds. This variability was brought about by non-isometric growth pattern captured by PC 1 and PC 2. In PC 1 for example, seed length increased more rapidly than the width producing slender seeds (fig. 2). This result deviates from the original scaled-up or isometric allometry elaborated by Shingleton *et al*. (2007); Feng *et al*. (2009) and Sadras and Egli (2008). Our result rather was similar to the one observed by Mazer and Wheelwright (1993), *i.e*. the two directional growth in fruits of *Ocotea tenera* (lauraceae) was not proportional.

On the other hand, the observed differences between seed samples from two provinces (fig. 2 and table 3), can be interpreted as manifestation of genetic variability of weedy rice expressed within limits of the canalized trait of seed shape.

Although weedy rice evolved to become the highly competitive intermediate form between the common rice (*Oryza sativa*) cultivar and the wild type Oryza, the shared canalized pattern of apical dominance in the panicle not only indicate close genetic relatedness with the rice cultivars but were used effectively to enhance reproductive success and optimize fitness. Trade-off must be in place to allow seeds at the distal and apical end of the panicle to gain more resources and increase chances of establishing itself (Zhu *et al*., 1988 and Murty and Murty, 1982) in a highly disturbed agro-ecosystem. Acquiring back the feral characteristics of weedy rice may even facilitate more on colonization, establishment and spread.

Here we conclude that the significant shape differences between distal seeds (superior grains) and mid-panicle and proximal seeds (inferior grains) along panicle length are a consequence of size differences brought about by differential grain filling. The pattern of shape differences is canalized phenotypic trait shared among members of the genus Oryza, and could be advantageous for weedy rice to enhance reproductive success and optimize fitness. Moreover, acquirement back of the feral characteristics such as seed shattering and dormancy could help in the colonization, establishment and spread of weedy rice.

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