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REGULAR ARTICLE

# Rice plant pattern as predictor of the milling and cooking quality in breeding programs

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## ABSTRACT

An appropriate characterization of agronomic and quality traits is a fundamental tool for selecting stable genotypes suitable to be registered as new varieties. The objective of this work was to evaluate agronomic and quality traits of rice germplasm tested in two consecutive years for 23 advanced lines of Portuguese Rice Breeding Program and 3 commercial varieties. The influence of genotype, year and their interaction on grain yield and milling yield, grain uniformity and selected quality indicators for rice was assessed as well as the correlations between the agronomic, biometric and quality parameters. Results showed a generally dominant influence of the genotype, but with some quality parameters significantly affected by year conditions, and with some genotypes more stable than others. Some accessions have also shown better grain biometric uniformity within a year and between years. Significant correlations between quality and agronomic parameters that were observed may mark a plant pattern that can be used as predictor of the milling yield. These analyses provide objective tools for selecting most promising genotypes in rice breeding programs.

**Keywords:** Industrial quality; Rice breeding; Screening tests; Stable genotypes

## INTRODUCTION

The world faces huge challenges, such as population expansion, climate change and degradation of environmental resources. Hence, more sustainable rice production systems with improved rice productivity and quality are a main objective for breeders across the globe (Global Rice Science Partnership, 2010).

The effect of climate change decreases rice yield and rice quality, is a challenge to increasing rice production needed to face growing world population (Peng et al., 2004; Welch et al. 2010). There is a constant increase in mean global air temperature by 0.3 to 0.6°C (Nichols et al., 1995). Higher minimum temperature reduces rice yield due to higher loss of carbon through increased respiration (Zisca and Bunce 1998). In opposition, higher maximum temperature has been shown to raise yield (Welch et al., 2010). High temperature will increase chalkiness, causing grains to break during dehulling and polishing, and consequently decreasing the amount of acceptable and marketable polished rice (Fitzgerald and Resurreccion, 2009), opposing any yield gain.

European and global breeding programs are focused on creating varieties able to fit the needs of rice farmers for good agronomic performance resulting on high yield, of industry for high milling yields and uniformity and consistency over the years, and of end consumers for cooking and sensory qualities. The trade liberalization has favored the change in consumption or cultivation of rice *Japonica* subspecies for imported *Indica* subspecies; and, in parallel, an increased demand for exotic types of rice and even for pre-prepared meals (Fernandes and Correia 2012). The trend has been seen in countries like Italy, Spain and Portugal. Portugal has the highest per capita rice consumption in Europe, with 15 kg/inhabitant/year (MADRP, 2007). The global market tends to favor the long-grain varieties *Indica*-type whereas European preferences include consumption and cultivation of *Japonica* varieties

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to cover the demand of traditional dishes like *paellas, arrozes, risottos* or *carolinos* (Calingacion et al. 2014). *Carolino* rices are commercially classified as long grains (type-A) and adapted varieties play a vital role for Portuguese rice chain sustainability (Oliveira et al. 2015). The marketing value of rice depends primarily on its physical integrity and chemical qualities after harvesting as well as consumer acceptance. Genotypes with strong uniformity and stability of grain biometrics, like grain length, also tend to be better considered in the rice market, and their suitability to be registered as new varieties will be greater too when they are evaluated by the DUS (distinctness, uniformity and stability) tests (Subba Rao et al. 2013; Pourabed et al. 2015). Frequency distribution analysis of biometric data can be a good indicator of the uniformity of a given rice genotype.

Several correlations among agronomic and quality traits have been found (Ahmad et al. 2009; Hasnain and Ali 2013; Arulmozhi and Muthuswamy 2013; Lee et al. 2013; Ali et al. 2014). Although they can vary significantly among different authors, depending on the tested varieties and the specificity of each environment, they can be used as indicator for quality preselection of rice populations (Moreira et al. 2014).

The present study was developed in the framework of the Portuguese Rice Breeding Program led by INIAV (National Institute for Agricultural and Veterinary Research) and COTARROZ (Rice Operative and Technological Center, Portugal) to create new adapted varieties with improved agronomic and nutritional traits suitable to local conditions, while also taking into account consumer preferences, an example of a modern breeding program with a whole chain approach from farm to fork.

The objective of the present study was to test new varieties for stability, with successful commercial ones used for comparison, illustrating how the methodology can be used to select advanced lines in breeding programs with a whole chain approach. This implies to ascertain the effect of agronomic characteristics viz. genotype, year and their interaction, on agronomic parameters (field data), grain biometrics (paddy and milled) and its uniformity (size distributions), and quality traits (amylose content and viscosity data) indicative of cooking behavior. Potential correlations between the parameters that could form the basis for predictors of potential yields were also assessed.

## MATERIALS AND METHODS

### Plant materials

Twenty six genotypes of rice (*Oryza sativa* L.) were selected for the present study (Table 1). Within these genotypes, 23 are advanced lines resulting from crosses made in the Portuguese Rice Breeding Program, framing four genotypic clusters and including sister lines. Each breeding advanced line was assigned its unique code. Three internationally available commercial varieties that are successful among Portuguese farmers were also tested for comparison: Ariete, Gladio and Ronaldo.

### Growth conditions

Rice genotypes were grown in field trials during 2012 and 2013 seasons at the same location, on the rice experiment station of COTARROZ, located at Salvaterra de Magos (CO) in Tejo/Sorraia region, in field plots with the same cultivation methods. The genotypes were sown in a randomized block design with three replicate plots per accession. The plots were 8 m length by 1.2 m width, so 9.6 $m^2$. The localization and description of experimental field and crop management conditions are detailed in Table 2.

Sowing was carried out on May 29$^{th}$ in 2012 and May 13$^{th}$ in 2013, with a density of 400 grains per $m^2$; while harvesting took place in October 30$^{th}$ and October 15$^{th}$, respectively. The total nitrogen applied was the same in both years: 115.5 kg N/ha. Also, two herbicide applications were carried out in June in both years. Continuous flooding was the irrigation method chosen.

With respect to environmental conditions in experimental site, the average temperature in rice season for 2012 was 20.1 °C,with a cumulative precipitation of 154.2 mm (Fig. 1a) while in 2013, the average temperature during rice season was 20.2 °C and the precipitation 75.4 mm (Fig. 1b).

### Data collection and measured parameters

The 26 genotypes from the three field replications and two years were characterized by 3 groups of traits: agronomic (including biometry of paddy grain), milling (including biometry of milled kernels) and cooking quality (potential indicators), as summarized in Table 3.

Agronomic and paddy grain biometric traits were chosen as per Yousefnia et al. (2012). TGW was counted on Numigral (Chopin Technologies, France). The mature crop was harvested manually and a sample of 500 g of grain paddy from each genotype was collected. Biometric analyses of the paddy rice grains were evaluated in 50 g by using an automatic S21 apparatus and software (Suzuki, Brasil). The digital images of an average of 700 grains for each sample were analyzed.

The paddy grain biometric uniformity was evaluated by the size distributions of length (pGL) and width (pGW). Each sample of 50 g was divided in 13 classes according to size (length or width), with each size being represented

**Table 1: Genotypes codes and germplasm genealogy**

| No | Genotype code | Genealogy (crossing origin) |
|---|---|---|
| 1 | OP 1001 | Basmati C. 621 x Lido |
| 2 | OP 1004 | Italpatna x Milyang 43 580/99 04BB3 |
| 3 | OP 1101 | Lido x VB 7 |
| 4 | OP 1102 | Lido x VB 7 |
| 5 | OP 1103 | Lido x VB 7 |
| 6 | OP 1104 | Lido x VB 7 |
| 7 | OP 1105 | Safari x VB 7 |
| 8 | OP 1109 | Estrela A x IR 72 |
| 9 | OP 1201 | (Regina x Delta 04BB) X (Estrela A x Suweon 285 x Basmati 621 04BB1) |
| 10 | OP 1202 | (Regina x Delta 04BB) X (Estrela A x Suweon 285 x Basmati 621 04BB1) |
| 11 | OP 1203 | (Regina x Delta 04BB) X (Estrela A x Suweon 285 x Basmati 621 04BB1) |
| 12 | OP 1205 | (Feronio x VB 7) x Zeus |
| 13 | OP 1206 | (Feronio x VB 7) x Zeus |
| 14 | OP 1207 | (Feronio x VB 7) x Zeus |
| 15 | OP 1209 | (Lido x VB 1-26) x Bravo |
| 16 | OP 1210 | (Lido x VB 7) x Arelate |
| 17 | OP 1211 | (Lido x VB 7) x Arelate |
| 18 | OP 1212 | (Valtejo x VB1-26) x (Onda x Estrela A 04 BB 3) |
| 19 | OP 1216 | Lido x VB 7 |
| 20 | OP 1217 | Lido x VB 7 |
| 21 | OP 1224 | Lido x VB 7 |
| 22 | OP 1225 | Lido x VB 7 |
| 23 | OP 1227 | Oscar x Suweon 285 319/88-4-11SM-01 |
| 24 | Ariete | Ariete |
| 25 | Gladio | Gladio |
| 26 | Ronaldo | Ronaldo |

**Table 2: Localization and description of experimental field and crop management details**

| Site | COTARROZ (Salvaterra de Magos) | | |
|---|---|---|---|
| Coordinates | 39°26' N, 008°78' W | | |
| Altitude (m asl) | 19 | | |
| **Soil conditions (0-20 cm, 2012)** | **Units** | **Results** | **Interpretation** |
| Texture | | Sandy loam | Coarse soil |
| Ext. P ($P_2O_5$) | mg/kg | 79 | Medium content |
| Ext. K ($K_2O$) | mg/kg | 34 | Low content |
| Ext. Mg | mg/kg | 106 | High content |
| O.M. | % | 0.81 | Low content |
| pH ($H_2O$) a) | | 5.7 | Slightly acid |
| Ca ($CaCO_3$) | t/ha | 0 | |
| Total N | % | 0.036 | Very low content |
| **Harvest year** | **2012** | | **2013** |
| Fertilizer application (kg N $ha^{-1}$) | 115.5 | | 115.5 |
| Seed bed | 60 | | 60 |
| Top dressing | 55.5 | | 55.5 |
| Sowing date | 29th May | | 13th May |
| Herbicide application (Propanol) | 11+9 l/ha | | 11+9 l/ha |
| Harvesting date | 30th October | | 15th October |
| Environmental conditions | | | |
| ETo (mm) | 704.8 | | 700.2 |
| PPT (mm) | 154.2 | | 75.4 |
| $T_{max}$ (°C) | 27.3 | | 27.8 |
| $T_{med}$ (°C) | 20.1 | | 20.2 |
| $T_{min}$ (°C) | 14.0 | | 14.1 |

ETo: cumulative evapotranspiration from sowing to harvesting during each year; PPT: cumulative precipitation from sowing to harvesting during each year; $T_{med}$: mean daily temperature; $T_{max}$ and $T_{min}$: average daily maximum and minimum daily temperatures

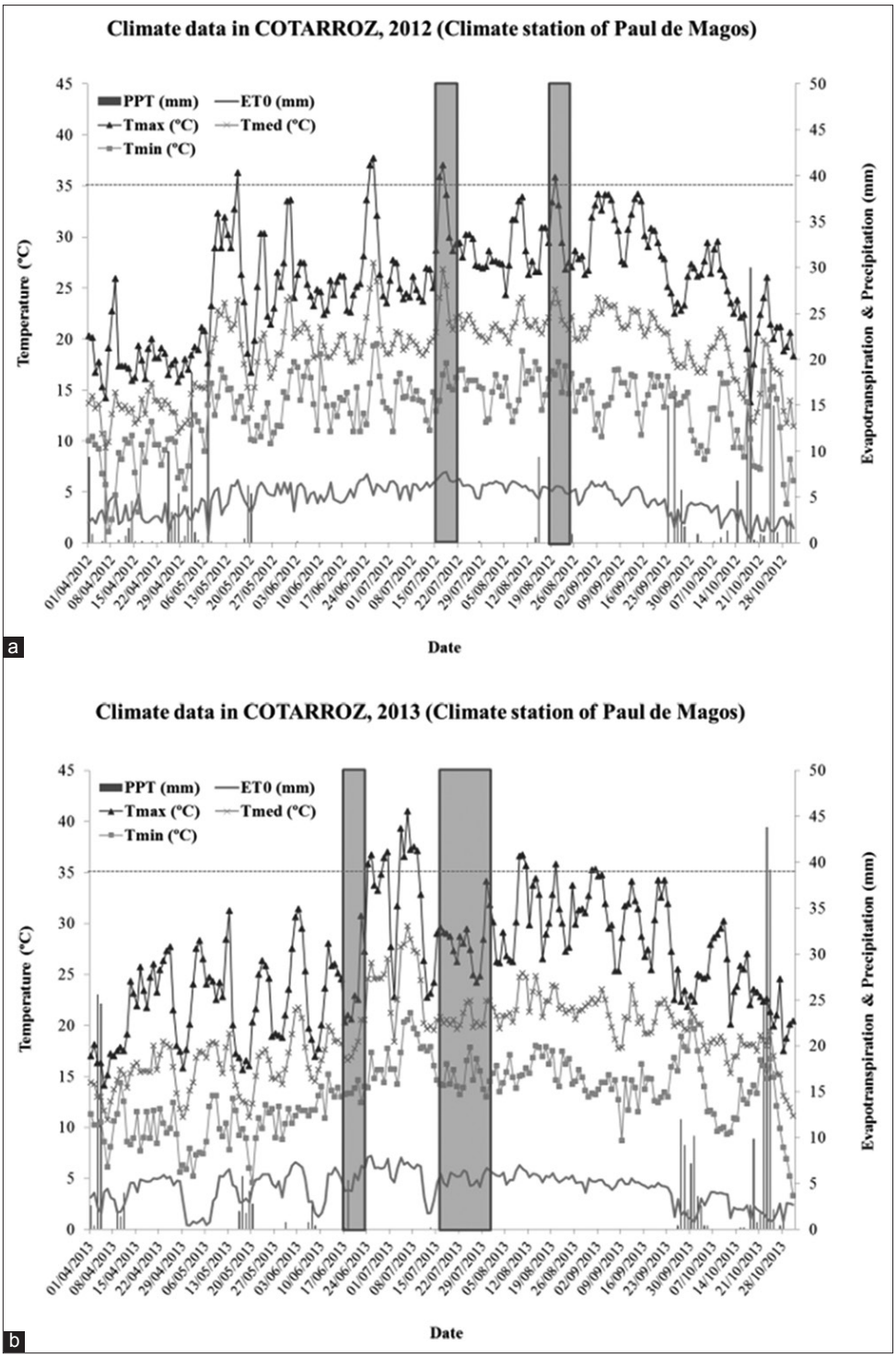



**Fig 1.** Distribution of temperature (ºC), evapotranspitation (mm) and precipitation (mm) during the years 2012 (a) and 2013 (b). Genotypes period for panicle initiation (left) and heading time (right) are highlighted by the two shaded areas. ETo: evapotranspiration; PPT: precipitation; $T_{med}$: mean daily temperature; $T_{max}$ and $T_{min}$: daily maximum and minimum temperatures.

by its middle point and the data composed by the weight in gram of each class.

The three field replicates were pooled for the milling and quality analysis. Paddy samples were dehusked in a Satake mill (THU, Satake, Taito, Japan) and polished (Suzuki MT-98, Santa Cruz do Rio Pardo, São Paulo, Brazil) for assessing milling yields, biometric data and quality traits of the milled rice. The potential yield of husked rice (MY) was determined according to ISO 6646, 2011. Biometric analysis of the polished grain was performed as for paddy samples. The amylose content was determined by

**Table 3: Agronomic, biometric and quality traits measured**

| Group | Code | Trait |
|---|---|---|
| Agronomic | PH | Plant height |
| | PM2 | Number of panicles per $m^2$ |
| | GP | Number of grains per panicle |
| | EG | Percentage of empty grains |
| | TGW | Thousand grain weight |
| | GY | Grain yield (kg/ha) |
| | pGL | Grain length of paddy grain |
| | pGW | Grain width of paddy grain |
| | pLW | Length to width ratio of paddy grain |
| Milling | kL | Grain length of milled kernel |
| | kW | Grain width of milled kernel |
| | kLW | Length to width ratio of milled kernel vitreous whiteness of the grain |
| | gW | Area of chalkiness of grains |
| | gC | Dehusking yield |
| | dY | Overall milling yield (dehusking and polishing) |
| | MY | Milling yield |
| Cooking quality | AC | Amylose content |
| | PV | Peak viscosity |
| | BD | Breakdown |
| | SB | Setback from peak |

a colorimetric technique and ISO 6647-2, 2015 method. The determination of rice paste gelatinization and viscosity characteristics was performed by a rapid viscosity analyzer (RVA-4, Newport Scientific, Warriewood, Australia) following AACC International Approved Method 61-02.01.

### Data analysis

A two-way Analysis of Variance (ANOVA) was used to test the significance of the main effects of genotype and year and of their interactive effect on the agronomic, milling and quality traits. For stability analysis, genotype is a control factor and year is a noise factor. The interactive effect is very important to select varieties, as when it is significant it means that some genotypes were more affected by the noise factor (year) than others. A one-way ANOVA for each variety in the case where interactive effects were significant was used to specify which varieties were stable (not affected by year variability).

The genotypes were also grouped with a post-hoc analysis (averages of both years) using Fisher's Least Significant Difference (LSD) to compare means and define homogeneous groups.

The paddy grain biometric uniformity of each genotype was evaluated by fitting standard frequency distributions (Normal and Weibull) to the pGL and pGW data and the goodness of fit was quantified by the coefficient of determination ($R^2$). The distribution parameters, namely mean, standard deviation, skewness (asymmetry around the mean - a value equal to 0 for a normal distribution) and kurtosis (peakedness of the distribution - the excess kurtosis is 0 for a normal distribution) were also calculated from the raw data. If a distribution is close to normal, then the best uniformity is quantified simply by the lowest standard deviation.

Correlations between the measured traits (Table 3) were first identified for every pair with Pearson's correlation coefficients. Principal Component Analysis was also applied to the milling and quality data to define a quality space to analyze stability (variability of quality traits over the years) by the Euclidean Distance (ED) between the 3 scores of PC1, PC2 and PC3 in the two years, this being the square root of the sums of the squared differences:

$$ED = \sqrt{\left(S1_{2012} - S1_{2013}\right)^2 + \left(S2_{2012} - S2_{2013}\right)^2 + \left(S3_{2012} - S3_{2013}\right)^2}$$

Where S1, S2 and S3 are the scores of PC1, 2 and 3, respectively.

All statistical analyses were performed with "Statistix 9.0".

## RESULTS

### Environmental conditions

Minimum temperatures during plants emergence period were considerable lower in 2013 (9.8°C) compared with 2012 (14.5°C), resulting in more constraints in plant emergence in 2013 and, consequently, on a lower density of emerged plants compared with 2012. The period between emergence and panicle initiation (Fig. 1) was colder in 2013 compared with 2012 (minimum temperatures average of 11.4°C and 14.5°C, respectively). Panicle initiation in 2012 occurred about 30 days later with higher maximum temperatures (30.2°C on average) compared to 2013 (27.9°C on average). Furthermore, in 2012 heading and anthesis occurred in a period with higher temperatures (30.2°C on average) compared with 2013 (27.9°C on average). Average daily maximum and

minimum temperatures during grain filling were higher in 2013 (30.6°C and 14.7°C) that in 2012 (28.3°C and 13.5°C). The two years were therefore considered sufficiently distinct, and representative of typical variability. Further details and implications regarding plant growth are discussed in section 4.

### Effects of Genotype, Year and GxY interaction on total variability of agronomic and paddy grain biometric traits

Genotype and season growth conditions explain most of the variability observed (Fig. 2), with less than 10% of the total sum of squares unexplained by these effects, except the GY, GP (in both cases, the error was around 1/3 of the total sum of squares) and EG (error under 30% of the total). It can also be seen that the genotype is the most dominant effect causing differences in agronomic and paddy characteristics, always showing a significant effect, except for GP, where year had a very significant effect, even higher on average than that of the genotype. The year had a statistically significant effect in all responses (except EG, pGL, but in these cases the interactive effect was significant), and only for PH and TGW this effect did not show a significant interaction with genotype. Results showed that the differences between the two years vary for the 26 genotypes.

A one-way ANOVA was then applied for each variety to ascertain which were stable, and exhibited the same response in both years. Results are shown in Table 4. Stable genotypes for a particular trait have a blank cell in the table. Those showing significant differences (at 95% confidence level) are marked with an arrow (upwards meaning that the response was significantly higher in 2013 than in 2012 and downwards otherwise).

In Table 4, the results show that PH, EG and TGW were stable for most genotypes and the significant, albeit small, average effect is due to those that were quite significantly affected by the year conditions. Considering PH, genotypes 4 and 10 were significantly smaller in 2013 year. Additionally, in 2013 genotypes 9, 15 and 25 showed significantly higher percentage of empty grains, and genotypes 2, 9, 14, 24, 25 and 26 a higher grain weight. The other characteristic where year had an average small effect, PM2 had a similar situation with only 3 genotypes not being stable, 5 and 20 with higher PM2 in 2013 and genotype 9 higher in 2012; this opposite behavior of these 3 genotypes explains the significance of the interactive effect.

The least stable characteristics were biometry, GP and GY. GP showed a significant interactive effect because although most genotypes had lower GP in 2013, genotypes 1, 7, 8, 9 and 25 were in fact stable. Regarding harvest yield, most genotypes had higher values in 2013, except genotype 5, which had lower, besides those that were stable. There were similar opposite behaviors in biometry of the paddy grains: genotype 25 was the only that was significantly longer and wider in 2013 than in 2012, genotype 8 was also longer in 2013, and genotype 7 the only to have a significantly lower length to width ratio in 2013. The only genotypes fully stable in biometry (similar pGL, pGW and pLW in both years) were 1, 3, 4, 6 and 10. These were lines containing the Basmati C. 621 and/or the Lido parentage (among others). Interestingly, none of the commercial varieties

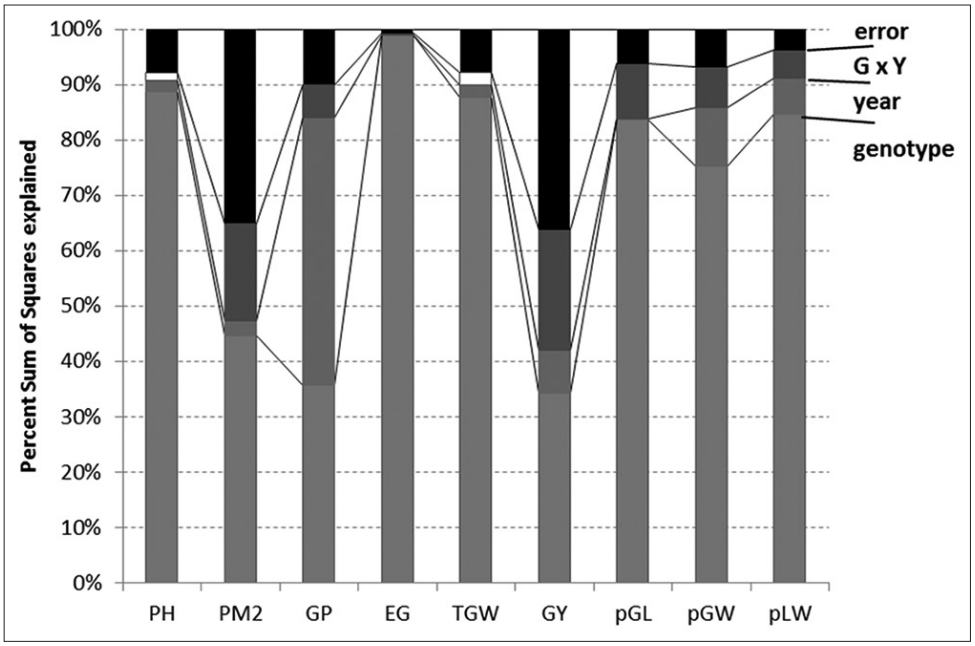



**Fig 2.** Decomposition of the total sums of squares with the 2-way ANOVAS of the agronomic and paddy data of the 26 genotypes in 2 years plantation in 3 field replicates in each year. Filled bars indicate statistically significant effects at 95% confidence level.

**Table 4: Stability of the agronomic and paddy grain biometry characteristics to different year growth conditions, assessed by one-way ANOVAs at 95% confidence level**

| Genotype code | | PH | PM2 | GP | EG | TGW | GY | pGL | pGW | pLW |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | OP 1001 | | | | | | ↑ | | | |
| 2 | OP 1004 | | | ↓ | | ↑ | | ↓ | ↓ | ↑ |
| 3 | OP 1101 | | | ↓ | | | ↑ | | | |
| 4 | OP 1102 | ↓ | | ↓ | | | ↑ | | | |
| 5 | OP 1103 | | ↑ | ↓ | | | ↓ | | | ↑ |
| 6 | OP 1104 | | | ↓ | | | | | | |
| 7 | OP 1105 | | | | | | | | | ↓ |
| 8 | OP 1109 | | | | | | ↑ | ↑ | | |
| 9 | OP 1201 | | ↓ | | ↑ | ↑ | | ↓ | | |
| 10 | OP 1202 | ↓ | | ↓ | | | | | | |
| 11 | OP 1203 | | | ↓ | | | | | | ↑ |
| 12 | OP 1205 | | | ↓ | | | ↑ | | | ↑ |
| 13 | OP 1206 | | | ↓ | | | | | ↓ | ↑ |
| 14 | OP 1207 | | | ↓ | | ↑ | | | ↓ | ↑ |
| 15 | OP 1209 | | | ↓ | ↑ | | | | ↓ | ↑ |
| 16 | OP 1210 | | | ↓ | | | | | ↓ | ↑ |
| 17 | OP 1211 | | | ↓ | | | ↑ | | ↓ | ↑ |
| 18 | OP 1212 | | | ↓ | | | | ↓ | | |
| 19 | OP 1216 | | | ↓ | | | | ↓ | ↓ | ↑ |
| 20 | OP 1217 | | ↑ | ↓ | | | ↑ | ↓ | ↓ | ↑ |
| 21 | OP 1224 | | | ↓ | | | ↑ | | ↓ | ↑ |
| 22 | OP 1225 | | | ↓ | | | | ↓ | ↓ | ↑ |
| 23 | OP 1227 | | | ↓ | | | | ↓ | ↓ | ↑ |
| 24 | Ariete | | | ↓ | | ↑ | | | | ↑ |
| 25 | Gladio | | | | ↑ | ↑ | | ↑ | ↑ | |
| 26 | Ronaldo | | | ↓ | | ↑ | | | | ↑ |

Arrows indicate statistically significant differences between the 2 years according to a one-way ANOVA of the respective genotype at 95% confidence level

performed so well regarding consistency of biometry, with genotype 25, the variety Gladio, being particularly variable. This is in agreement with local knowledge, as it is considered that this variety shows biometry of indica grains in some years, but not in others.

Genotypes 1, 6 and 7 were quite stable, the former only had a significantly higher yield in 2013 than 2012, the middle one had just a lower number of grains per panicle in 2013 and the latter a significantly lower length to width ratio of the paddy grains in 2013 than 2012, all other responses were consistent. Genotype 1 had Basmati and Lido parentage, the latter variety being a parentage of genotype 6 as well, together with VB7, a parent shared by genotype 7 (plus Safari), but many others with these parentages did not show such a high consistency. It is also noted that genotypes 3, 4, 5, 6, 19, 20, 21 and 22 all have the same parents but different stability of the agronomic and biometric characteristics over different years. Therefore, there is an element of chance in the combination of the DNA of parents in reaching a particular consistency to growth conditions.

Genotypes 2, 4, 5, 9, 10 and 20 can be considered the most variable because they show significant differences in the 2 years even in traits where most other genotypes were stable. It is noted that this analysis of stability is not desirability, as it gives equal weight to all responses and the biometry is analysed only in terms of the average sizes, whereas uniformity in size is very important for quality. High production yields and high uniformity as well as stability of sizes are more desired traits than others. These were; therefore, need to be considered in more detail.

### Variance of means according to the Genotype

Tables 5(a), 5(b) and 5(c) show the means for all agronomic and biometric paddy grain traits for the 26 genotypes, with the homogeneous groupings according to Fisher's Least Significant Difference (L.S.D.) used to identify significant differences. The greatest variation was observed for EG with a coefficient of variation (C.V.) of 37%. Paddy grain length has shown the lowest variation, with a C.V. of just over 1%. It is noted that this table pools together the values of the two different years, and therefore the subsequent comments are particularly accurate the more stable genotype. This pooling is however fairly accurate as the only case where the year has a very significant impact, which is the GP value, showed all genotypes with a similar trend, a lower value in 2013 (except those that were stable, as identified previously).

There were some sister lines as identified in Table 1, and just as they showed different stability, they also show significant differences between them in the actual values

**Table 5(a): Means, their comparison with the Fisher's least significant difference (LSD) and coefficient of variation (CV) for GY (kg/ha), pGL (mm) pGW (mm) and pLW**

| | Genotype | GY | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|---|
| 9 | OP 1201 | 8049 | X | | | | | |
| 18 | OP 1212 | 7811 | X | X | | | | |
| 11 | OP 1203 | 7393 | X | X | X | | | |
| 23 | OP 1227 | 7252 | X | X | X | X | | |
| 2 | OP 1004 | 7022 | X | X | X | X | X | |
| 10 | OP 1202 | 6919 | X | X | X | X | X | |
| 21 | OP 1224 | 6763 | X | X | X | X | X | X |
| 20 | OP 1217 | 6757 | X | X | X | X | X | X |
| 15 | OP 1209 | 6672 | | X | X | X | X | X |
| 26 | Ronaldo | 6536 | | X | X | X | X | X |
| 24 | Ariete | 6414 | | X | X | X | X | X |
| 1 | OP 1001 | 6408 | | X | X | X | X | X |
| 22 | OP 1225 | 6215 | | | X | X | X | X |
| 14 | OP 1207 | 6194 | | | X | X | X | X |
| 17 | OP 1211 | 6135 | | | X | X | X | X |
| 7 | OP 1105 | 6015 | | | X | X | X | X |
| 25 | Gladio | 6014 | | | | X | X | X |
| 16 | OP 1210 | 5958 | | | | X | X | X |
| 3 | OP 1101 | 5957 | | | | X | X | X |
| 6 | OP 1104 | 5948 | | | | X | X | X |
| 8 | OP 1109 | 5865 | | | | | X | X |
| 4 | OP 1102 | 5769 | | | | | X | X |
| 19 | OP 1216 | 5714 | | | | | X | X |
| 13 | OP 1206 | 5553 | | | | | X | X |
| 12 | OP 1205 | 5504 | | | | | | X |
| 5 | OP 1103 | 5270 | | | | | | X |
| Mean | | 6426 | | | | | | |
| L.S.D. (95%) | | 1341.1-1642.5 | | | | | | |
| C.V. (%) | | 18.24 | | | | | | |

| | Genotype | pGL | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | OP 1207 | 9.54 | X | | | | | | | | | | | | |
| 17 | OP 1211 | 9.54 | X | | | | | | | | | | | | |
| 19 | OP 1216 | 9.39 | | X | | | | | | | | | | | |
| 4 | OP 1102 | 9.37 | | X | | | | | | | | | | | |
| 10 | OP 1202 | 9.31 | | X | X | | | | | | | | | | |
| 21 | OP 1224 | 9.23 | | | X | | | | | | | | | | |
| 11 | OP 1203 | 9.03 | | | | X | | | | | | | | | |
| 3 | OP 1101 | 9 | | | | X | | | | | | | | | |
| 13 | OP 1206 | 9 | | | | X | | | | | | | | | |
| 7 | OP 1105 | 8.94 | | | | X | X | | | | | | | | |
| 25 | Gladio | 8.91 | | | | X | X | | | | | | | | |
| 16 | OP 1210 | 8.84 | | | | | X | X | | | | | | | |
| 12 | OP 1205 | 8.83 | | | | | X | X | X | | | | | | |
| 1 | OP 1001 | 8.82 | | | | | X | X | X | | | | | | |
| 5 | OP 1103 | 8.82 | | | | | X | X | X | | | | | | |
| 2 | OP 1004 | 8.76 | | | | | | X | X | X | | | | | |
| 9 | OP 1201 | 8.75 | | | | | | X | X | X | | | | | |
| 6 | OP 1104 | 8.71 | | | | | | | X | X | X | | | | |
| 23 | OP 1227 | 8.66 | | | | | | | | X | X | X | | | |
| 24 | Ariete | 8.61 | | | | | | | | | X | X | X | | |
| 18 | OP 1212 | 8.56 | | | | | | | | | | X | X | | |
| 8 | OP 1109 | 8.53 | | | | | | | | | | X | X | | |
| 26 | Ronaldo | 8.49 | | | | | | | | | | | X | X | |
| 20 | OP 1217 | 8.39 | | | | | | | | | | | | X | X |
| 22 | OP 1225 | 8.38 | | | | | | | | | | | | X | X |

(*Contd...*)

**Table 5(a): (*Continued*)**

| | Genotype | pGL | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | OP 1209 | 8.35 | | | | | | | | | | | | | X |
| | Mean | 8.88 | | | | | | | | | | | | | |
| | L.S.D. (95%) | 0.13 | | | | | | | | | | | | | |
| | C.V. (%) | 1.31 | | | | | | | | | | | | | |
| | **Genotype** | **pGW** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** | **K** | **L** | |
| 9 | OP 1201 | 3.02 | X | | | | | | | | | | | | |
| 23 | OP 1227 | 3.02 | X | | | | | | | | | | | | |
| 8 | OP 1109 | 2.97 | X | X | | | | | | | | | | | |
| 10 | OP 1202 | 2.93 | | X | X | | | | | | | | | | |
| 19 | OP 1216 | 2.93 | | X | X | | | | | | | | | | |
| 13 | OP 1206 | 2.91 | | X | X | X | | | | | | | | | |
| 14 | OP 1207 | 2.9 | | X | X | X | | | | | | | | | |
| 21 | OP 1224 | 2.9 | | X | X | X | | | | | | | | | |
| 2 | OP 1004 | 2.88 | | | X | X | X | | | | | | | | |
| 4 | OP 1102 | 2.87 | | | X | X | X | | | | | | | | |
| 7 | OP 1105 | 2.87 | | | X | X | X | | | | | | | | |
| 11 | OP 1203 | 2.86 | | | X | X | X | | | | | | | | |
| 18 | OP 1212 | 2.86 | | | X | X | X | | | | | | | | |
| 16 | OP 1210 | 2.84 | | | | X | X | X | | | | | | | |
| 26 | Ronaldo | 2.82 | | | | | X | X | X | | | | | | |
| 22 | OP 1225 | 2.78 | | | | | | X | X | X | | | | | |
| 12 | OP 1205 | 2.76 | | | | | | | X | X | | | | | |
| 20 | OP 1217 | 2.74 | | | | | | | | X | X | | | | |
| 24 | Ariete | 2.72 | | | | | | | | X | X | X | | | |
| 15 | OP 1209 | 2.7 | | | | | | | | X | X | X | | | |
| 3 | OP 1101 | 2.67 | | | | | | | | | X | X | | | |
| 17 | OP 1211 | 2.66 | | | | | | | | | | X | | | |
| 6 | OP 1104 | 2.65 | | | | | | | | | | X | | | |
| 5 | OP 1103 | 2.55 | | | | | | | | | | | X | | |
| 1 | OP 1001 | 2.32 | | | | | | | | | | | | | X |
| 25 | Gladio | 2.3 | | | | | | | | | | | | | X |
| | Mean | 2.78 | | | | | | | | | | | | | |
| | L.S.D. (95%) | 0.08 | | | | | | | | | | | | | |
| | C.V. (%) | 2.41 | | | | | | | | | | | | | |

Different letters represent significant differences ($p < 0.05$). Thick lines divide groups that are significantly different from the highest or lowest value, or that are significantly different from others (no overlapping of homogeneous groups).

of the agronomic and paddy biometry traits. The biometric parameters pGL, pGW and pLW had a high heterogeneity among genotypes obtained from the same cross. The pLW values of 8 genotypes derived from "Lido x VB 7" parents ranged from 3.03 to 3.47 which were significantly different from each other. OP1202 and OP1203 (genotypes 10 and 11) were the only sister lines with similar pGW and pLW values.

Genotypes 2, 9, 10, 11, 18 and 23 would be the most attractive from the production point of view. As shown in Tables 5(b) and 5(c), these all happened to have been genotypes that were also consistent over the 2 years. However, in terms of biometry genotype 10 (OP1202) was both significantly longer and significantly wider grains with stability in both years, with genotypes 2 (OP1004) and 23 (OP1227) having shown significantly different dimensions in the 2 years. Thus, in terms of the most important field characteristics and stability, the OP1202 variety would have been identified as the best performer, despite their high plant height when compared with commercial varieties.

### *Uniformity of paddy grain length (pGL)*

Figure 3(a) shows the uniformity of sizes of the paddy grain length in 2 years. Each sample was fitted to a Normal and a Weibull distribution, in case it would be found that the normal distribution was not applicable. However, the normal distribution was indeed appropriate for all but exceptions are OP1201 and OP1212 in the 2012 harvest. Sizes will be more uniform as standard deviation was lower and kurtosis was higher, although it should be noted that standard deviations would be higher for larger sizes simply due to the effect of shift of mean.

**Table 5(b): Parameters' means - Comparison with the Fisher's least significant difference (LSD) and coefficient of variation (C.V.) for pLW, PH (cm) and PM2**

| | Genotype | pLW | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Gladio | 3.88 | X | | | | | | | | | | | | | | | | | |
| 1 | OP 1001 | 3.8 | | X | | | | | | | | | | | | | | | | |
| 17 | OP 1211 | 3.59 | | | X | | | | | | | | | | | | | | | |
| 5 | OP 1103 | 3.47 | | | | X | | | | | | | | | | | | | | |
| 3 | OP 1101 | 3.38 | | | | | X | | | | | | | | | | | | | |
| 6 | OP 1104 | 3.3 | | | | | | X | | | | | | | | | | | | |
| 14 | OP 1207 | 3.29 | | | | | | X | X | | | | | | | | | | | |
| 4 | OP 1102 | 3.27 | | | | | | X | X | X | | | | | | | | | | |
| 12 | OP 1205 | 3.21 | | | | | | | | X | X | | | | | | | | | |
| 19 | OP 1216 | 3.21 | | | | | | | X | X | X | | | | | | | | | |
| 10 | OP 1202 | 3.18 | | | | | | | | | X | X | | | | | | | | |
| 21 | OP 1224 | 3.18 | | | | | | | | | X | X | | | | | | | | |
| 24 | Ariete | 3.17 | | | | | | | | | X | X | X | | | | | | | |
| 11 | OP 1203 | 3.16 | | | | | | | | | X | X | X | X | | | | | | |
| 7 | OP 1105 | 3.12 | | | | | | | | | | X | X | X | X | | | | | |
| 16 | OP 1210 | 3.11 | | | | | | | | | | X | X | X | X | X | | | | |
| 13 | OP 1206 | 3.1 | | | | | | | | | | | X | X | X | X | X | | | |
| 15 | OP 1209 | 3.09 | | | | | | | | | | | | X | X | X | X | | | |
| 20 | OP 1217 | 3.07 | | | | | | | | | | | | | X | X | X | X | | |
| 2 | OP 1004 | 3.04 | | | | | | | | | | | | | | X | X | X | X | |
| 22 | OP 1225 | 3.03 | | | | | | | | | | | | | | | X | X | X | |
| 26 | Ronaldo | 3.01 | | | | | | | | | | | | | | | | X | X | |
| 18 | OP 1212 | 2.99 | | | | | | | | | | | | | | | | | X | |
| 9 | OP 1201 | 2.9 | | | | | | | | | | | | | | | | | | X |
| 8 | OP 1109 | 2.88 | | | | | | | | | | | | | | | | | | X |
| 23 | OP 1227 | 2.87 | | | | | | | | | | | | | | | | | | X |
| | Mean | 3.2 | | | | | | | | | | | | | | | | | | |
| | L.S.D. (95%) | 0.07 | | | | | | | | | | | | | | | | | | |
| | C.V. (%) | 2.02 | | | | | | | | | | | | | | | | | | |

| | Genotype | PH | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | OP 1004 | 105.6 | X | | | | | | | | | | |
| 23 | OP 1227 | 100.2 | | X | | | | | | | | | |
| 11 | OP 1203 | 93.3 | | | X | | | | | | | | |
| 7 | OP 1105 | 91.7 | | | X | | | | | | | | |
| 18 | OP 1212 | 90.5 | | | X | | | | | | | | |
| 10 | OP 1202 | 89.8 | | | X | X | | | | | | | |
| 24 | Ariete | 85.1 | | | | X | X | | | | | | |
| 9 | OP 1201 | 84.9 | | | | X | X | | | | | | |
| 15 | OP 1209 | 81.5 | | | | | X | X | | | | | |
| 3 | OP 1101 | 80.8 | | | | | X | X | | | | | |
| 6 | OP 1104 | 77.7 | | | | | | X | X | | | | |
| 5 | OP 1103 | 77.3 | | | | | | X | X | | | | |
| 13 | OP 1206 | 75.3 | | | | | | | X | X | | | |
| 14 | OP 1207 | 75.2 | | | | | | | X | X | X | | |
| 12 | OP 1205 | 73.9 | | | | | | | X | X | X | | |
| 20 | OP 1217 | 71.6 | | | | | | | | X | X | X | |
| 22 | OP 1225 | 70.9 | | | | | | | | X | X | X | |
| 21 | OP 1224 | 70.6 | | | | | | | | X | X | X | |
| 8 | OP 1109 | 70.3 | | | | | | | | | X | X | |
| 4 | OP 1102 | 68.2 | | | | | | | | | | X | |
| 26 | Ronaldo | 67.8 | | | | | | | | | | X | |
| 19 | OP 1216 | 67.2 | | | | | | | | | | X | |
| 17 | OP 1211 | 66.8 | | | | | | | | | | X | |
| 25 | Gladio | 66.7 | | | | | | | | | | X | |

(*Contd...*)

**Table 5(b): (*Continued*)**

| | Genotype | PH | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | OP 1210 | 59.4 | | | | | | | | | | | X |
| 1 | OP 1001 | 58 | | | | | | | | | | | X |
| Mean | | 77.7 | | | | | | | | | | | |
| L.S.D. (95%) | | 4.98 | | | | | | | | | | | |
| C.V. (%) | | 5.59 | | | | | | | | | | | |

| | Genotype | PM2 | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | OP 1210 | 390 | X | | | | | | | | | |
| 11 | OP 1203 | 306 | X | | | | | | | | | |
| 25 | Gladio | 308 | X | X | | | | | | | | |
| 10 | OP 1202 | 318 | X | X | X | | | | | | | |
| 1 | OP 1001 | 365 | X | X | X | | | | | | | |
| 17 | OP 1211 | 356 | X | X | X | | | | | | | |
| 26 | Ronaldo | 288 | X | X | X | X | | | | | | |
| 20 | OP 1217 | 299 | X | X | X | X | X | | | | | |
| 5 | OP 1103 | 321 | | X | X | X | X | X | | | | |
| 6 | OP 1104 | 393 | | X | X | X | X | X | X | | | |
| 22 | OP 1225 | 423 | | X | X | X | X | X | X | X | | |
| 19 | OP 1216 | 293 | | | X | X | X | X | X | X | | |
| 15 | OP 1209 | 279 | | | X | X | X | X | X | X | X | |
| 21 | OP 1224 | 290 | | | | X | X | X | X | X | X | X |
| 9 | OP 1201 | 338 | | | | | X | X | X | X | X | X |
| 4 | OP 1102 | 433 | | | | | | X | X | X | X | X |
| 18 | OP 1212 | 389 | | | | | | X | X | X | X | X |
| 3 | OP 1101 | 310 | | | | | | | X | X | X | X |
| 2 | OP 1004 | 341 | | | | | | | X | X | X | X |
| 8 | OP 1109 | 377 | | | | | | | | X | X | X |
| 23 | OP 1227 | 330 | | | | | | | | | X | X |
| 12 | OP 1205 | 351 | | | | | | | | | X | X |
| 14 | OP 1207 | 294 | | | | | | | | | X | X |
| 7 | OP 1105 | 287 | | | | | | | | | X | X |
| 24 | Ariete | 399 | | | | | | | | | X | X |
| 13 | OP 1206 | 379 | | | | | | | | | | X |
| Mean | | 340.76 | | | | | | | | | | |
| L.S.D. (95%) | | 56.4 | | | | | | | | | | |
| C.V. (%) | | 14.45 | | | | | | | | | | |

Different letters represent significant differences ($p < 0.05$). Thick lines divide groups that are significantly different from the highest or lowest value, or that are significantly different from others (no overlapping of homogeneous groups)

Figure 3(a) shows standard deviations versus length: the longer the grain and smaller the standard deviation, the more uniform are sizes. Given that genotype OP1211 is among the longest (see Table 5a), its low standard deviation quantifies a particularly good uniformity. One should also consider the kurtosis, and for this variety it is not significantly different from a normal distribution, so this is a very uniform variety. However, it has significantly lower yields than OP1202, the most stable variety with high yield. OP1202 also shows a good uniformity, with low (2012) to average (2013) standard deviation compared to size and among the lowest kurtosis, but still close to that of a normal distribution. The distribution is also very symmetrical, with skewness values close to 0.

Of the smallest varieties, genotypes OP1225 and OP1217 were also consistent in providing low spreads (standard deviations) in both years, however, both showed a fairly high kurtosis in 2013, although not in 2012 - however, as shown in Table 5, the actual average sizes were significantly different in 2 years, so these varieties do not show uniformity with consistency.

### Uniformity of paddy grain width (pGW)

The fit of experimental values of the size distributions of paddy grain width to a normal distribution were all acceptable. Figure 3(b) shows the actual values of standard deviation, skewness and kurtosis.

The commercial variety Ariete shows smallest spread in both years. Gladio shows the most deviated behaviour

**Table 5(c): Parameters' means - Comparison with the Fisher's least significant difference (LSD) and coefficient of variation (C.V.) for GP, EG and TGW (g)**

| | Genotype | GP | A | B | C | D | E | F | G | H | I | J | K | L | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | OP 1209 | 106 | X | | | | | | | | | | | | |
| 23 | OP 1227 | 100 | X | X | | | | | | | | | | | |
| 6 | OP 1104 | 97 | X | X | X | | | | | | | | | | |
| 24 | Ariete | 92 | | X | X | X | | | | | | | | | |
| 3 | OP 1101 | 91 | | X | X | X | | | | | | | | | |
| 12 | OP 1205 | 90 | | X | X | X | X | | | | | | | | |
| 2 | OP 1004 | 89 | | | X | X | X | | | | | | | | |
| 5 | OP 1103 | 89 | | | X | X | X | | | | | | | | |
| 13 | OP 1206 | 87 | | | X | X | X | X | | | | | | | |
| 18 | OP 1212 | 85 | | | | X | X | X | X | | | | | | |
| 4 | OP 1102 | 80 | | | | | X | X | X | X | | | | | |
| 20 | OP 1217 | 79 | | | | | X | X | X | X | | | | | |
| 7 | OP 1105 | 78 | | | | | | X | X | X | X | | | | |
| 22 | OP 1225 | 77 | | | | | | X | X | X | X | | | | |
| 1 | OP 1001 | 75 | | | | | | | X | X | X | | | | |
| 14 | OP 1207 | 75 | | | | | | | X | X | X | | | | |
| 21 | OP 1224 | 72 | | | | | | | | X | X | X | | | |
| 25 | Gladio | 72 | | | | | | | | X | X | X | | | |
| 19 | OP 1216 | 71 | | | | | | | | X | X | X | | | |
| 26 | Ronaldo | 70 | | | | | | | | X | X | X | | | |
| 8 | OP 1109 | 68 | | | | | | | | | X | X | X | | |
| 17 | OP 1211 | 63 | | | | | | | | | | X | X | X | |
| 11 | OP 1203 | 57 | | | | | | | | | | | X | X | X |
| 16 | OP 1210 | 54 | | | | | | | | | | | | X | X |
| 10 | OP 1202 | 53 | | | | | | | | | | | | X | X |
| 9 | OP 1201 | 52 | | | | | | | | | | | | | X |
| | Mean | | | | | | | 77.73 | | | | | | | |
| | L.S.D. (95%) | | | | | | | 10.74 | | | | | | | |
| | C.V. (%) | | | | | | | 12.06 | | | | | | | |

| | Genotype | EG | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | OP 1102 | 28.2 | X | | | | | | | | | | |
| 7 | OP 1105 | 24.6 | X | X | | | | | | | | | |
| 12 | OP 1205 | 21.8 | | X | X | | | | | | | | |
| 19 | OP 1216 | 19.2 | | X | X | X | | | | | | | |
| 21 | OP 1224 | 18.8 | | | X | X | X | | | | | | |
| 13 | OP 1206 | 18.1 | | | X | X | X | | | | | | |
| 1 | OP 1001 | 17.9 | | | X | X | X | X | | | | | |
| 9 | OP 1201 | 16.8 | | | X | X | X | X | | | | | |
| 14 | OP 1207 | 16.7 | | | X | X | X | X | | | | | |
| 18 | OP 1212 | 14.5 | | | | X | X | X | X | | | | |
| 25 | Gladio | 14.2 | | | | X | X | X | X | X | | | |
| 6 | OP 1104 | 13.4 | | | | | X | X | X | X | X | | |
| 15 | OP 1209 | 11.8 | | | | | | X | X | X | X | X | |
| 23 | OP 1227 | 11 | | | | | | | X | X | X | X | X |
| 26 | Ronaldo | 10.4 | | | | | | | X | X | X | X | X |
| 3 | OP 1101 | 10.1 | | | | | | | X | X | X | X | X |
| 5 | OP 1103 | 9.5 | | | | | | | X | X | X | X | X |
| 10 | OP 1202 | 8.9 | | | | | | | | X | X | X | X |
| 11 | OP 1203 | 8.6 | | | | | | | | | X | X | X |
| 17 | OP 1211 | 8.5 | | | | | | | | | X | X | X |
| 20 | OP 1217 | 8.5 | | | | | | | | | X | X | X |
| 2 | OP 1004 | 7.7 | | | | | | | | | | X | X |
| 22 | OP 1225 | 7.7 | | | | | | | | | | X | X |
| 24 | Ariete | 7.6 | | | | | | | | | | X | X |

(*Contd...*)

**Table 5(c): (*Continued*)**

| | Genotype | EG | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | OP 1109 | 6.6 | | | | | | | | | | X | X |
| 16 | OP 1210 | 5.8 | | | | | | | | | | | X |
| Mean | | | | | | | 13.3 | | | | | | |
| L.S.D. (95%) | | | | | | | 5.63 | | | | | | |
| C.V. (%) | | | | | | | 36.92 | | | | | | |

| | Genotype | TGW | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | OP 1202 | 36.4 | X | | | | | | | | | |
| 19 | OP 1216 | 34.7 | | X | | | | | | | | |
| 9 | OP 1201 | 34.5 | | X | | | | | | | | |
| 11 | OP 1203 | 34.5 | | X | | | | | | | | |
| 4 | OP 1102 | 34 | | X | | | | | | | | |
| 21 | OP 1224 | 33.8 | | X | X | | | | | | | |
| 14 | OP 1207 | 32.3 | | | X | X | | | | | | |
| 8 | OP 1109 | 32.2 | | | | X | | | | | | |
| 23 | OP 1227 | 32.2 | | | | X | | | | | | |
| 26 | Ronaldo | 31.5 | | | | X | | | | | | |
| 18 | OP 1212 | 31 | | | | X | | | | | | |
| 7 | OP 1105 | 30.9 | | | | X | | | | | | |
| 16 | OP 1210 | 30.8 | | | | X | | | | | | |
| 2 | OP 1004 | 29 | | | | | X | | | | | |
| 24 | Ariete | 29 | | | | | X | | | | | |
| 13 | OP 1206 | 28.4 | | | | | X | X | | | | |
| 17 | OP 1211 | 28.3 | | | | | X | X | | | | |
| 12 | OP 1205 | 27.4 | | | | | | X | X | | | |
| 3 | OP 1101 | 27.2 | | | | | | X | X | | | |
| 6 | OP 1104 | 26.7 | | | | | | | X | X | | |
| 15 | OP 1209 | 26.1 | | | | | | | X | X | X | |
| 5 | OP 1103 | 25.2 | | | | | | | | X | X | |
| 25 | Gladio | 25.1 | | | | | | | | | X | |
| 20 | OP 1217 | 25 | | | | | | | | | X | |
| 22 | OP 1225 | 24.9 | | | | | | | | | X | |
| 1 | OP 1001 | 23 | | | | | | | | | | X |
| | Mean | | | | | | 29.75 | | | | | |
| | L.S.D. (95%) | | | | | | 1.55 | | | | | |
| | C.V. (%) | | | | | | 4.54 | | | | | |

Different letters represent significant differences ($p < 0.05$). Thick lines divide groups that are significantly different from the highest or lowest value, or that are significantly different from others (no overlapping of homogeneous groups).

from a normal distribution, with high skewness and kurtosis.

OP1203 was the genotype with highest pGW (3.18 mm) and uniformity and OP1212 showed greater width variability (Fig. 3b). The OP1202 genotype, so far considered the best, balanced between high yield, uniformity and stability, had a high standard deviation in 2012 and low kurtosis, so it is not the most uniform variety regarding width.

### Quality traits of polished grains

Dehusking yield (DY, percentage of brown rice after dehusking) was higher for OP1201, 1202, 1210 and 1227 (over 82%). OP1205 and OP1206 significantly performed poorly with yields of 77%.

Total milling yield after polishing (MY, after removing bran and kernels with defects) was highest for the commercial Gladio variety, even though within the year variability, only 8 of the advanced lines have significantly lower values. MY over 70% are considered good locally, with about 20% loss from dehusking and 10% from bran removal. The other commercial varieties performed equally well, and the significantly lower values (compared to Gladio) of OP's 1102, 1201, 1206, 1207, 1209, 1216, 1217, 1224, 1227 would be a concern for farming yields, specially the latter two.

With respect to biometric analysis, OP1217 had minimum kL (5.53 mm) whereas OP1202 had maximum kL (6.33 mm). The shorter grains OP1217, OP1225, OP1209

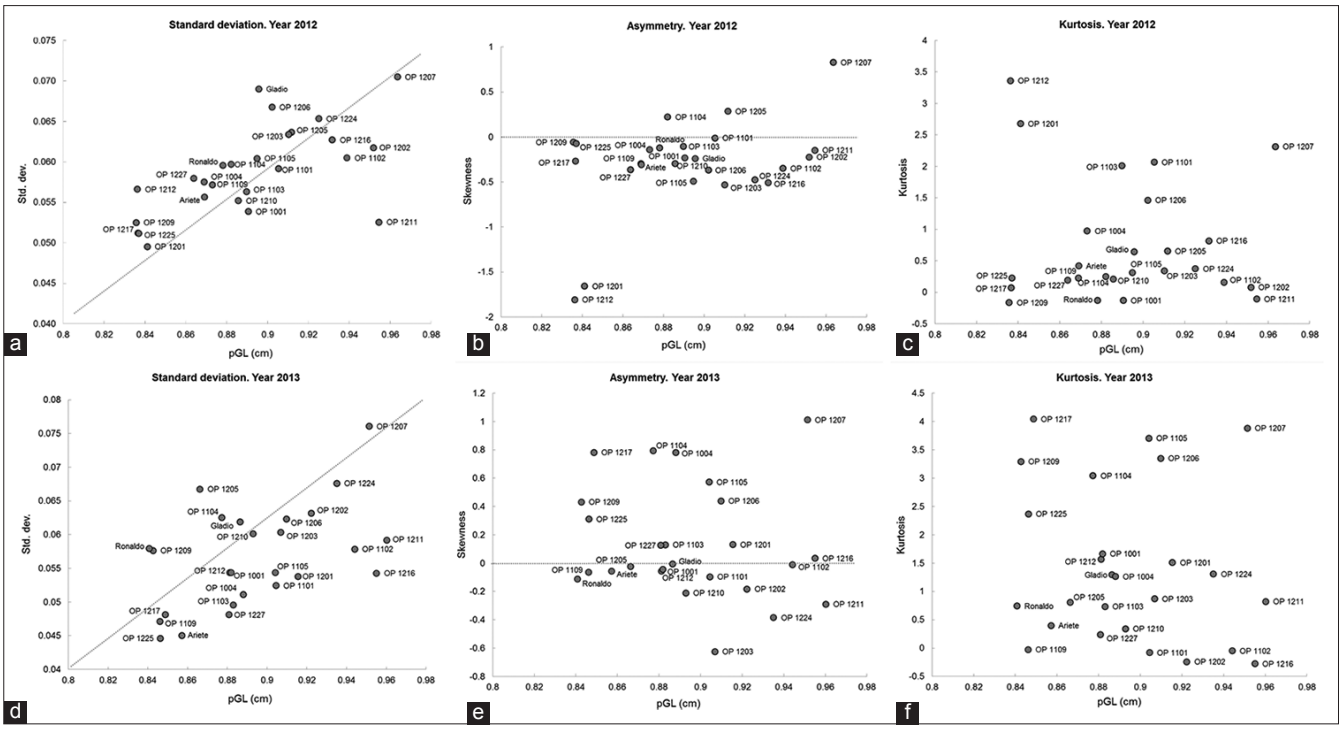



**Fig 3a.** Frequency distribution parameters of the 26 genotypes for paddy grain length (pGL) in years 2012 and 2013: standard deviation (a,b), skewness (c,d) and kurtosis (e,f).

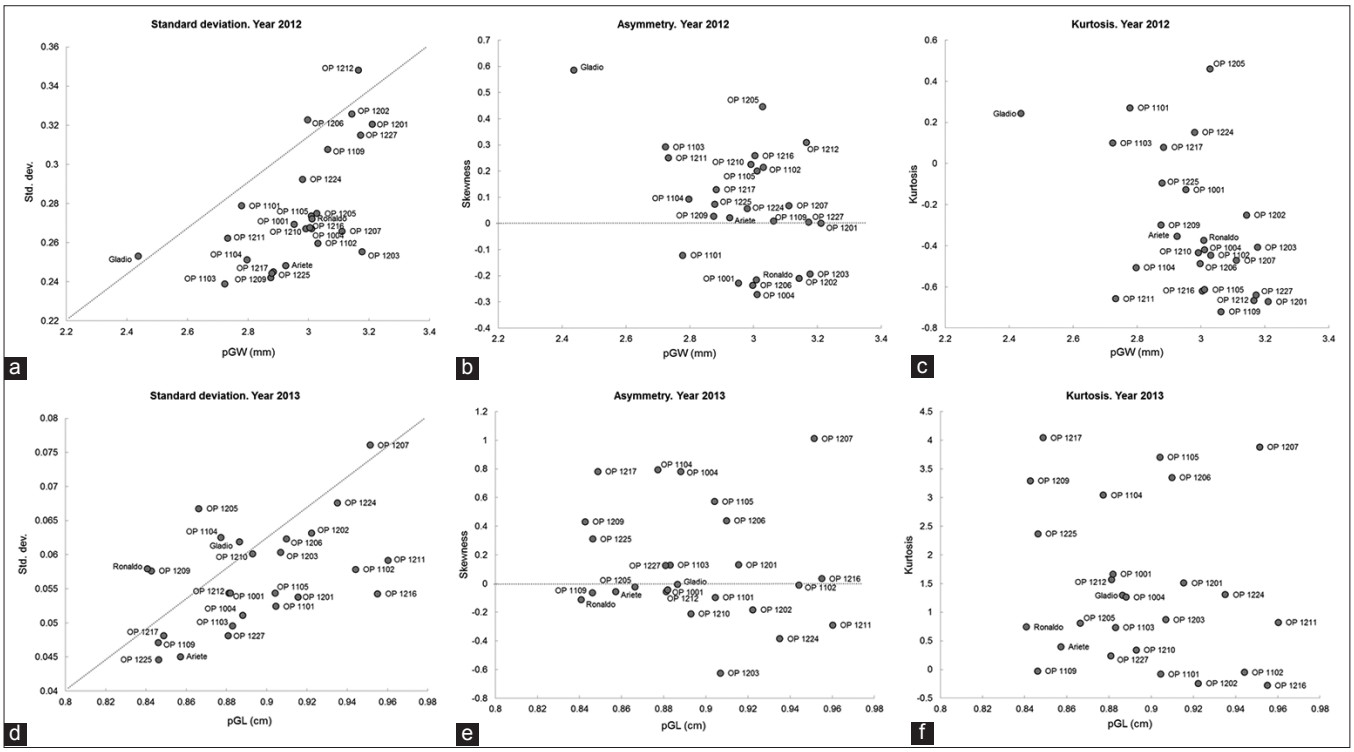



**Fig 3b.** Frequency distribution parameters of the 26 genotypes for paddy grain width (pGW) in years 2012 and 2013: standard deviation (a,b), skewness (c,d) and kurtosis (e,f).

and OP1109 were close to Ronaldo (5.64 mm). Gladio was among the longest (6.18 mm), like OP1203 (6.19 mm) and OP1216. Ariete showed a medium kL (5.85 mm), similar to OP1103 and OP1104. According to the grain length data, ten of the advanced lines could be classified as long grain varieties and thirteen as medium.

Another important biometric parameter for classification of rice varieties is L/W ratio. According to European classification of rice, only one advanced line, OP1001, is a long B variety (3.06), surpassed only by Gladio (3.20), while other advanced lines belonged to round category. Ronaldo had the lowest L/W, with OP1109 and 1227 similarly round. A critical quality attribute indicator of cooking behaviour is amylose content. Gladio outnumbered all the advanced genotypes with 30% amylose content. Most advanced lines exhibited AC lower than 20%, but OP 1001, 1004, 1105, 1201 and 1209 had means over 24% that were similar to Gladio when taking the yearly variability as source of error.

Pasting behavior of 26 genotypes depicted that Ronaldo and OP1210 have highest PV (3798 cP and 3651 cP, respectively) and BD (2153 cP and 2089 cP), and minimum SB (-808 cP and -802 cP). Gladio and OP1001 showed similar trends with high SB (1417 cP and 1591 cP, respectively) and low PV (2532 cP and 1964 cP) and BD (1118 cP and 786 cP).

### Principal component analysis of quality traits

A Principal Component Analysis with normalised Varimax rotation indicated that only 3 principal components had an eigenvalue above 1 (Scree plot not shown), explaining almost ¾ of the total variance of the data. Factor loadings (Table 6) show that the dominant parameters in the first principal component (PC) were the viscosity parameters and the amylose content; the second PC was dominated by width, length-to-width ratio and total milling yield and the third by the kernel length.

The almost orthogonal relation between kernel length and L/W ratio is an interesting feature: the important L/W value is mostly dominated by width, not length. It is also generally expected that rice with high amylose content tends to have high length and L/W ratio but within these genotypes, that broad relation was not verified, amylose content relates quite well to the viscosity parameters, and very poorly with the biometric parameters. Individual correlations will be analysed later in more detail with PLS regressions.

**Table 6: Factor loadings of the 3 first principal components of a PCA of the quality and milling data with normalised Varimax rotation. Dominant factors are highlighted in bold**

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| kL | –0.01718 | 0.023512 | **–0.94896** |
| kW | 0.369055 | **–0.79499** | 0.010999 |
| kLW | –0.36394 | **0.717071** | –0.51394 |
| AC | **–0.78762** | 0.023014 | –0.07885 |
| PV | **0.817547** | –0.04218 | 0.143565 |
| BD | **0.929507** | –0.07175 | 0.05531 |
| SB | **–0.94515** | 0.152233 | 0.041622 |
| DY | 0.26437 | 0.522683 | 0.28852 |
| MY | 0.006651 | **0.736879** | –0.07185 |
| Variance explained | 37.6% | 22.2% | 14.3% |
| Cummulative | 37.6% | 59.8% | 74.0% |

This pooling of the quality parameters with the linear combinations defined by the scores in Table 6 permits a representation of the quality space with two simple plots of the scores of the 3 PC for the different genotypes in the 2 different years in order to identify similarities and differences as well as stability (Fig. 4). The 3 commercial varieties are very distinctive, especially Gladio. The similarity between Gladio and OP1001 is evident in the fact that they are the only genotypes occupying the left upper quadrant in PC2 vs. PC1 graph, it is noted that this is the most meaningful of the 2 graphs, because PC2 and PC3 are both dominated by biometric parameters. The Ariete variety (24) is the closest to the center of both graphs. Advanced line OP1104 (6) is quite similar in both graphs, whereas OP1109 (8), OP1217 (20) and OP1225 (22) are very close in the PC2 vs. PC1 space. Variety Ronaldo is the furthest away from Gladio, in the diametrically opposite quadrant, and showing a significant variability of PC2 in the two years.

### Correlations between agronomic, milling and quality parameters

Considering first the one-to-one relations, Pearson's correlation coefficients among agronomic and quality traits for the 26 genotypes in 2012 and 2013 are presented (Table 7). PH correlated significantly ($p < 0.05$) with other agronomic parameters and had positive correlation with GP, pGW and kW and was negatively correlated with PM2 and EG. PM2 and GP were negatively correlated (-0.4903, $p < 0.001$). As expected, pGL, pGW, kL and kW correlated positively with TGW. However, pLW had negative correlation with TGW values.

There were only six statistically significant one-to-one correlations between quality parameters, as depicted in Table 7. AC correlated positively with kLW ($p < 0.05$) and SB ($p < 0.001$), and negatively with PV ($p < 0.001$) and BD ($p < 0.001$). BD had a positive correlation with PV ($p < 0.001$) and kW ($p < 0.05$). A positive correlation was observed between SB and kLW ($p < 0.01$). With regards to PV values, an increase in kW increases PV by 0.3930 ($p < 0.01$). However, PV is negatively correlated with kLW and an increase in kLW decreases PV by a value of 0.4914 ($p < 0.001$). kLW was significantly correlated with all the quality parameters, followed by kW. This suggests the importance of kLW in rice varietal distinctions.

AC only significantly correlated with one of the agronomic parameters: EG (0.3098, $p < 0.05$). EG correlated with other quality parameters, with a positive effect on AC kL

**Table 7: Pearson`s correlations coefficient among agronomic, biometric and quality traits of data obtained from 26 genotypes in years 2012 and 2013**

| Traits | PH | PM2 | GP | EG | GY | pGL | pGW | pLW | TGW | AC | kL | kW | kLW | PV | BD | SB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PM2 | −0.3592* | | | | | | | | | | | | | | | |
| GP | 0.2877* | −0.4903*** | | | | | | | | | | | | | | |
| EG | −0.3148* | −0.1404$^{NS}$ | −0.0276$^{NS}$ | | | | | | | | | | | | | |
| GY | 0.1584$^{NS}$ | 0.2116$^{NS}$ | −0.3462* | −0.1641$^{NS}$ | | | | | | | | | | | | |
| pGL | 0.0697$^{NS}$ | −0.0044$^{NS}$ | −0.0065$^{NS}$ | 0.1069$^{NS}$ | −0.1950$^{NS}$ | | | | | | | | | | | |
| pGW | 0.3484* | −0.4338** | −0.0316$^{NS}$ | −0.0832$^{NS}$ | 0.1426$^{NS}$ | 0.0968$^{NS}$ | | | | | | | | | | |
| pLW | −0.2578$^{NS}$ | 0.3768** | 0.0224$^{NS}$ | 0.1534$^{NS}$ | −0.2228$^{NS}$ | 0.5264*** | −0.7897*** | | | | | | | | | |
| TGW | 0.2692$^{NS}$ | −0.1269$^{NS}$ | −0.3675* | 0.0647$^{NS}$ | 0.2492$^{NS}$ | 0.2960* | 0.5844*** | −0.3242* | | | | | | | | |
| AC | 0.2330$^{NS}$ | 0.0165$^{NS}$ | 0.2508$^{NS}$ | 0.3098* | −0.0534$^{NS}$ | −0.0980$^{NS}$ | −0.2742$^{NS}$ | 0.2120$^{NS}$ | −0.1718$^{NS}$ | | | | | | | |
| kL | 0.1037$^{NS}$ | −0.0294$^{NS}$ | −0.2890* | 0.3215* | −0.0899$^{NS}$ | 0.4237** | 0.1048$^{NS}$ | 0.1859$^{NS}$ | 0.4386** | 0.1454$^{NS}$ | | | | | | |
| kW | 0.4624*** | −0.3040* | −0.0450$^{NS}$ | −0.1718$^{NS}$ | 0.1986$^{NS}$ | 0.1166$^{NS}$ | 0.7324*** | −0.5766*** | 0.7999*** | −0.2361$^{NS}$ | 0.0919$^{NS}$ | | | | | |
| kLW | −0.3601* | 0.2794$^{NS}$ | −0.1265$^{NS}$ | 0.3321* | −0.2092$^{NS}$ | 0.1239$^{NS}$ | −0.6215*** | 0.6385*** | −0.4678*** | 0.3248* | 0.4551** | −0.8402*** | | | | |
| PV | −0.0255$^{NS}$ | 0.1330$^{NS}$ | −0.1845$^{NS}$ | −0.3622* | 0.0906$^{NS}$ | −0.0460$^{NS}$ | 0.2497$^{NS}$ | −0.2826$^{NS}$ | 0.3281* | −0.6334*** | −0.2039$^{NS}$ | 0.3930** | −0.4914*** | | | |
| BD | −0.2079$^{NS}$ | 0.2243$^{NS}$ | −0.2293$^{NS}$ | −0.2995* | 0.0107$^{NS}$ | −0.0470$^{NS}$ | 0.1802$^{NS}$ | −0.2216$^{NS}$ | 0.3231* | −0.6561*** | −0.1490$^{NS}$ | 0.3324* | −0.3995** | 0.9539*** | | |
| SB | 0.1967$^{NS}$ | −0.1367$^{NS}$ | 0.3135* | 0.2285$^{NS}$ | −0.0168$^{NS}$ | −0.0532$^{NS}$ | −0.2983* | 0.2609$^{NS}$ | −0.4846*** | 0.7039*** | 0.0325$^{NS}$ | −0.4575** | 0.4482** | −0.8936*** | −0.9459*** | |
| MY | 0.0615$^{NS}$ | 0.4275** | −0.6010*** | −0.1600$^{NS}$ | 0.4848*** | −0.0617$^{NS}$ | −0.0794$^{NS}$ | 0.0224$^{NS}$ | 0.0670$^{NS}$ | −0.1384$^{NS}$ | −0.0252$^{NS}$ | −0.1154$^{NS}$ | 0.0898$^{NS}$ | 0.1451$^{NS}$ | 0.0607$^{NS}$ | −0.0527$^{NS}$ |

PH: plant height, PM2: number of panicles per square meter, GP: number of grains per panicle, EG: percentage of empty grains, GY: grain yield, pGL: paddy grain length, pGW: paddy grain width, pLW: paddy length/width ratio, TGW: thousand grain weight, AC: amylose content, kL: kernel length, kW: kernel width, kLW: kernel length/width ratio, BD: breakdown, PV: peak viscosity, SB: setback, MY: milling yield. *, **, ***: Significant at 5%, 1% and 0.1% levels; NS: Not-significant.

and kLW and negative for BD and PV. GP was positively correlated with SB (0.3135, $p < 0.05$) whereas a negative correlation was observed with kL (-0.2890, $p < 0.05$) and MY (-0.6010, $p < 0.001$). As expected, the paddy and polished biometric parameters were highly correlated and the positive relation between pGW and kW was the most significant ($p < 0.001$). TGW was an indicator of biometric and pasting behavior significantly influencing pGL (0.2960), pGW (0.5844), kL (0.4386), kW (0.7999), kLW (-0.4678), PV (0.3281), BD (0.3231) and SB (-0.4846). However, MY and AC were not directly correlated to TGW.

## DISCUSSION

The genotype was found to be the dominant influence on total variability of agronomic parameters, with maximum effect on PH (88%), higher than the value of 73% obtained by Negrão et al. (2013). Notwithstanding, the year had a significant influence in most of agronomic parameters, in most cases the interactive effect indicating a different behavior between gentoypes. Singh et al. (2013) also detected a significant effect of Genotype x Environment interaction on plant height, but not for panicle length.

The 2013 season was characterized by a cold and rainy spring compared to 2012, conditions that had consequences on plant emergence, which was worst in 2013. The prevalence of low temperatures at sowing generally results in poor rice seed germination, seedling establishment and plants vigor (Chen et al. 2005).

Furthermore, several other significant temperature differences, favorable to 2012, between the two years for traits like PM2, TGW or GY, could be explained in part by differences in maximum values reached between the two seasons (Fig. 1). Temperatures above 35 °C (heat stress) that occurred in 2013 during the period between panicle initiation and heading can represent a constraint to number of panicles and subsequently to yield. Higher maximum temperatures during grain filling period in 2013, especially in the final phase of grain filling, could also negatively influence grain weight. In addition, minimum temperatures during those periods were also quite high. Several studies (Peng et al. 2004; Fitzgerald and Resurreccion 2009; Jagadish et al. 2010; Welch et al. 2010; Kim et al. 2011) concluded that abnormally high maximum and minimum temperatures during the flowering phase can produce some infertility problems in rice spikelets. Furthermore, heading and anthesis time in 2012 occurred in a period with higher temperatures (30.2 °C, in average) compared with 2013 (27.9 °C, in average), which in addition to the temperatures peaks (above 35 °C) that arose in 2012, could have been an environmental constraint (heat stress) to

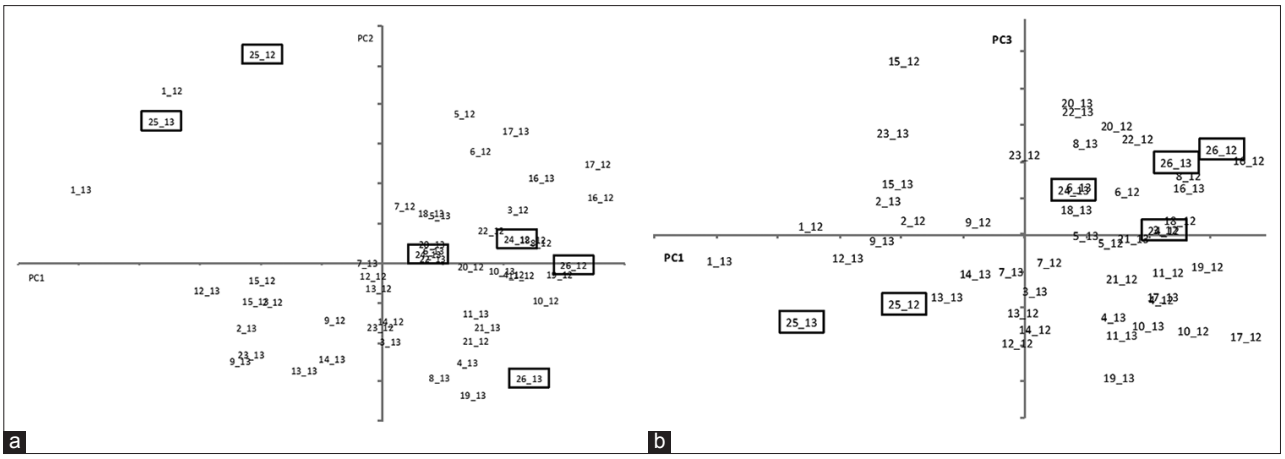



**Fig 4.** Quality space defined by the 3 principal components of the quality and milling parameters. Scores of the PC values of the commercial varieties are highlighted. The first digits indicate the genotype as per table 1 and the latter 2 digits the harvest year. (a) Scores of the first 2 PCs; (b) Scores of the 1st and 3rd PCs.

rice plants with consequences on spikelet fertility, namely number of grains per panicle (Jagadish et al. 2007).

The results obtained are coherent due to the cereals crop compensatory mechanisms of yield components (PM2 and GP), which mean that less panicle resulted in more grains per panicle and consequently lower grain weight (TGW). Zhu et al. (2002) also observed that with an increase in panicle per hill, the spikelet per panicle decreased. In 2013 season, genotypes in average obtained more grains per panicle, resulting in a lower grain weight. This season was marked by a cold and rainy spring compared to 2012, conditions that had consequences on plants emergence, which was worst in 2013, resulting in fewer plants per surface and consequently less PM2. The prevalence of low temperatures at sowing generally results in poor rice seed germination, seedling establishment and plants vigor (Chen et al. 2005).

Despite the highlighted crop season effects on grain yield and yield components, responses are mostly genotype dependent (Jeng et al. 2006). However, it was observed that the differences between genotypes that are sister lines could be almost as high as the differences between genotypes with different genealogy.

Advanced lines OP1001, OP1203 and OP1212 were considered overall to show better phenotypic traits under field environment than all the others, as well as the best agronomic and quality traits for their inclusion in the rice market. OP1001 denotes shorter plant height, significant advantage compared with taller OP1203 and OP1212 more susceptible to lodging, leading frequently to grain yield losses (Aguilar, 2010). In fact, breeding programs aim to obtain new genotypes that are shorter than the parents, but as long as these shorter lines have other outstanding features at the same time (Guimarães et al. 2006). Of these, OP1001 was also very stable, with only the yield being affect by the different years with significance. The other 2 were stable in terms of yield in the two years, but showed a bit less consistency in biometry (OP1203 had significantly higher length to width ratio of the paddy grain in 2013, and OP1212 a lower paddy grain length). The latter two were also among the highest yields, which suggest their greater interest for farmers compared to OP1001. However, OP1202 had both high yield and a better size uniformity between the different years, and is therefore more stable to the difference in growing conditions of the different years.

Several significant correlations within agronomic and quality traits were observed and expected such as the yield components, the paddy and polished biometric parameters and the viscosity parameters. The amylose content related more closely to the viscosity parameters than the biometric data. A strong relationship between amylose content and the rice grain Indica biometric pattern has already been proven (Yadav et al. 2007; Biselli et al. 2014), but this relation was not found with these genotypes that contain more japonica germplasm and thus also lower amylose and L/W values. The correlation between amylose content (AC) and viscosity parameters has been extensively reported by other authors (Wang et al. 2013; Kaur et al. 2014).

The grain yield (GY) is only correlated with number of grains per panicle (GP) and milling yield (MY) and not with grain weight (TGW), probably due to an unusual weather in 2013 having been able to mask or minimize some expected correlations. More years of study could bring to light correlations among the traits considered more clearly.

The correlations between the paddy and polished biometric parameters are obvious but it is noted that the polished

grain length/width ratio depends basically of paddy width and correlates quite poorly with just the length.

Contrarily to other studies (Ahmad et al. 2009; Arulmozhi and Muthuswamy 2013; Hasnain and Ali 2013; Lee et al. 2013), no relationship between AC and GY was detected, which also has occurred in the study of Ali et al. (2014) for basmati rice.

The correlation among the data suggests that the milling yield (MY) and the biometric grain parameters are quality traits more related to agronomic parameters. The number of panicles (PM2), number of grains per panicle (GP) and grain yield could be good indicators of the milling yield (MY) and the grain width can be determined by plant height (PH) and number of grains per panicle.

## CONCLUSION

The genotype has greater influence on total variability of agronomic parameters than year and interaction but weather conditions during the crop season have an important impact on traits such as grain yield and components, namely, plant height, number of grains per panicle and grain weight. Some accessions show more grain biometric uniformity than others when studying some important traits like grain length and width. Advanced lines OP1001, OP1109, OP1203 and OP1212 would be considered the best performers in terms of field behavior, agronomic and quality traits, for their inclusion in the rice market. However, OP1202 was the most stable variety, with much greater consistency both of agronomic parameters, high yields and quality traits in both years. It therefore indicates a better resistance to the variability of growing conditions caused by different weather patterns, and thus the best overall quality potential in terms of consistency. Rice breeding programs should give special focus on selecting for rice grain yield and uniformity in order to obtain new varieties adapted to local environmental conditions aiming resilience to climate change and in line with the market needs.

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### Author's contributions

Clemente Ortiz-Romero analysed the data and wrote the manuscript. Ana Sofia Almeida developed the advanced lines under the Portuguese Rice Breeding Program. Shivani Pathania wrote the manuscript. Cátia Silva and Diana Lourenço performed the physic-chemical analysis experiments, Jorge Oliveira analysed and discussed the data, Carla Brites designed and coordinated the research project experiments.

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