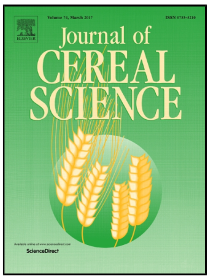

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RICE QUALITY PROFILING TO CLASSIFY GERMPLASM IN BREEDING PROGRAMS

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Highlights

- A quality space was defined with five dimensions (water absorption, composition, visual inspection, texture and rheology)
- The selection of axis of the space was based on measuring 50 different parameters of 11 varieties, which included the widest variability of behaviour of long grain rice, and applying variable reduction techniques
- The scores of a rice variety in the proposed quality space provides a fingerprint of behaviour that can be used in breeding programmes to identify directions for improvement, which is illustrated with 4 new varieties under development

Abstract

The objective of this work was to define a quality space for assessing rice varieties. Eleven long grain varieties, seven commercial and four new advanced lines were assessed to obtain complete quality profile considering appearance, physicochemical parameters, water absorption behaviour, pasting profile and textural attributes. Commercial varieties were chosen to provide the widest variation in properties, applying the variability analysis concepts of the Taguchi method, including *Japonicas*, *Indicas*, hybrids and aromatics. Quality parameters were measured in five different dimensions of quality space (totalling 50). Variable reduction techniques were applied to chose 3 parameters in each dimension (totalling 15 quality indicators) that would describe the whole space with greatest orthogonality, accuracy and yet explaining a significant proportion of the whole variance of data. The analysis of the quality space thus defined and similarities between varieties is illustrated with the conclusion of how the 4 new advanced lines perform in terms of quality behaviour, where it is concluded that one of them is very promising as an improvement over European (*Indica*) towards the behaviour of a pure Guyana (*Indica*), whereas 3 others have significant shortcomings in various aspects of the quality space compared to all others, albeit their greater closeness to *Japonicas*.

Keywords: Rice, Quality Profile, Multivariate analysis, Breeding

1. Introduction

Rice is the principal staple food for half of the world's population is consumed from ancient times and there are several varieties grown around the world, which have been adapted to cooking and consuming styles in various cultures. The quality of rice is a multidimensional concept including nutritional composition, cooking, physical and textural characteristics (Bocevska et al., 2009). Eating and cooking qualities are recognised as important traits affecting consumer acceptability of rice (Bao, 2014). Due to growing consumer demands, the global rice breeding programs target improvement in grain quality (Calingacion et al., 2014).

Many aspects of grain quality, such as milling behaviour, appearance, nutritional properties and cooking qualities, have been routinely evaluated. Starch is one of the main components in rice grain and so its physicochemical parameters such as apparent amylose content, gelatinization temperature, gel consistency, and pasting viscosity have been used as indicators of cooking and eating quality (Kong et al., 2015). Rice is consumed largely as cooked whole grains and consequently, texture is an essential attribute for palatability (Zhou et al., 2002). The elongation of grains, volume expansion as well as water absorption characteristics, are also important characteristics of cooked rice quality (Ge et al., 2005). Protein and lipid content are also important factors used to define rice quality (Zhou et al., 2002).

There is, therefore, no standard index of rice grain quality because there are many characteristics to be considered. The objective of this work was to provide a simple fingerprint of the multidimensional nature of rice quality that could be used to compare different varieties objectively. Different aspects of rice quality behaviour were deemed relevant: (i) appearance; (ii) chemical composition; (iii) water absorption; (iv) pasting parameters, and; (v) texture. In order to define a quality space with axis in each of these dimensions, it is important to define the possible variability that may exist so that the scales are sufficiently representative of the wide variety of targets that could be defined, depending on the variety and cooking uses of the rice itself. Therefore, this is not a problem that is addressed properly by using a large number of varieties chosen randomly, because this could give excessive weight to the average behaviour and not capture sufficiently well the extremes. An efficient way to quantify maximum variability within a system has been proposed by the Taguchi method, which is a popular method for robust engineering design (Ross, 1988). In order to obtain an effective assessment of variability, replicates are planned with outer arrays to ensure that the system is tested under possible extremes of variability, and with a balance that does not give more weight to the average behaviour that would bias the results towards the trivial solution. Even with just two conditions, provided they are extreme, the assessment of variability is more likely to be correct then by testing a large number of conditions that occur randomly. It is noted that estimating variability with concepts like standard deviation or variance is more difficult than estimating an average, and if observations were randomly obtained, one might need at least 50 for a normal distribution, up to 100 for other distributions, which is not effective. Although the Taguchi suggestion of outer arrays and testing the system under extremes of the noise factors is not based on statistical proof (Montgomery, 2009), it has a very good body of evidence at this stage of success in analysing (and handling) variability in engineering systems (Taguchi, Chowdhury and Wau, 2005). Giving an example of what this could mean in practice, if we wished to describe amylose content variation, for instance, instead of testing the amylose content by analysing 100 different varieties of rice to obtain a standard deviation; analysing only two, a very low and a very high variety, would define the scale of variability efficiently. As the quality domain, in this case, is multidimensional and several characteristics that can vary significantly among varieties are important, a larger number of extremes was considered. *Basmati* (from India)*, Indica* (from Guyana)*, Indica* (from a European variety), *Japonica* (Ariete variety), Japonica (Carnaroli variety) and *Jasmine* (from Thailand) were considered to provide the widest variation of behaviour for commercially relevant cultivars in Europe. It was further decided to add a processed grain to this mix of extremes, parboiled (from European *Indica*), simply because from a consumer point of view, this is a rice that can be bought instead of any of the others as if it was a different type. Due to the parboiling process causing a pre-gelatinisation of starch, it is known that these grains will show a very different behaviour, but from a cooking point of view, relevant to the user, they are important extremes, so the scale of the quality space to be defined needed to be sure of covering this behaviour as well.

In order to illustrate how a quality space defined by extremes of behaviour of the rice varieties that the European consumer chooses from can be used, four advanced lines from a breeding program, ongoing in Portugal, were added, totalling 11 different varieties to be characterised.

The selection of a limited number of parameters that can define the quality space comprehensively, yet effectively, was done by profiling quality in its possible totality by measuring all parameters that could be used to represent the behaviour of the rice samples in all dimensions of the quality space. In each dimension, parameters were then selected considering their differentiating capacity of the different rice varieties (generating a high number of homogenous groups), their repeatability (low variation between replicate measurements), and that in each dimension the selected parameters would have a high orthogonality between them and describe a high portion of the total variance of the data (high loadings in different principal components).

The data analysis techniques used varied depending on the needs for the quantity of data under analysis in each case, including partial correlations, Analysis of Variance, multilinear regression and principal component analysis.

2. Materials and methods

2.1 Materials

Different commercial types of rice (*Oryza sativa*) available in the domestic market but from different sources were obtained via the international supply chain of the Ernesto Morgado company (Figueira da Foz, Portugal): two aromatics (a *Basmati* from India and a *Jasmine* from Thailand), two *Indica* (a pure *Indica* from Guyana, and a European *Indica* grown in Portugal, variety Gladio), and two *Japonica* (an Ariete and a Carnaroli, both grown locally - the former is the most widely used cultivar for the commercial "carolino" type and the latter for *risotto* cooking). All varieties complied with the requirements for long grain classification (grains in excess of 6 mm long). A seventh commercial type was added, parboiled rice, grown and processed in Greece from European *Indica*.

For comparison and illustration of the use of the quality space in breeding programs, four varieties under development by the Instituto Nacional de Investigação Agronomica e Veterinaria (INIAV, Oeiras, Portugal) were added. These are denoted by the code numbers OP1001, OP1109, OP1212, OP1203. The former is a variety to be registered under the name Maçarico and the latter is Ceres (the other two are not in the process of being registered). These varieties result from crossbreeding, containing the genotypes Basmati x Lido, Estrela x IR72, Valtejo x Onda x Estrela, and Regina x Delta x Estrela x Suweon x Basmati, respectively

2.2 Methods

2.2.1 Grain appearance

Rice grain length (L), rice grain width (W), their ratio (L/W), chalkiness (C), total whiteness (TW) and vitreous whiteness (VW) were evaluated in 50 g samples by image processing (S21 model and software, Suzuki, Brazil).

2.2.2 Chemical composition

Starch, protein, fat and ash content were assessed by a Near Infrared (NIR) analyser (MPA, Bruker) and calibrations provided by Bruker. The amylose content (in percent) was determined by a colorimetric technique according to the ISO 6647-2:2015 method.

2.2.3 Water absorption behaviour

Weight variation in soaking (WVS) was based on the method used by Medcalf and Gilles (1965) with slight modifications. Ten g of rice were immersed in 150 mL of water in an agitated container, without heating, for 20 hours at room temperature. Weight variation in cooking (WVC) was measured with a similar method but for temperature and time. Samples of rice (150 g) were placed in 500 mL of boiling water in an agitated container for 20 min. Rice was blotted with absorbent paper to remove excess water prior to weighing.

Weight variation in adapted cooking (WVAC) refers to the quality cooking test used in the industrial company Ernesto Morgado SA. For real culinary purposes, rice must be cooked in an appropriate quantity of water and for the appropriate time to obtain a similar end result. The cooking time and the amount of water added in each case were that considered by the industry mentioned to provide a similar standard of sensory quality, as advised to consumers for home cooking. For *Basmati*, Guyana (*Indica*), European (*Indica*), Ariete (*Japonica*) and Thai (*Jasmine*) the proportion rice:water was 1:2, and the cooking time 14 minutes. For Parboiled (*Indica*) the proportion rice:water was 1:2.5, and the cooking time 17 minutes. For Carnaroli (*Japonica*)) the proportion rice:water was 1:3, and the cooking time 17 minutes.

The weight variation is just the difference between the final and the initial weights divided by the initial. It is noted that weight variation is not a measure of water absorption alone, as the rice may also leach out starch and other soluble solids. Volume-based analysis of water absorption was performed with the standard Borasio apparatus to measure the hydration capacity (MIAG BUHLER-spA). The Borasio test (Borasio, 1935) gives the volume increase (VI, in percent) of the rice due to water absorption and the volume of water that was absorbed (WA (%)) by the rice.

2.2.4 Pasting parameters

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. Viscosity curves determined 8 parameters: peak viscosity (VP1), trough (VT1), time for 1st peak (VPt), temperature at 1st peak (VPT), setback of 1st peak (VS1), setback of 2nd peak (cooling) (VS2), breakdown (VB) and final viscosity (VF).

2.2.5 Texture attributes

The texture profile analysis (TPA) of 5g samples of rice cooked in excess water (as in WVC measurements) was determined using a Texture Analyser TA-XT2 (Stable Micro Systems, London, UK). A two-cycle compression, the force-versus-time program was used with a test speed of 2 mm/s and a rate of 80% strain using a cylinder plunger with a 20 mm diameter. Twenty-five parameters were taken from each test: Peak force of 1st (P1) and 2nd (P2) positive cycles and 1st negative cycle (P-1); Area under the curve for the 1st (A1) and 2nd (A2) positive cycle and the first negative cycle (A-1); time to 1st positive (tP1) and 1st negative (tP-1) peaks; Peak ratios 1st negative / 1st positive (RP-1/1) and 2nd positive / 1st positive (RP2/1); Area ratios 1st negative / 1st positive (RA-1/1) and 2nd positive / 1st positive (Ac1)**;** Cohesiveness (Coh); Chewiness (Che); Apparent modulus (AM); Adhesiveness (Adh); Work done to hardness of 1st cycle (WH1) and of 2nd cycle (WH2); Recoverable work done in 1st cycle (RW1) and in 2nd cycle (RW2); Gumminess (Gum); Chewiness index (C Ind); Springiness (Spr); Springiness index (Spr I); Mean load (ML).

2.2.6 Statistical analysis

All analysis were made using the Statistica software (v7, Statsoft, Tulsa OK, USA). Pair-wise partial correlations were assessed with Pearson's correlation coefficients. Homogenous groups were determined with post-hoc analysis of one-way ANOVA for the parameter in question, with Tukey's Honest Significant Differences. Multiparametric correlations were determined with least squares regression. Principal Component Analysis (PCA) was done with normalised Varimax rotation. Data were normalised prior to processing to ensure equal weights to each parameter:

$$X_{norm,i} = \frac{X_i - X_{min}}{X_{max} - X_{min}}$$

where Xi is one sampling point of parameter X; X_{min} the minimum of all X value readings; X_{max} the maximum of all X value readings, and thus $X_{norm,i}$ is the normalised value, necessarily having a value between 0 and 1.

The overall similarity between different varieties was quantified from the mean squared deviation (SD) of the selected quality parameters, by calculating the Euclidean distance (ED) of the normalised parameters chosen:

$$ED = \frac{\sqrt{\sum_{k=1}^{n_p} \left(X_{norm,k,j} - X_{norm,k,,ref}\right)^2}}{n_p}$$

where $X_{norm,k,j}$ denotes the normalised value of chosen parameter k for rice variety j and $X_{norm,k,ref}$ the corresponding value for the same parameter for the variety against which the others are being compared, and n_p is the total number of parameters chosen to describe the 5 dimensions of the quality space (n_p=15).

3. Results and discussion

3.1 Water absorption

The number of Tukey homogeneous groups (95% confidence level) and the average standard deviation for each parameter are shown in table 1. The partial correlation coefficients between all pairs are not shown, the lowest correlation (0.022) was between WVC (weight variation in cooking) and WVS (weight variation in soaking). These were also the parameters with greater distinguishable capacity (higher Tukey groups) and repeatability (lower average standard deviation) and therefore were chosen to define this quality dimension. WVAC (weight variation in adapted cooking) was highly correlated with these 2: a simple multilinear model WVAC(normalised) = a + b*WVS(normalised) + c*WVC(normalised) had a 0.91 correlation coefficient with the experimental data, therefore WVAC can be considered largely defined by the other 2 parameters and removed. VI (volume increase) and WA (water absorption) had a strong correlation between them (0.73 correlation coefficient). VI had a lower correlation with WVS and WVC (0.49 correlation coefficient for a multilinear model) than WA (0.70) and a lower average standard deviation, and therefore VI was chosen as a 3rd parameter needed to define this quality dimension fully, and WA can be excluded: a multilinear model for WA as a function of WVS, WVC and VI has a high correlation coefficient (0.83).

In conclusion, weight variation in soaking and in cooking and volume increase in the Borasio test are the parameters needed to describe the behaviour regarding water absorption. These parameters can be used to identify similarities and differences between cultivars. For instance, figure 1 shows the 2-D projections of the quality space thus defined, where it can be seen that 3 of the new varieties, OP1109, 1203 and 1212, are very different from all others, whereas OP1001 is quite similar to European indica (Gladio variety).

It can also be seen that commercial varieties and advanced lines showed mean water absorption of 30% and 28% of their weight in cold water, and indica (Parboiled) particularly had high WVS, which could be due to hydrothermal treatment leading to lower glass transition temperature of rice, hence more hydration. Water binding during soaking depends on diffusion properties of rice, which rely on grain structure, composition (protein and amylose content), post-harvest processing, soaking temperature and initial moisture content of rice (Oli, Ward, Adhikari, & Torley, 2014). The presence of proteins in surface layers and their higher water diffusion coefficient than starch aids in water absorption during soaking, however, it has been reported that high protein rice requires more water and time to cook (Husain, Hwa, & Ahmad, 1981).

Water absorption increases significantly when the temperature exceeds gelatinization temperature, with WVC values being about 10 times greater than WVS. The longer varieties Basmati, Jasmine (Thai) and Indica (Guyana) (L = 6.82mm, 6.46mm, 6.83 mm) were characterised by higher WVC values of 2.48, 2.44 and 2.12, respectively. High amylose content has been related to high WVC in literature (Juliano, 1993), however, in this study effect of length was more pronounced than effect of amylose content (amylose content = Basmati: 31.19%; Jasmine (Thai): 16.73%; Indica (Guyana): 29.43%) on WVC values. It has been reported that high amylose rice has a higher capacity to absorb moisture during cooking (Juliano, 1993; Yadav and Jindal, 2007). Yadav and Jindal (2007) observed that the changes in dimensions were greater in lower amylose content rice varieties than the higher amylose content types. Hydration properties of high amylose varieties Indica (Guyana) and Japonica (Carnaroli) are different irrespective of similar amylose content which is attributable to the potential effect of amylopectin structure and other factors (Tran et al. 2011; Traore, McClung, Fjellstrom, & Futakuchi, 2011). High protein rice has been shown to absorb water at a slower rate during cooking. It was also reported that high protein rice required more water and longer time to cook (Husain, Hwa, & Ahmad, 1981). Protein is supposed to form a thicker barrier around the starch granule, thus slowing water uptake, retarding gelatinization and swelling (Bocevska, Aldabas, Andreevska, & Ilieva, 2009).

3.2 Pasting parameters

The typical RVA test applied subjects the sample to a gradual heating, holds it at the high temperature for some time, and then cools it down gradually again, measuring the apparent viscosity continuously, which will show a first peak during the heating part of the cycle, then a trough and a second peak with the cooling part of the cycle. From these viscosity curves 8 different parameters can be determined: Value of the 1st (heating) viscosity peak (VP1), value of the 1st (heating) trough (VT1), time at which the 1st peak occurs (VPt), temperature when the 1st peak occurred (VPT), setback of the 1st peak (VS1), setback of the 2nd peak (cooling) (VS2), breakdown (VB) and final viscosity (VF). Each variety was tested 3 times

In order to seek a minimum number of parameters with high orthogonality and high probability of explaining a high degree of the total variance of all data, the first approach was to cluster the parameters with a Principal Component Analysis. The results are shown in table 2, together with the number of Turkey groups at 95% confidence level in one-way ANOVA for each parameter and the normalised average standard deviation. Parameters with high loadings in one principal component have a high degree of interdependency whereas parameters with high loadings in different PC's will have a high degree of orthogonality. In problems with a large number of parameters, such as chemometry, it is common that the individual parameters are replaced by the scores of the principal components. However, it was not desired to do so in this case because it is important to have parameters that have a low error (high repeatability). Scores of principal components will inevitably have a much higher error than individual parameters for two reasons, the propagation of error of all parameters into the score, and the error of the loading factors themselves. Thus, it was considered more appropriate to use the PCA to select key parameters from each PC, with a high loading factor and a low standard deviation, and from among each group consider also the distinguishability capacity (number of Tukey groups) and a low pairwise correlation coefficient between all parameters thus selected (high orthogonality). It can be seen that just 3 principal components explain most of the variance of the data (96%). VP1 is the most repeatable parameter (lowest standard deviation), but it is the only that does not have a clear allocation to a PC, which means that it is bound to generate too high correlations with others. This was indeed verified with the partial correlation coefficients (above 0.5 for all parameters, except 0.27 for VS1). A multilinear model using VP1, VS1 and the lowest correlated in the PC1 group with these two (that would be VF) was indeed found to be unable to explain a sufficiently high variance of two parameters, (VPT and VPt), thus, VP1 is not a good choice for reasons of orthogonality with others.

The maximum orthogonality will be obtained by selecting first one of the parameters with high loadings in PC1, the best choice is VT1 which has the highest loading factor (hence the greatest probability of high orthogonality with others) and high differentiating capacity (highest number of Tukey groups). The next choice from PC2 will be VS1 which also has a high differentiating ability, low error, and lowest partial correlation coefficient with VT1 (0.25). Finally, there is only one parameter in the 3rd PC, VPt, and it has lower correlation coefficients with VT1 (0.5) and VS1 (0.067) than VP1 would have (0.81 and 0.27, respectively).

Table 2 also shows the percent of the variance of the data of each parameter that is explained by a simple multilinear model of the 3 chosen parameters, VT1, VS1 and VPt, with are respectively the trough of peak 1 (heating), the setback of peak 1 (heating) and the time when peak 1 (heating) occurs. The % variance explained is the square of the correlation coefficient in the case of the other parameters, and the explained variance of the one-way ANOVA in the case of the chosen parameters. It can be concluded that this choice of 3 provides a very good description of the whole set of data of all 8 parameters, with high orthogonality.

The locations of the 11 varieties in this dimension of the quality space are shown in fig. 1.

The effect of amylose content on peak viscosity is not evident. Works have been reported where amylose content is positively (Singh et al., 2006; Tran et al., 2001) and negatively (Chung, Liu, Lee, & Wei, 2011; Tan & Corke, 2002) correlated with peak viscosity. Differences in the viscosity profiles are also attributed to characteristics of the starch granules i.e. amylose/amylopectin ratio and chain length distribution of amylopectin. Basmati had the highest value for trough, final viscosity and set back. Japonica varieties showed higher values for breakdown and lower values for final viscosity and setback. The low final viscosity in intermediate to low amylose varieties is explained by fewer amylose chains. The linear nature of the amylose molecule allows it to retrograde, forming a gel matrix in which amylopectin molecules and starch granules are embedded and sometimes take several days to retrograde. Non-waxy varieties retrograde faster, resulting in higher final viscosity than the waxy varieties as seen in this study (Cueves & Fitzgerald, 2012). High values for breakdown and low values for setback are desirable for processed rice and are indicative of high cooking quality since neither the cooked rice retrogrades nor becomes stiff upon cooling (Kong, Zhu, Sui, & Bao, 2015). For commercial varieties, setback viscosity increased with amylose content which is not due to peak viscosity, but rather because final viscosity is affected by amylose content. Advanced lines (except OP1001) had significantly different behaviour as they showed high peak viscosity, breakdown, intermediate holding strength (trough) and low set back values. OP1001, however, had a pasting profile similar to Basmati, Indica (Guyana) and Indica (European). Parboiled rice had minimum values for all the pasting parameters. Elevation of final viscosity and the decline in the breakdown and total setback time are the major changes due to parboiling (Oli, Ward, Adhikari, & Torley, 2014).

3.3 Texture attributes

Texture is a multidimensional attribute that governs palatability of cooked rice. A very comprehensive test is a two-cycle compression test resulting in a TPA (texture profile analysis), which provides a large number of parameters, 23 in total. Table 2 shows the results of a principal component analysis of the normalised values of all parameters, as well as the normalised standard deviations of repeats and the number of homogenous groupings with one-way ANOVAs in each case. The peak force of the 1st cycle (P1) had the highest loading in PC1 and is also a quantification of hardness, perhaps the most fundamental texture characteristic, and, therefore, was chosen as a key parameter. Considering then a key parameter from PC2, the highest loading was for Adhesiveness, but this parameter did not have a very good capacity to differentiate between varieties, having generated only 3 Tukey groups in its one-way ANOVA and therefore the negative peak force in the release part of the 1st cycle was preferred as key parameter for the 2nd PC. It also has a clear physical meaning, being the maximum adhesive force, and was called "stickiness" for further reference. As expected, the partial correlation coefficient between these 2 chosen parameters is very low (0.071). A third parameter needs to be chosen, but those with high loadings in the 3rd and the 4th PCs have a very poor capacity to differentiate between varieties, with only 2 Tukey groups for springness and springness index and no significant differences detected between any variety in the case of the ratio of the 2nd to the 1st peak. Thus, it was preferred to choose as a 3rd parameter one of those without a high loading factor in any of the PCs. The mean load also had no capacity for differentiation, so cohesiveness and ratio of the negative to positive areas of the first cycle would be better options. However, cohesiveness had a high partial correlation coefficient with one of the parameters already chosen (0.722 with P-1), whereas the area ratio between negative and positive peaks of the first cycle had low correlations (0.271 and 0.13 with P1 and P-1, respectively). It is, therefore, a good choice for a 3rd key parameter with high orthogonality and differentiating capacity. It quantifies the ratio of energies involved with adhesion and compression (energy released by the mechanical recovery over the energy spent in the initial compression) and was thus designated "recovery" for future reference.

In order to assess whether 3 parameters would suffice to quantify all the texture data, a polynomial 2nd order surface was used to fit the data for each of the other parameters. There were only 4 were a simple combination of the chosen 3 key parameters could not explain satisfactorily the variance of the data: springiness, springiness index, ratio of 2nd to 1st peak forces and cohesiveness. However, these are also parameters with a very poor usability to differentiate between varieties, with only 2 or 1 Tukey groups, and therefore it was considered that the added complexity of bringing a 4th parameter to accommodate this data better was not worthwhile.

Fig. 1 shows the location of the 11 varieties in the quality space defined by the 3 parameters chosen.

Hardness is the most commonly measured parameter (Zhout, Robards, Helliwell, & Blanchard, 2002). *Indica* (Parboiled) and *Indica* (Guyana) showed the highest values with lowest being *Jasmine* (Thai) and *Basmati* varieties. Several studies have demonstrated that parboiling improves textural properties such as hardness, cohesiveness, gumminess, chewiness and resilience in cooked grain (Bello, Baeza, & Tolaba., 2006; Zhout, Robards, Helliwell, & Blanchard, 2002). Bello et al. (2006) reported harder texture in cooked parboiled rice than the untreated counterpart. The hardness value of other varieties was in a similar range, yet the results had some significant difference among them. Bocevska, Aldabas, Andreevsha, & Ilieva (2009) stated that amylose content has a strong influence on rice texture. Higher amylose content corresponds to harder texture in general. This was not observed in these data, as basmati, which was the softest, had a high amylose content. Generally, rice varieties with high amylose content (over 25%) cook dry, are less tender and become hard upon cooling while those with low amylose content (below 20%) cook moist and are sticky (Dipti, Bari, & Kabir, 2003). Low protein rice samples are tender than high protein samples of the same cultivar (Hamaker and Griffin, 1993). It suggested that the existence of a protein barrier and the hydration of the protein together influenced viscosity curves, which would determine the texture of the cooked rice (Derycke et al., 2005).

There is a clear differentiation in cohesiveness values for commercial varieties, *Japonica* (Ariete) and the advanced lines. Cohesiveness values were positively correlated with the grain length, L/W, protein content ($p<0.01$) and amylose content ($p<0.05$). Results were similar to works documented by Mohapatra & Bal, 2006. However, the values of springiness are similar for almost all the samples. Odenigbo et al., (2014) reported that springiness of cooked parboiled and non-parboiled rice was similar. Gumminess is the energy required to disintegrate a semi-solid food to a readiness for swallowing. The value of gumminess for parboiled rice was substantially higher than commercial and advanced lines. Non-parboiled high amylose rice has been known to have low gumminess with a high value for adhesiveness (Odengibo et al., 2014), which is similar to the results shown here. Grain length, L/W and amylose content were found to have a positive correlation with adhesiveness ($p<0.01$). However, Mohapatra and Bal (2006) reported that raw rice having high amylose content had lower adhesiveness values compared to low amylose and medium amylose varieties. This was attributed to the fact that high amylose rice has a dry and fluffy texture when cooked, compared to a lower amylose variety (Mohapatra and Bal, 2006).

3.4 Grain appearance

There were 6 parameters measured for visual appearance: 3 biometric - length (L), width (W), the length-to-width ratio (L/W) and 3 colour - total whiteness (TW), vitreous whiteness (VW) and chalkiness (C). Obviously, the 3 biometric parameters are linearly dependent (from 2 of them the 3rd is immediately given).

A Principal Component Analysis showed that the 6 parameters clustered in just 2 principal components explaining 87% of the total variance of the data, with the 3 colour parameters having high loadings in the first principal component, and the 3 biometric ones with high loadings in the second. Size and white/chalk parameters are therefore highly orthogonal. Length showed the lowest correlations with total and vitreous whiteness (0.04 and 0.008) and also a very low correlation with chalkiness (0.175) and was, therefore, the first choice. Vitreous and total whiteness were very highly correlated (0.97). Vitreous whiteness had the lowest correlation with the biometric parameters and therefore either one is a possible choice for the colour parameters. However, choosing only 2 parameters (one from each principal component) would not describe the data sufficiently (a multilinear model with only these 2 gives low correlation coefficients for chalkiness, 0.58). All combinations were tried and the best was to choose as defining parameters of the visual appearance the length, the width and the total whiteness. A multilinear model of these 3 has a high correlation coefficient with chalkiness (0.81), and obviously also with L/W and with vitreous whiteness. Thus, these 3 parameters represent the full set very well. Figure 2 shows how the 11 varieties spread in this dimension of the quality space.

Consumer preference of rice is invariably dependent on appearance and includes whiteness, chalkiness, grain length, grain width and length-to-width (L/W). Whiteness and translucency (vitreousness) are important parameters as it has been studied that consumers prefer white and translucent grains and are prepared to pay a premium for it (Rani et al., 2006). The rice varieties studied had whiteness values ranging from 76.99 (Indica (Parboiled)) to 163.60 (Japonica (Carnaroli)). In general, Indicas had lower values than Japonicas for whiteness, vitreousness and chalkiness. The total whiteness of advanced lines was higher and vitreousness was comparable to Indicas. White and translucent grains are rated more highly by consumers (Fofana et al., 2011; Adu-Kwarteng, Ellis, Oduro, & Manful, 2003). Chalkiness is a major concern in rice breeding because it is one of the key factors in determining quality and price. The chalky endosperm consists of loosely packed, round and large compound starch granules while the translucent endosperm comprises tightly packed, polyhedral and small single starch granules. The chalky grains show significantly different physicochemical, morphological, thermal, cooking and textural properties from translucent grains. Percentage of grains with chalkiness is one of the main indices of rice-determining appearance quality (Bao, 2014).

The range for grain length, grain width and L/W ranged from 5.56 mm to 6.83mm, 2.26 mm to 4.31 mm and 2.26 to 4.31 respectively. Indicas are characterised by longer grains and slender appearance. Grain appearance is an important parameter but it has been stated that it is not easy to recognise which rice varieties grains really belong to according to their appearance (Bocevska, Aldabas, Andreevska, & Ilieva, 2009).

3.5 Chemical composition

Six compositional parameters were measured: the contents of amylose, starch, moisture, ash, fat and protein. A principal component analysis clustered in the first component with high loadings starch, moisture and protein content, and in a second component the fat and amylose contents. Ash was not strongly bound to either. Amylose and moisture contents had the best repeatability, a good number of Tukey groups and a low correlation between themselves (0.36), being in different PC's. Thus, they are good choices for this quality dimension. These two parameters would replace starch, fat and protein content as a simple multilinear model of these as a function of the chosen 2 has a good correlation (0.86, 0.72 and 0.75, respectively), but not ash (correlation coefficient of the multilinear model 0.37). Ash was also not clustered in the two principal components and had very low correlation with either moisture or amylose content. Thus, it is necessary to choose this parameter as well to cover a high percentage of the variance of the data. A multilinear model of moisture, amylose and ash content explains a high percentage of the variance of the data of the other 3 parameters (correlation coefficients of 0.72, 0.86 and 0.91 for fat, starch and protein contents, respectively).

Different rice varieties encompass varied physicochemical properties which are the basis of rice grain quality and influence advanced quality attributes in relation to pasting and textural profiles. Within the compositional data, amylose content and protein content are most important parameters. Earlier studies have reported that amylose content and protein content directly influence the cooking, eating, pasting and textural qualities of milled rice (Champagne et al., 1997; Fofana et al., 2011; Kong, Zhu, Sui, & Bao, 2015; Bagchi, Sharma, & Chattopadhyay, 2016). Rice varieties had a wide variation in amylose content, ranging from 16.73% to 31.19% for the seven commercial varieties (19.42% to 33.70% for advanced lines). Previous studies reported that apparent amylose content of different rice genotypes ranged from 1.5% to 31.6% (Singh, Kaur, Sodhi & Sekhon, 2005; Yadav and Jindal, 2007; Kaur, Panesar, Bera, & Kumari, 2014) and Lawal et al. (2011) classified varieties as waxy (0-2 % amylose), very low amylose (2 -12 %), low amylose (12 -20 %), intermediate amylose (20-25 %) and high amylose (25-33 %). The range obtained with the chosen 7 commercial varieties is, therefore, a good representation of rice with intermediate to high amylose content. As depicted in figure 2, Jasmine (Thai) with OP1109 and OP1212 had the lowest values for amylose content while OP1001 had maximum amylose content (33.70%). It is considered that amylose content is mainly linked with sensory properties of cooked rice while protein in rice is associated with textural properties of cooked rice (Juliano, 2003). The mean value of protein content in commercial varieties was 7.64%. With respect to milled rice, protein is the second most abundant constituent (after starch) ranging from 4 to 11% (Champagne, Wood, Juliano, & Bechtel, 2004). According to the classification given by Matsue and Ogata (1998), Japonica varieties usually have 6.0-7.0 % protein whereas Indica varieties have protein content in the range of 7.1-8.9 %. Protein content in Indica rice was reported to be 10.00–20.00% higher than Japonica varieties (Sun, Hou, & Zhang, 2008).

In spite of protein content having a very significant importance in rice behaviour, it is not part of the chosen parameters to define the compositional space, as its values are well predicted by the 3 chosen parameters (a simple linear combination of the values of moisture, ash and amylose content had a correlation coefficient for protein content of 0.91, and a simple quadratic surface would explain 93.9% of the variance of the protein data).

3.6. Overall quantification of differences

All scores of the chosen quality parameters were normalised between their observed minimum and maximum, and show a variability measured with 3 repeats. The overall similarity between different varieties can thus be quantified by a mean square difference, giving equal importance to each parameter. The mean square error is the same as the average Euclidean distance (ED) in the quality space, defined by:

This quantitative measure of similarity of the quality behaviour can be measured for one dimension of the space (3 parameters) or for the whole space, (15 parameters). The significance of the number obtained can be compared to the maximum ED between different repeat measurements of the same variety: when ED between two varieties is smaller than the maximum ED between repeats, there is no significant difference between the varieties. Triangular plots can be used to detail the difference.

The use of this analysis of the quality space for breeding programs is illustrated with the analysis of the 4 new varieties denoted with the codes OP. As the trials took place in Portuguese fields where the commercial varieties that are known to grow well are European Indica and Japonica (ariete), would the new varieties just be more of the same (in which case better yields would be their only interest if verified)? Or would any of them show a quality profile more similar to more interesting varieties, like a pure Indica (Guyana), or a basmati, or a carnaroli, in which case they would show potential for particular culinary uses?

There are of course many comparisons that could be shown. Figure 3 shows an example, comparing all varieties to the Guyana Indica variety. The bar chart at the centre shows that all varieties are significantly different overall (the error between repeats is just about 4% and the closest ED is 6%), with basmati being closest, followed by OP1001. This new advanced line has a closer quality space location to the pure Guyana indica than even the European indica, whereas the other 3 advanced lines are all much more different. The radar plots show the details for the 5 dimensions of the quality space. The darker lines (full for Guyana, dashed for basmati) show clearly that the main difference between these 2 is the texture behaviour, with basmati having much lower hardness, stickiness and recovery than Guyana, indeed, among the lowest in the first 2 cases observed for all varieties, whereas Guyana showed among the highest hardness and medium stickiness measured. Another significant difference is in the appearance parameters, where basmati showed the lower width. The OP1001 new variety (dashed grey line) is more similar to European Indica (full grey line) except in compositional data where it showed among the highest amylose contents and much lower ash content than European indica, and in texture, where it had a much lower recovery, lower even than that of basmati. This analysis shows in a very visual way that OP1001 is an improvement over European indica towards a closer quality behaviour to that of pure Guyana indica, and also shows the main areas of further improvement: higher water absorption (especially in cooling) and volume increase, lower moisture content.

It might be thought that maybe the other varieties would be similar to japonicas, but that was actually not the case. The other 3 show significant overall differences to carnaroli, and although they would be closer to ariete, even a European indica is closer even, so these varieties do not show a promising quality behaviour compared to any of the existing varieties. This is summarised by the mean squared deviations to the japonicas in figure 4. It may also be seen in the previous figures showing locations in the quality space that in many cases these 3 varieties were almost in a class of their own, significantly different from all others. It is also shown in figure 4 that OP1001 is the closest to a European indica, as already hinted by figure 3.

4. Conclusions

A quantification of quality attributes of rice varieties was proposed that allows to visually and speedily compare different varieties, using parameters that can be measured with accuracy and that represent with conciseness a very complex quality space.

The definition of the rice quality profiling was based on grain appearance (grain length, width, and vitreous whiteness) chemical composition (moisture, ash, amylose), water absorption behaviour (absorption in soaking and cooking, volume increase), pasting profile (trough, setback and peak time) and textural attributes (hardness, stickiness, recovery) of different commercial varieties that had been chosen in order to provide a sufficiently wide variation of the quality parameters. Illustrating the use of this quality profiling in analysing breeding program outcomes, 4 advanced lines were compared, where it could be concluded that one of the four (Maçarico, coded OP1001) had unique characteristics with regard to the others, showing a behaviour that goes from that a European (*Indica*) towards a pure Guyana (*Indica*). Other advanced lines would be closer to Ariete (*Japonica*), but with many quality traits completely different from any other variety, so they cannot be expected to perform so well the existing varieties and for consumer acceptance.

This illustrates the applicability of a quality space analysis such as this in identifying which traits are more likely to produce a rice closer to commercially valuable varieties. This is particularly important as consumers are typically loyal to specific quality traits of the existing varieties, and often the result of a breeding program could be a hybrid that satisfies no one for not being either one or another of the existing varieties. Therefore, it is important to ensure that a new variety has quality behaviour that is sufficiently close to that of commercially successful varieties.

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Table 1
Homogenous groups and average standard deviations of the values obtained for the 6 and 5 parameters defining compositional data and water absorption behaviour of 11 varieties

Parameter	Number of homogeneous groups	Average standard deviation (normalised)
Amylose	6	0.01
Starch	5	0.0706
Moisture	7	0.0111
Ash	5	0.0889
Fat	5	0.0432
Protein	7	0.029
WVS	8	0.0207
WVC	5	0.0224
WVAC	3	0.0672
VI	4	0.0785
WA	4	0.0989

Table 2
Factor loadings of a PCA with normalised varimax rotation, homogenous groups and average SD of the values defining pasting behaviour and texture profile of varieties

	Factor loadings				N. Tukey	Normalised	Variance
	PC1	PC2	PC3	PC4	groups	st.dev.	explained
Rheological behaviour							
VT1	**0.951**	**-0.264**	**0.053**		**9**	**0.0128**	**99.4%**
VF	**0.933**	-0.067	0.353		7	0.0064	96.6%
VS2	**0.779**	0.129	0.584		7	0.0114	89.5%
VB	0.146	**-0.945**	0.190		8	0.0125	89.9%
VS1	**0.476**	**0.825**	**0.294**		**9**	**0.0119**	**99.8%**
VPT	-0.240	**0.817**	-0.358		5	0.0334	74.1%
VPt	**-0.315**	**0.521**	**-0.761**		**6**	**0.0354**	**99.8%**
VP1	0.642	-0.748	0.150		8	0.00567	95.8%
% Var.exp	40.0%	39.5%	16.5%				
Texture profile							
P1 (hardness)	**0.988**	**0.029**	**0.072**	**-0.077**	**5**	**0.115**	**94.10%**
P2	**0.981**	0.032	0.061	-0.150	4	0.130	99.8%
WH2	**0.956**	0.221	0.174	0.020	4	0.154	97.6%
Ac1	**0.955**	0.124	0.078	0.075	4	0.143	93.7%
A2	**0.945**	0.263	0.167	0.048	3	0.148	97.6%
WH1	**0.944**	0.068	0.057	0.053	4	0.130	92.7%
A1	**0.944**	0.063	0.058	0.073	4	0.131	92.2%
Gum	**0.939**	0.159	0.147	-0.069	5	0.156	98.2%
AM	**0.931**	0.024	0.086	-0.232	5	0.170	96.0%
RW2	**0.911**	0.349	0.149	0.107	3	0.125	96.4%
RW1	**0.899**	0.356	0.159	0.161	3	0.122	95.6%
Che	**0.838**	0.181	0.436	0.015	3	0.109	91.4%
CInd	**0.838**	0.181	0.436	0.015	3	0.109	91.4%
tP1	**0.799**	-0.140	-0.006	0.290	3	0.176	91.6%
Adh	0.009	**0.937**	-0.109	-0.031	3	0.055	82.4%
P-1 (stickiness)	**0.082**	**0.920**	**0.153**	**-0.131**	**5**	**0.053**	**95.6%**
A-1	0.049	**0.854**	0.254	0.077	6	0.053	97.8%
tP-1	0.432	**0.768**	0.167	0.036	2	0.084	81.9%
RP-1/1	-0.499	**-0.768**	-0.226	0.095	5	0.090	99.6%
Spr	0.236	0.186	**0.924**	0.066	2	0.114	53.4%
Spr.I	0.236	0.186	**0.924**	0.066	2	0.114	53.4%
RP2/1	-0.016	0.068	-0.104	**-0.942**	1	0.130	48.0%
ML	0.105	-0.182	-0.277	0.207	1	0.243	49.0%
RA-1/1 (recovery)	**-0.171**	**-0.741**	**-0.270**	**-0.089**	**4**	**0.103**	**89.6%**
Coh	0.215	0.454	0.358	-0.122	5	0.093	76.4%
% Var.exp	48.8%	12.8%	13.6%	9.5%			

Parameters clustered in each PC are highlighted in bold and chosen ones are underlined. The % variance explained column refers to the ability of the 3 parameters chosen to explain the variance of each of the parameters with a simple multilinear model (rheological behaviour) and a quadratic surface (texture profile).

List of figures:

Fig. 1. Location of the 11 varieties in the quality space defined with the 3 parameters chosen for water absorption, rheological data and texture of cooked samples.

(The dotted lines summarise statistically significant differences expressed by the Tukey groups. 0% represents the minimum value observed of the parameter (0.2 for WVS, 1.35 for WVC, 140 for VI, 560 cP for VT1, -439 cP for VS1, 5.53 min for VPt, 351 N for P1, -10.5 N for P-1 and 0 for RA-1/1) and 100% the maximum observed (0.49 for WVS, 2.49 for WVC, 220 for VI, 2479 cP for VT1, 1732 cP for VS1, 7 min for VPt, 1930 N for P1, -74.5 N for P-1 and 0.016 for RA-1/1)

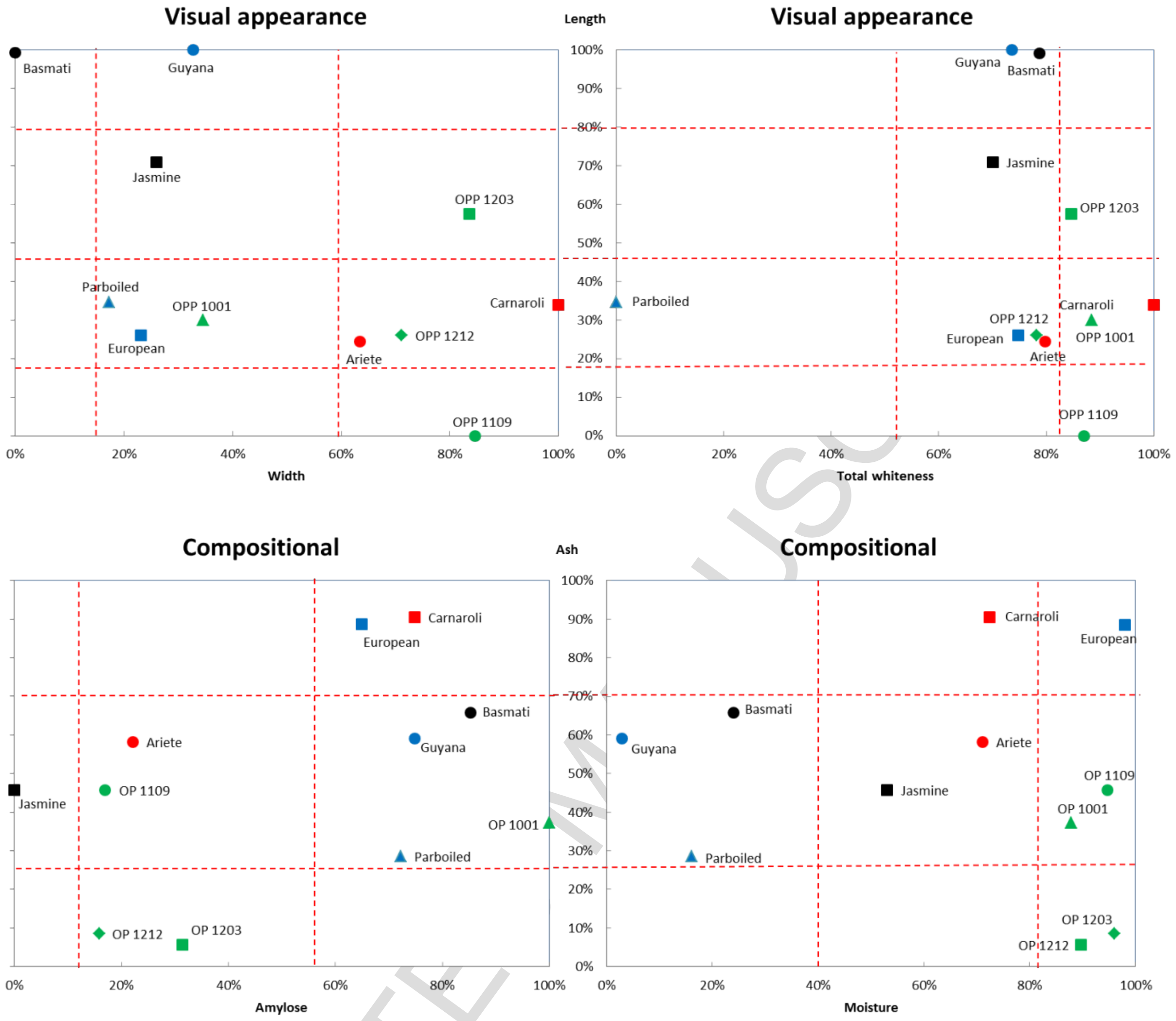


Fig. 2. Location of the 11 varieties in the quality space defined with the 3 parameters chosen for visual appearance and chemical compositional data.

(The dotted lines summarise statistically significant differences expressed by the Tukey groups. 0% represents the minimum value observed of the parameter (5.56 for L, 1.58 for W, 76.99 for TW, 16.7 for amylose, 12.7 for moisture and 0.29 for ash) and 100% the maximum observed (6.83 for L, 2.62 for W, 163.69 for TW, 33.7 for amylose, 15.3 for moisture and 0.64 for ash))

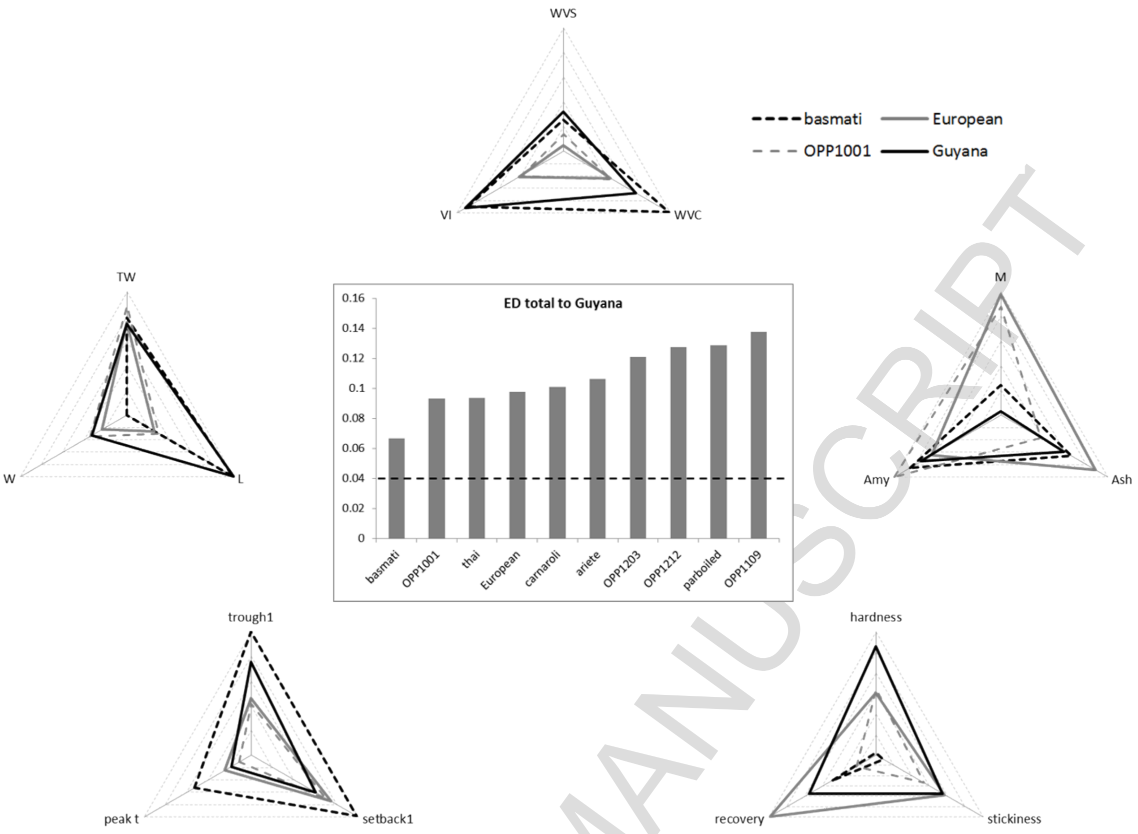
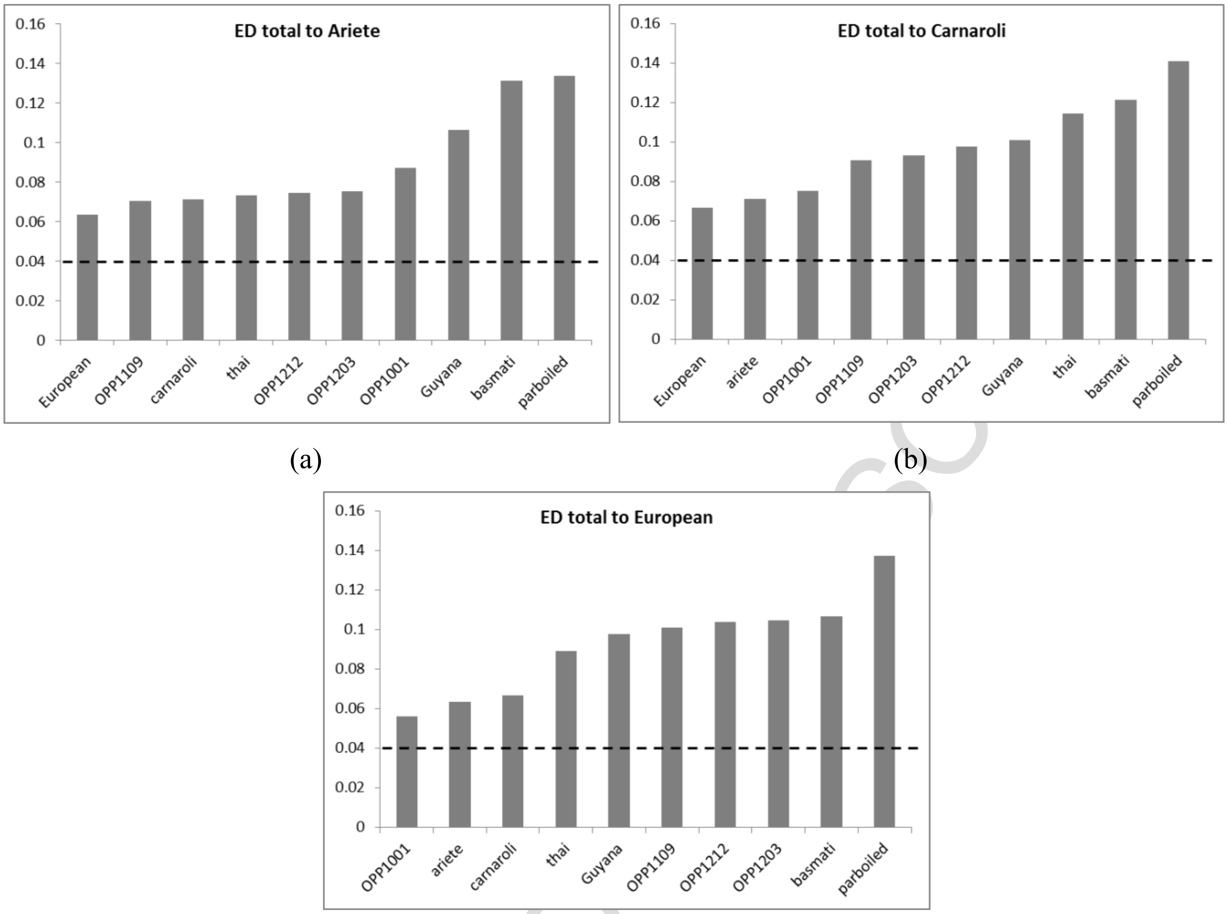


Fig. 3. Analysis of similarities comparing to pure Guyana (*Indica*).

(The dashed line in the bar plot indicates the maximum ED (mean squared deviation) between repeats. The radar plots show the average scores of 4 of the varieties being compared in detail (legend on the top right) in the 5 dimensions of the quality space defined. The radar plot gridlines mark 20% of the normalised scale, from minimum observed (0%) to maximum observed (100%) of the respective quality parameter.)



(a) (b)

(c)

Fig. 4. Mean squared deviations (ED) of all 15 quality parameters chosen for the 5 dimensions of the quality space between all varieties and the 2 *Japonicas* and the European (*Indica*). (a) Ariete, (b) Carnaroli, (c) European (*Indica*)