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SEED SIZE AND ALLOMETRIC RELATIONSHIPS AMONG LOCAL MAIZE VARIETIES IN GALICIA (NW SPAIN)

Óscar GARCÍA TUESTA[1]*, Jessica GROBA[1], Laura PIÑA[1], Seyla GIL[1], Emilio ANDÚJAR[1], Carmela DARRIBA[1], Pablo GONZÁLEZ NOVOA[1], Yaiza SAN MIGUEL[1], Francisco MORA[1,2], Javier MONTALVO[1,3]

[1]The Matrix Foundation, Research and Sustainable Development, Spain
[2]Department of Statistics and Operations Research & [3]Department of Ecology and Animal Biology, University of Vigo, Spain
*Corresponding author: oscargarcia793@gmail.com

ABSTRACT

The AGRIECO research project promotes organic farming with local varieties of maize (*Zea mays* L.). Conservation of agrobiodiversity is important because local varieties show different traits that are valuable for adaptation to climate change. Seed size is a relevant trait for germination and seedling growth, fundamental processes for successful crop production. We analyse the differences in grains of 20 maize local varieties from Galicia (NW Spain), from a seed bank (Phytogenetic Resources Center, CRF-CSIC-INIA) and from local farmers. Multivariate characterization of grain samples from these varieties showed relatively high phenotypic variability in average grain size and other grain physical traits. A measure of grain size, Thousand Kernel Weight (TKW) showed a range of 2.4 times among varieties, from 203 to 497 g, with statistically significant differences between some of them. In addition, TKW showed a 2-fold variation range in the coefficients of variation (CV) among varieties. The average and CV of TKW showed a relatively high negative intervarietal correlation. In addition, intervarietal and intravarietal allometric relationships between grain weight and volume were identified. The results demonstrate evidence of phenotypic differences in reproductive attributes among maize local varieties. They underline the feasibility and importance of a characterization of the components of biodiversity in order to promote their value and agricultural use.

Keywords: *agrobiodiversity, allometry, grain size, Galicia, sustainability*.

INTRODUCTION

Production of maize (*Zea mays* L. subsp. *mays*) is one of the most important worldwide among cereal crops. It is grown for human and animal food, and this crop is also a valuable renewable natural resource for many industrial processing facilities (Zhang et al., 2021; Anuada et al., 2022). Maize was domesticated from a wild grass in southwest Mexico, being a good example of how human selective breeding and seed saving allow for adaptation to agronomic environments (Stitzer & Ross-Ibarra, 2018). Maize moved to Europe, Africa and Asia, and thousands of traditional open-pollinated varieties were developed although nowadays are rapidly being replaced by modern more productive commercial hybrid varieties. Maize is one of the most variable and adaptable species of the Poaceae family. Adaptation in maize means good performance with respect to yield and other agronomic traits in a given environment (Brown et al., 1985; Stitzer & Ross-Ibarra, 2018). Maize crops present high diversity in grain size, form and biochemical composition due to genetic and environmental factors, including the grain location in the cob (Pérez de la Cerda et al., 2007). Maize grain size is a relevant trait for germination, seedling growth and yield (Kara, 2011; Akinyosoye et al., 2014). In the National Inventory of Phytogenetic Resources of Spain there are 2,459 traditional maize varieties, 30% of which come from the region of Galicia, located in the NW of the Iberian Peninsula (CRF-CSIC-INIA, 2020). The coexistence of different local maize varieties within the same region reflects their agronomic adaptation to different environments and uses (Magdaleno-Hernández et al., 2020). Local maize varieties are more tolerant to drought periods, show greater resistance to pests and diseases, or exhibit advantages for traditional processing methods and hence they are generally considered tastier. Risk of maize crop failure in hybrid varieties is higher than in local open-pollinated varieties if conditions are substandard (Andersen et al., 2022). Seed Banks are important facilities for phytogenetic resource conservation of crop varieties, as well as assuring food security. It is paramount that the genetic and phenotypic characteristics of maize varieties are known by the farmers for their conservation and use (Fuentes López, 2008). Global warming is a significant threat to crop yields, causing declines in production, even in the most productive areas. Due to the susceptibility to extreme heat of maize crops, switching varieties is a farmer option of adaptation to climate change (Roberts & Schlenker, 2011). Food needs are expected to double by 2050, and agrobiodiversity helps meeting this demand in a socially and ecologically sustainable way (Kremen and Miles, 2012). Organic farming is associated with greater biodiversity (species richness), probably due to the semi-natural characteristics of this type of production (Wickramasinghe et al., 2003) and the reduced use of chemical fertilizers and pesticides (Paoletti et al., 1992; Hole et al., 2005). Organic farming increases biodiversity by 30% on average compared to conventional farming in temperate climates of the northern hemisphere (Tuck et al., 2014). Besides, organic farming is valuable because more biodiversity implies more resilience to climate change (Arnés et al., 2013). The aim of this paper is to characterize a set of local maize varieties from different localities of Galicia region based on physical variables of grain. This work results from AGRIECO research project, which promotes agrobiodiversity conservation and organic farming using local maize varieties in Northern Spain.

MATERIALS AND METHODS

Grains of 20 local maize varieties from different locations of Galicia region (NW Spain) were characterised in the Applied Ecology Laboratory of the University of Vigo in late spring 2022. Fifteen varieties samples were supplied by a Seed Bank (Phytogenetic Resources Center, CRF-CSIC-INIA) and the rest by local farmers (Fig. 1). The following methodology was used to estimate eight grain physical variables of each maize variety. First, kernels (grains) that were broken, had insect holes or signs of a fungal infection were removed from the samples. Secondly, length (L), width (W) and thickness (T) were measured in a sample of 20 grains of each variety using a digital calliper reading to 0.01 mm. The geometric mean diameter (D_g), surface area (A), volume (V) and sphericity index (Φ) of grain samples were calculated using equations (Karababa & Coskuner, 2007). Thirdly, individual kernel fresh weight was measured in a sample of 100 grains using an analytical balance. Thousand Kernel Weight (TKW) was used for description of grain weight (Wu et al., 2018; Paulsen et al., 2019).

The mean value and the coefficient of variation (CV) of the grain physical variables of each variety were calculated; CV expresses the intravarietal variability. Tukey's test was used for pairwise comparisons of TKW means among varieties.

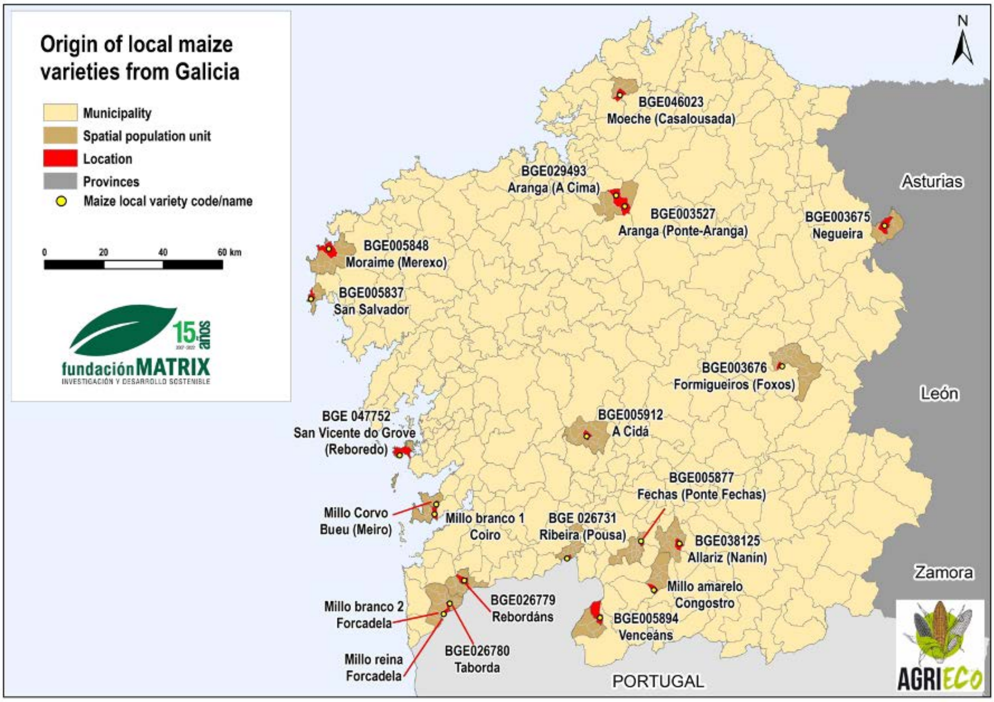

Figure 1. Provenance of 20 local maize varieties of Galicia region (NW of Spain) supplied by CRF-CSIC-INIA (codes are indicated) and local farmers (local name in Galician).

The allometric relationship between TKW and V was examined by a potential regression model (TKW = aV^b) for each variety and for all varieties at the same time. Tukey's test was also used for pairwise comparisons of scaling exponent (b) among varieties. To examine the variability of the local maize varieties according to the mean values of the set of grain physical variables, the scaling exponent, as well as the CV of the physical variables, a Principal Component Analysis (PCA) was used, after data standardisation. An agglomerative hierarchical clustering of varieties was also performed using the same variables, based on the Euclidean distance and Ward's method. Software R (version 4.2.0 Patched) was used for statistical analyses.

RESULTS AND DISCUSSION

Grain weight spectrum of local maize varieties

The grain weight spectrum showed a wide variation range of more than 2.4 times among the 20 maize varieties (Fig. 2). There were statistically significant differences in TKW means between many of them. For example, Millo Reina, with a TKW = 497 g outperforms 19 varieties, and this variety along with five others outperform the TKW of the remaining 14 varieties. On the opposite side, grain weight of the variety BGE003527 was the lowest, TKW = 203 g, significantly less than all the others. The phenotypic differences among varieties in this reproductive trait suggest a wide spectrum of adaptation to local environment.

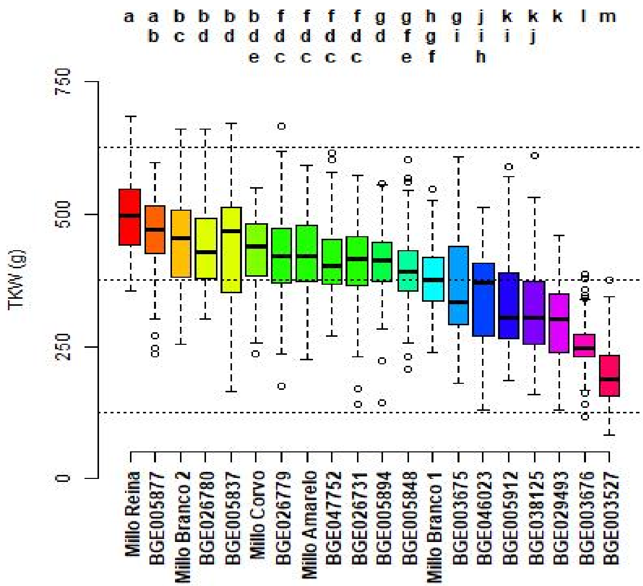

Figure 2. Box-plot of TKW of local maize varieties. Different letters or groups of letters denote statistically significant differences between pairs of varieties ($p<0.05$).

Allometry in maize grain size

Figure 3 plots TKW versus grain volume for 20 maize varieties (20 grains of each variety). An intervarietal negative allometry between both variables was identified. For the whole set of varieties, grain weight scales roughly as the square root power of grain volume rather than isometrically (TKW = $18.5V^{0.549}$; $p<0.01$; $R^2 = 0.44$; N = 400). An explanation could be that grain bulk density decreases among varieties with increasing grain volume (Niklas, 1994).

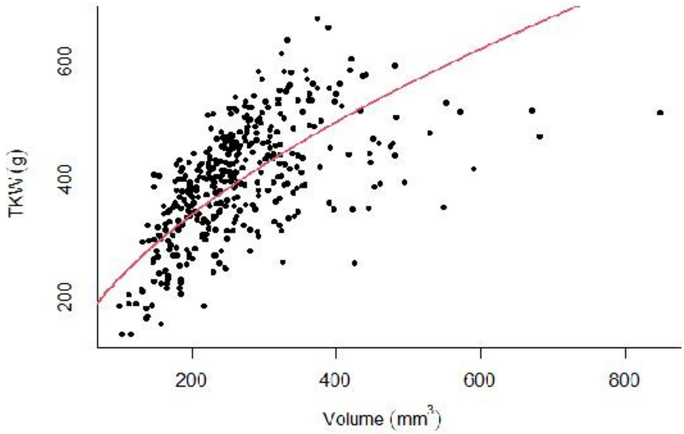

Figure 3. Allometric relationship between TKW and volume (V) of grains of 20 local maize varieties. The line expresses the regression fit to an allometric equation (TKW = aV^b).

The allometric relationship was statistically significant for every variety considered separately (intravarietal allometry; $p<0.01$, except in the variety BGE004858, which is $p<0.05$; R^2 varied between 0.29 and 0.90; N = 20). This size-dependent relationship showed that grain weight grows proportionally less than grain volume in all maize varieties, with a variation range among varieties in scaling exponent from 0.299 to 0.853. However, there were no statistically significant differences between varieties in scaling exponent.

Maize intervarietal and intravarietal allometry in grain weight versus grain volume suggest a relationship between ontogeny of seed size and changes in size among mature individual grains, i.e., between ontogenetic and static allometries (Pélabon et al., 2013). We interpret that pressure of selective breeding in different environments during the development of different maize varieties in Galicia was closely linked to environmental constraints of growth and development of grains and, therefore, their size and shape in mature stage.

Multivariate characterization of maize varieties

The result of the PCA (Fig. 4) shows that two components explain almost 80% of the variability among the 20 varieties based on 9 physical features of grains. Component 1 described the main trend of variation, which explains 57.7% and

arrange the varieties according to numerous grain size traits, including two perpendicular grain dimensions. This trend segregates at the negative end the varieties with lower values of D_g, S, V, L, W and TKW (e.g., the variety BGE003527, with L = 7.71 mm and TKW = 203 g), and at the positive end the varieties with the highest values of these physical variables (e.g., Millo Corvo, with L = 15.67 and TKW = 430 g).
This trend coincides with a climatic geographic gradient associated with continentality, with grain size being smaller in the varieties from inland Galicia, located at a distance of between 20 and 100 km from the coastline and at a higher altitude (200-800 m asl), and larger in the varieties of provenances near the sea (< 20 km) and lower altitude (< 200 m asl).

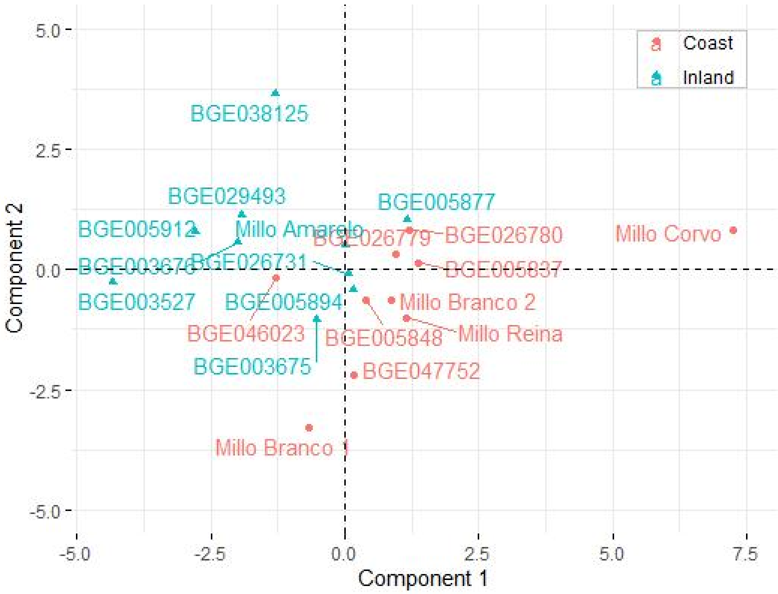

Figure 4. Distribution of local maize varieties in the ordination space defined by the two components of a PCA based on 9 physical traits. Code/name and origin of varieties are indicated (see Fig. 1).

The second trend (Component 2), which is independent of the previous one, explained 21.8% of the variability among maize varieties. It orders them according to two grain traits: thickness and sphericity. The negative end of this Component 2 segregated the varieties with less thickness and sphericity (e.g., Millo Branco 1, with a T = 5.16 mm and Φ = 66%); on the positive end were varieties with coarser, more spherical grains, e.g., the variety BGE038125, with a T = 6.81 mm and Φ = 103%). This secondary trend was not associated with a clear geographic variation pattern.

The scaling exponent did not contribute to explain the variability among varieties, which is consistent with described results on allometry of grain size.
The dendrogram of the classification (Fig. 5) allows us to identify three different groups of maize varieties, that roughly coincide with the groups defined by their origin (Fig. 4), designated as Group 'Inland-M' with 9 varieties of small grains, with an average volume of 228 mm^3 (average L = 9.34 mm); Group 'Coast-L' with 10 varieties of big grains, with an average volume of 276 mm^3 (average L = 10.70 mm), and Group 'Coast-XL', with a single variety from the coast (Millo Corvo) and more voluminous grains than the previous group, with V = 489 mm^3 (L = 15.68 mm).

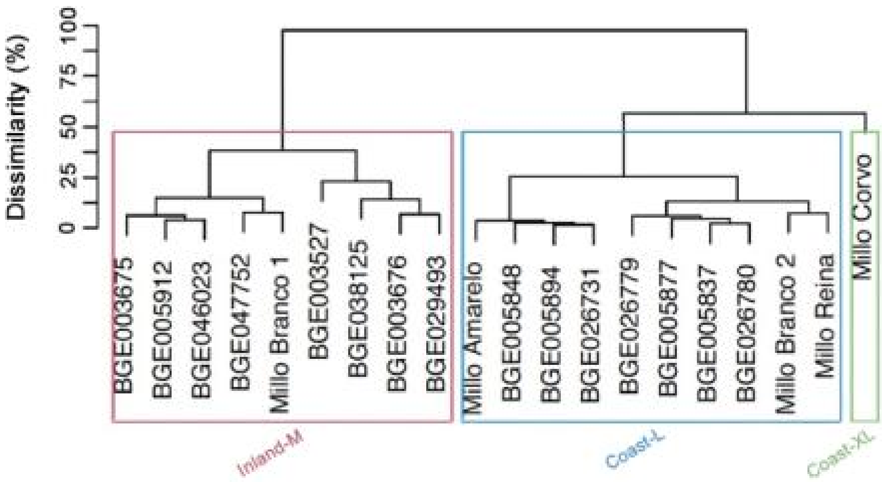

Figure 5. Hierarchical clustering dendrogram of local maize varieties using 9 physical variables of grains at once, which defines three groups: Inland-M (9 varieties), Coast-L (10 varieties) and Coast-XL (1 variety). Code/name of varieties are indicated (see Fig. 1).

The result of a PCA using eight additional traits that correspond to the coefficient of variation of physical variables, which express the intravarietal variability, revealed that varieties with a smaller grain size tended to present greater intravarietal heterogeneity in TKW, D_g, L, S and W, and those varieties with larger grain size showed greater intravarietal uniformity of their grain physical traits. The TKW variable can be highlighted and at the same time illustrate this fact. CV of TKW showed a 2-fold variation range among varieties. The average and CV of TKW showed a relatively high negative intervarietal correlation ($r = -0.66$; $p<0.01$). Phenotypic variance of maize grain traits is attributed to genotypic effects (Chen et al., 2016; Zhu et al., 2018).
Intravarietal differences in grain size dimensions could be related due to their position on the cob, since apical grains are smaller and rounder than others (Pérez

de la Cerda et al., 2007). However, differences in grain size can also be of genetic or environmental origin.

Maize grain size effect on germination is complex. In vitro germination is positively correlated with grain size (Akinyosoye, 2014). Smaller maize grains show faster germination than larger ones in drier soils (Iowa State University, 2020). This is consistent with their higher amylase activity, which is relevant for seed vigour and seedling emergence (Sulewska et al., 2014). The prevalence of small-sized grain in maize varieties from inland areas of Galicia could be interpreted as a selection pressure on this trait of less favourable environmental conditions than in coastal areas.

Within the same maize variety, a higher TKW is associated with lower yield in a temperate climate (Sulewska et al., 2014). Nevertheless, under other climates, lower TKW is associated with lower maize yield and water stress (Çakir, 2004; Kara, 2011; Alemayehu et al., 2017; Anuada et al., 2022). Soil moisture conservation promotes higher TKW (Magdaleno-Hernández et al., 2020). The length of the growing period is greater on the coast –and also at lower altitudes– than in inland locations; the thermal amplitude shows an opposite pattern. These different environmental conditions could allow larger plants with larger grain size on the coast than in inland areas. This suggests a selective value of TKW in the differentiation of local maize varieties across Galicia localities. This hypothesis is consistent with experimental evidence of the effect of growing period length on seed weight in some plants (e.g., Winn & Gross, 1993).

The absence of differences among varieties in the scaling exponent of the relationship between TKW and grain volume suggests an ontogenetic nature of this intervarietal negative allometry. This hypothesis means that grain size spectrum across maize varieties would express a specific adaptation effect (the outcome of environmental differences linked to its geographical provenance) compatible with an environmental modulation of gene expression (the outcome of an interaction between maize variety and its growing environment).

CONCLUSIONS

The study showed phenotypic differences among traditional maize varieties from Galicia in the physical characteristics of the grain. There was a wide variation range in grain weight spectrum, expressed as TKW, of up to 2.4-fold. A multivariate characterization of maize varieties based on grain physical traits allows to identify a variation trend associated to their origin, segregating a group of them from the inland of Galicia, and two groups of varieties from the coast land areas, with smaller and larger grains, respectively. This empirical evidence suggests that continentality determines differences in adaptation to the local environment among traditional maize varieties.

Greater length of growing period and thermal amplitude might be the underlying climatic factors associated to differences in grain size among Galician maize varieties. This hypothesis is also supported by the maize intervarietal and intravarietal allometry, which indicates that grain weight grows proportionally less than grain volume. This fact suggests that development of traditional maize varieties in Galicia was clearly constrained by local environment conditions. Being maize grain size and yield two traits expected to be strongly correlated in Galicia, results highlight the relevance of increasing knowledge, conservation efforts and use of traditional maize varieties in this region to meet the needs of organic farming and challenges of climate change.

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