



Ingeniería de alimentos



MORPHOMETRIC CHARACTERIZATION OF CHALKINESS IN MEXICAN RICE VARIETIES BY DIGITAL IMAGE ANALYSIS AND MULTIVARIATE DISCRIMINATION

CARACTERIZACIÓN MORFOMÉTRICA DE LA MANCHA OPACA BLANCA (MOB) EN VARIETADES MEXICANAS DE ARROZ MEDIANTE ANÁLISIS DIGITAL DE IMÁGENES Y DISCRIMINACIÓN MULTIVARIADA

G.A. Camelo-Méndez, P.E. Vanegas-Espinoza, A.R. Jiménez-Aparicio,
L.A. Bello-Pérez and A.A. Del Villar-Martínez*

Instituto Politécnico Nacional, CEPROBI, Apartado postal 24 C.P., 62731, Yautepec, Morelos, México.

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Abstract

The opaque spot in the rice endosperm is called chalkiness; this characteristic has been recognized as a grain quality parameter for milling, and it is related to water retention. There is little scientific information about image analysis application (IAA) to characterize chalkiness development as pattern of the rice grain quality. In this work, chalkiness in the transversal section of polished grain of five rice varieties, using different parameters (form factor, fractal dimension concepts, angular second moment, lacunarity, entropy and color) was identified. The multivariate analysis indicated that the studied varieties presented morphometric characteristics that enabled it to be classified with 99.24% of accuracy. The results allowed the grouping by dendrogram analysis in two groups: 1) MorA-92, MorA-98, MorA-06, and 2) MF and MC, distinguishing between varieties, and demonstrating similar morpho-colorimetric chalkiness characteristic patterns between the analyzed rice varieties.

Keywords: Mexican rice cultivars, chalkiness, image analysis application, morpho-colorimetric features, multivariate analysis.

Resumen

La formación opaca en el endospermo del arroz es llamada mancha opaca blanca (MOB) o panza blanca; esta característica ha sido reconocida como parámetro de calidad del grano para procesos de molienda y retención de agua. Existe poca información científica sobre la aplicación del análisis de imágenes (IAA) para caracterizar la formación de la MOB, como patrón de calidad del grano de arroz. En este trabajo se analizó la formación de la MOB en el corte transversal del grano pulido de cinco variedades de arroz con diferentes parámetros (factor de forma, conceptos de dimensión fractal, segundo momento angular, lagunaridad, entropía y color). El análisis multivariado indicó que las variedades estudiadas presentaron características morfométricas que les permitieron ser clasificadas con 99.24% de exactitud. Los resultados fueron agrupados mediante un dendrograma que generó dos grupos: 1) MorA-92, MorA-98, MorA-06, y 2) MF y MC esto permitió distinguir entre las variedades que mostraron similares patrones morfo-colorimétricos característicos de la MOB entre las variedades de arroz estudiadas.

Palabras clave: variedades mexicanas de arroz, mancha opaca blanca, análisis de imágenes, características morfo-colorimétricas, análisis multivariado.

*Corresponding author. E-mail: adelvilarm@ipn.mx

1 Introduction

Rice (*Oryza sativa* L.) is one of the leading food crops in the world and the staple food for more than half of the world's population (Ohtsubo & Nakamura, 2007). In Mexico, rice cultivars are classified in three types, Morelos, Milagro and Sinaloa quality. Morelos grains are long with at least 20% of chalkiness or white belly relative to the total grain size; Milagro grains are short grains with up to 10% chalkiness; Sinaloa are translucent long grains. The most prestigious Mexican-rice type is Morelos due to its physical, milling, cooking and nutritional characteristics, which have been internationally recognized (Hernández & Tavitas, 2005; Chávez-Murillo *et al.*, 2011; Camelo-Méndez *et al.*, 2012; Espinosa-Mendoza *et al.*, 2012).

Chalkiness development is influenced by environmental factors, particularly those that interrupt normal grain filling (Patindol & Wang, 2003) such as insufficient supply of photosynthetic products from source to sink organs (Seo & Chamura, 1979), high temperature during certain stages of grain development (Jia *et al.*, 1992; Candole *et al.*, 2000), rice blast or sheath blight infection (Webb, 1991), harvesting at too high moisture content or at non uniform stages of maturity (Sakai *et al.*, 1996) and physiological maturation during the filling process (Espinosa-Mendoza *et al.*, 2012).

The rice varieties with chalkiness contain individual starch granules mainly, poorly packed, spherical shape, and with air spaces. While in the translucent varieties, starch granules are polygonal in shape and densely packed (Patindol & Wang, 2003). The textural parameters assessment in cooked Mexican rice varieties showed that Mor-A06 presented the highest hardness, and Mor-A96 and Mor-A98 had the highest stickiness (Chávez-Murillo *et al.*, 2011), this quality could be attributed to presence of white belly, this has been consider as a desirable characteristic for quality grain (Hernández & Tavitas, 2005).

Recently, image analysis application (IAA) has been widely used in studies of size, shape and color measurements of rice (Candole *et al.*, 2000; Yadav & Jindal, 2001, 2007; Shimizu *et al.*, 2008; Shen *et al.*, 2009), and its different quality requirements (Jinrose *et al.*, Courtois *et al.*, 2010; van Dalen, 2004; Kim *et al.*, 2000). However, there is not scientific literature that reported the use of IAA to study chalkiness in transverse section of rice. Jinarose *et al.* (2010) developed algorithms that could be used to assess some dimensional parameters

such as the major axis, minor axis, and projected area of rice kernels effectively, suggesting that the color feature could be used to evaluate various kernel defects. Kim *et al.* (2000) studied Korean rice grains; chalky in the kernel was smaller than vitreous area, maximum diameter, minimum diameter, and perimeter because its starchy endosperm had lower amylose content and higher water absorption index than kernel with vitreous endosperm. The aim of this study was to analyze chalkiness morphology of five rice varieties, measuring geometric dimensions (area, perimeter and Feret diameter), shape factors (circularity and compactness), fractal dimension, textural parameters (entropy and angular second moment), color measurements and perform a multivariate analysis, on transverse section of rice grains.

2 Materials and methods

2.1 Samples

Dehulled and polished rice grains (THU-35, Satake Corporation, Hiroshima, Japan and McGill Miller #2, Rapsco, Brookshire, TX) of varieties Morelos A-06 (MorA-06), Morelos A-92 (MorA-92), Morelos A-98 (MorA-98), Milagro Campechano (MC) and Milagro Filipino (MF) were used. They were obtained from the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) in Zacatepec, Morelos, México. Varieties were grown in INIFAP experimental field during 2007 spring/summer crop cycle on clay loam soil at maximum temperature of 33.6°C and minimum of 17°C (Chávez-Murillo *et al.*, 2011; Camelo-Méndez *et al.*, 2012).

2.2 Digital image acquisition

Chalkiness images were acquired on black background, using stereoscopic microscope with 1.0 X objective (SMZ1500; Nikon, Tokio, Japan) and digital camera MTI-3CCD (Nikon, DC330, C-0.35X lens, Japan) connected to interface card (pci flash bus, Photo documentation v3.15) adapted to capture system (V2.3 Metamorph, Universal Imaging Co, USA) on a personal computer (Pentium Centrinium dual processor, 4 GB of RAM). The capture was done maintaining a constant focal distance and standard conditions of light (halogen illuminating 5.100 K). Captured images had of 1024 x 960 pixels (200 dpi) resolution and were stored in color *.tiff format (32 bit) and 0.024 mm/px resolution.

For this study chalkiness image of each cultivar was analyzed, transverse section (1 mm) was made of the axial side, and it was used to determine morphological and colorimetric chalkiness characteristics. Chalkiness images were analyzed with ImageJ program (V2.31, NIHUSA). Initially the images were converted to gray scale (8 bits) and then applied the “Smooth” filter to smooth the object boundaries. Chalkiness formations were binarized (black and white), using the “Threshold” tool, manually adjusted to a range of grayscale, the obtained image was superimposed on the original image.

2.3 Digital image analysis: lineal dimensions, area and morphological features

Morphological characteristics evaluated were based on the following size parameters (Fig. 1A): area, perimeter and Ferets diameter, shape factors (Meraz-Torres *et al.*, 2011; Quintanilla-Carvajal *et al.*, 2011; Tapia-Ochoategui *et al.*, 2011; Zheng *et al.*, 2006; Du & Sun, 2004): circularity (eq. (1)) and compactness (eq. (2)):

$$\text{Circularity} = \frac{\text{Perimeter}^2}{\text{Area}} \quad (1)$$

$$\text{Compactness} = \frac{4\pi\text{Area}}{\text{Perimeter}^2} \quad (2)$$

Angles measurement of MorA-92 and MorA-98 cultivars was performed with ImageJ program. First, binarized chalkiness image was skeletonized with “Skeletonize” tool (Camacho-Díaz *et al.*, 2010), then by means of the plugin “Analyze skeleton (2D/3D)” left and right angle were measured from the vertex or junction to the edge of each side.

2.4 Texture description methods

Fractal dimension of texture (FDT) (entropy and angular second moment) from the chalkiness-grain relation (CGR) (Fig. 1B) was evaluated using the SDBC (Shifting Differential Box Counting) tool of ImageJ software (Espinosa-Mendoza *et al.*, 2012). Fractal dimension (FD) of chalkiness (Fig. 1A) and CGR (Fig. 1B) was evaluated from the stereomicroscope images of the axial view of the grain, using the FracLac 2.5 Release 1b5j tool of the ImageJ program (Camacho-Díaz *et al.*, 2010). The box-counting method, based on the number of boxes

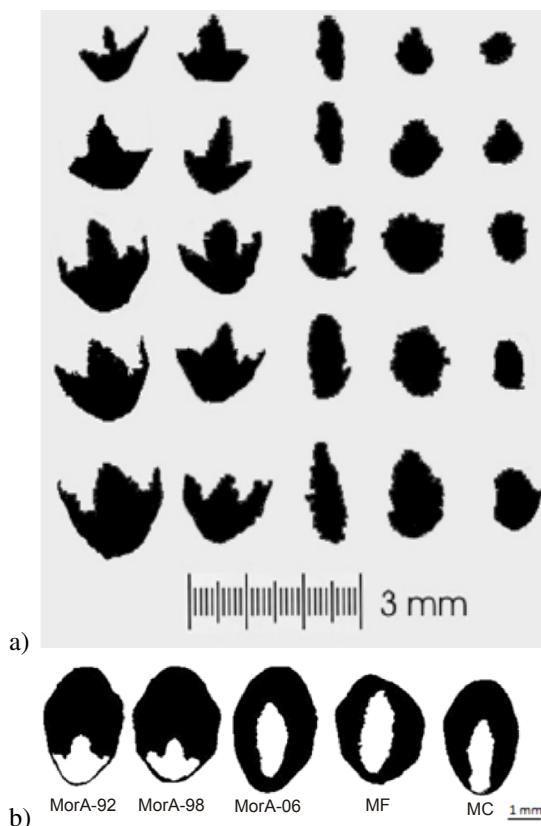


Fig. 1. Binary images of chalkiness formation in Mexican rice cultivars. A. Morphological differences in chalkiness characteristic. B. Chalkiness-grain relation image (CGR).

$N(\varepsilon)$ of size ε required to fill the entire curve (or area) of chalkiness rice seed, was used. Changing the size of ε means that the number of boxes $N(\varepsilon)$ also changes. The smaller the size of ε , the greater the number of boxes, while the larger the size of ε (*i.e.*, resolution), the smaller the number of boxes $N(\varepsilon)$ (Medina *et al.*, 2010). The box-counting method defines fractal dimension (D_f) by Eq. (3):

$$D_f = \frac{\log N(\varepsilon)}{\log \varepsilon} \quad (3)$$

2.5 Color measurement

Color evaluation was performed on chalkiness images using plugin “Color inspector 3D”. Generated histogram was represented at the L^*a^*b coordinates with the minimum number of cells per image.

2.6 Hierarchical analysis and principal component analysis

Hierarchical cluster analysis was performed using Euclidean distances generated by obtained values from the grain side view; results of such analysis were represented by a dendrogram (Medina et al., 2010).

This method was used to analyze the data set of 5 x n average measurements obtained of 5 rice cultivars and n chalkiness and chalkiness-grains relation parameters. We used a standardized data correlation matrix to calculate the eigenvalues (loadings), eigenvectors and principal components related to the original variables. In this study, three principal components were plotted to show and highlight the similar data in a scatter plot on three axes.

For multivariate statistical treatment, standardized data of quantitative seed traits were taken for calculations. Cluster analysis was calculated using Outweighed Pair Group Method with Arithmetic Mean (UPGMA) based on Euclidean similarity matrix defined by the Euclidean distance formula:

$$\text{Euclidean distance } (x, y) = \left\{ \sum_i (x_i - y_i)^2 \right\}^{1/2} \quad (4)$$

Further, three quantitative parameters (CCC, ΔA (0.5) and ΔA (1.0)) evaluated Goodness-of-Fit of hierarchical UPGMA clustering (Hintze, 2007; Wiesnerová & Wiesner, 2008).

- a) CCC (Cophenetic Correlation Coefficient): which is by definition the correlation between original distances of cultivars and those distances that result from the cluster configuration. As a general criterion of Goodness-of-Fit, dendrograms with $CCC \geq 0.750$, are accepted as statistically significant clustering (Hintze, 2007; Wiesnerová & Wiesner, 2008).
- b) ΔA (Delta Coefficient): Goodness-of-Fit of a dendrogram: $\Delta(0.5)$, and $\Delta(1.0)$, Delta Coefficient at $A=0.5$, and $A=1.0$, represent the statistics, which measure the distortion degree of generated clusters in a dendrogram instead of the degree of resemblance (as Cophenetic Correlation). Coefficient ΔA is given by the following formula:

$$\Delta_A = \left[\frac{\sum_{j < k}^N |d_{jk} - d_{jk}^*|^{1/A}}{\sum_{jk}^N (d_{jk}^*)^{1/A}} \right]^A \quad (5)$$

Where A is either $A = 0.5$ or $A = 1.0$, and d_{ij}^* is the distance obtained from cluster configuration in a dendrogram. The closer to zero are the values of ΔA , more significant is the clustering in generated dendrogram (Hintze, 2007; Wiesnerová & Wiesner, 2008).

2.7 Statistical analysis

For this study 70 polished rice grains of each cultivar were used. Geometric dimensions, morphological features and colorimetric results were expressed as means \pm standard deviation (SD). Statistical analysis was performed comparing by ANOVA with a confidence level of 95% ($P > 0.05$) with a multiple comparison Tukey's test (Statgraphics Centurion XVI software).

3 Results and discussion

3.1 Chalkiness morphometric parameters

Descriptors of chalkiness using image analysis measurement are shown in Table 1. The values for Mexican rice cultivars ranged in area (0.96 - 1.62 mm²), perimeter (5.23 - 7.30 mm), Feret diameter (1.46 - 2.25 mm), compactness (0.39 - 0.52) and circularity (25.06 - 37.24). Morelos varieties had higher area and perimeter than Milagro varieties; however, the other parameters did not show a defined pattern. The statistical analysis indicated that MorA-06 chalkiness showed higher values for the morphometric parameters than the other varieties. In addition, two form factors were used for this analysis (compactness and circularity), which indicated that varieties identified as MorA-92 and MorA-98 have high value of compactness and low value of circularity; these results showed that the chalkiness portion occupied in transverse section of different varieties were: MorA-92 (22.59%), MorA-98 (23.48%), MorA-06 (26.61%), MC (23.04%) and MF (18.53%).

Images from chalkiness of five rice cultivars indicated that only two varieties (MorA-92 and MorA-98) showed trilobular shape with characteristic angles (Fig. 1A). Angles from MorA-92 variety were 61.45 ± 14.12 and 64.80 ± 15.59 degrees, and values obtained from MorA-98 were 58.02 ± 15.40 and 58.10 ± 17.04 degrees for left and right angle, respectively. This feature could be used as special recognition pattern of "Morelos" variety.

Table 1. Chalkiness measurement descriptors of Mexican rice cultivars

Cultivar	Area (mm ²)	Perimeter (mm)	D. Feret (mm)	Compactness	Circularity
Mor-A98	1.25±0.38 ^b	5.50±1.17 ^{a,b}	1.62±0.16 ^{a,b}	0.52±0.11 ^d	25.06±0.91 ^a
Mor-A92	1.39±0.46 ^{b,c}	6.34±1.38 ^{b,c,d}	1.80±0.28 ^b	0.43±0.09 ^{a,b}	28.96±30.44 ^b
Mor-A06	1.62±0.82 ^c	7.30±2.40 ^d	2.25±0.59 ^c	0.39±0.14 ^{a,b}	37.24±15.31 ^c
MC	1.15±0.63 ^{a,b}	5.64±2.32 ^{b,c}	1.74±0.65 ^b	0.46±0.14 ^b	29.90±0.68 ^b
MF	0.96±0.70 ^a	5.23±3.05 ^a	1.46±0.61 ^a	0.45±0.15 ^{b,c}	33.58±18.34 ^{b,c}

The results are presented as mean ± SD (n=70). Values with the same letter in each column indicate no significant differences (Tukey's $P < 0.05$).

This is a first approach to implement a technique for pattern recognition in this type of product, avoiding subjectivity (Yokota *et al.*, 1999; Dubey *et al.*, 2006).

3.2 Chalkiness fractal dimension

Surface fractal dimension ($Df_{surface}$) of Mexican rice chalkiness was in range of 1.657-1.679, while $Df_{outline}$ was between of 1.148-1.205. $Df_{surface}$ values of the varieties studied were not significant different ($P < 0.05$). However, $Df_{outline}$ value of Milagro varieties was different to those of Morelos varieties. Similar results were obtained by Medina *et al.* (2010), where fractal dimension was not a parameter to classify seeds of quinoa varieties. However, these parameters have been used for food quality evaluation, being most statistical approaches, including the pixel-value run length method (Du & Sun, 2004). Texture description method of chalkiness-grain relationship (CGR) of five rice varieties were $Df_{surface}$ from 1.67 to 1.73, but MorA-06 and MF were different; lacunarity was from 0.27 to 0.34, which do not show statistical difference ($P > 0.01$), while entropy (0.63-0.67) and 2nd angular moment (0.58-0.61) showed similar values for the rice varieties, indicating that these parameters were not enough for morphometric characterization of these five rice varieties in relation to chalkiness.

3.3 Color measurement

There are few scientific reports of the image analysis application for the colorimetric characterization (CIEL) in polished rice grains (Camelo-Méndez *et al.*, 2012), but there are not reports about the use of IAA to characterize the color of chalkiness. Results indicated that chalkiness showed two main color parameters: L (86), a* (0), b* (0) and L (85), a* (-8), b* (43). Due to the color parameters of the chalkiness, these Mexican varieties can be classified as yellow as reported by Jinorose *et al.* (2010).

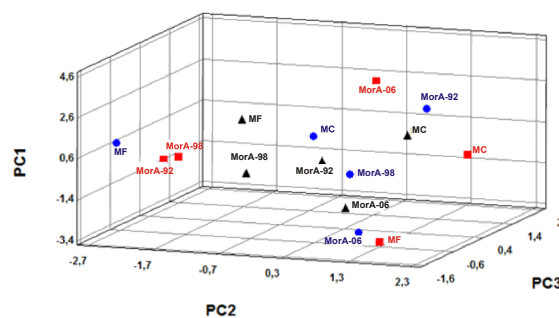


Fig. 2. Graphic matrix results of PC1, PC2, PC3 correlation. Red: all values matrix, Blue: chalkiness matrix, Black: chalkiness-grain relation (CGR) matrix.

3.4 Principal components analysis (PCA)

The principal component analysis (PCA) showed three dimension matrixes of 5 by n average measurements for all analyzed data; chalkiness geometric values, shape factor, and texture description features were determined. The calculated eigenvalues from the total data matrix generated three principal components, indicating that it is possible to describe them with 98.56% and with 98.32% chalkiness matrix. The CGR generated a matrix of two principal components described with 99.24% of the total variation between cultivars for each matrix. The PCA chalkiness results were placed in a scatter plot of three axes for the studied cultivars (Fig. 2). The most sensitive parameters described above are those with values above 0.1 (Medina *et al.*, 2010). Similar values of angles formation between MorA-98 and MorA-92 cultivars in morphometric chalkiness characteristics were observed. Total values are represented in red color, while blue was chalkiness matrix and black represented chalkiness-grain relation (CGR) matrix. This work is a first approach of clustering multivariate morphometric chalkiness characteristics in rice grains based on size, shape and second order features.

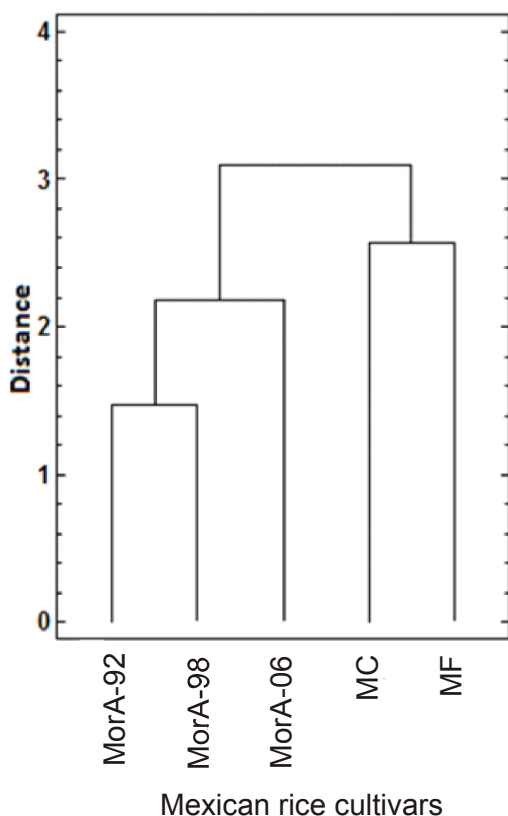


Fig. 3. Texture description of chalkiness-grain relation dendrogram.

3.5 Hierarchical cluster analysis

Cluster analysis of the five Mexican rice cultivars was carried out from matrixes used in the ACP. It was calculated by the Cophenetic Correlation Coefficient (CCC) of the generated dendrograms from the three matrixes by cluster analysis, obtaining values of 0.63 (total values), 0.60 (chalkiness values) and 0.83 (CGR values). The CGR dendrogram presented a value greater than 0.75, which is an acceptable correlation coefficient (Hintze, 2007). The generated CCC dendrogram from transverse section had a correlation coefficient of 0.83 that was supported by low average values of $\Delta 0.5 = 0.10$ and $\Delta 0.1 = 0.15$. The CCC values were higher than those reported for selection of seeds of quinoa (Medina *et al.*, 2010) but lower correlation coefficients than for linseed (Medina *et al.*, 2020; Wiesnerová & Wiesner, 2008). This difference could be attributed to different shape factors used in each study, characteristics of each grain from different cultivars. These results showed that cultivars could be classified according to size and shape of chalkiness. The identification of similarities between cultivars

produced two groups 1) MorA-92, MorA-98, MorA-06 and 2) MF and MC (Fig. 3), indicating that morphometric characteristics of the chalkiness can be used as a distinctive attribute of “Morelos” and “Milagro” varieties.

Conclusions

Morelos varieties presented larger area and perimeter than Milagro varieties; the other descriptors of the chalkiness did not show a defined pattern. MorA-92 and MorA-98) showed trilobular shape with characteristic angles. $Df_{surface}$ values of the Mexican rice varieties were not significant different ($P < 0.05$), but $Df_{outline}$ values of both varieties (Morelos and Milagro) were different. The principal component analysis (PCA) showed that it is possible to describe the chalkiness geometric values, shape factor, and texture description with 98.56% and with 98.32% chalkiness matrix. The hierarchical cluster analysis showed that rice cultivars could be classified according to size and shape of chalkiness. The usability of the technique in industry may represent lower cost, hassle-free with no need of skilled worker and easy to implement for rapid analysis.

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