Omonrice 12: 157-161 (2004)

SHORT COMMUNICATION

PROMISING NEW TECHNOLOGIES FOR CLASSIFYING AROMATIC RICES

Gay, F.[1], Mestres, C.[1], Cao Van Phung[2], Nguyen Thi Lang[2], Do Khac Thinh[3], Laguerre, M.[1], Boulanger, R.[1], Davrieux, F.[1]

ABSTRACT

Semi-quantitative methods based on Solid Phase Micro Extraction/Mass Spectroscopy (SPME/MS) and Near Infrared Spectrometry (NIRS) were used to evaluate the aroma of the grain of 53 samples including 48 samples of aromatic rices (Nang Thom Cho Dao and OM3536) collected in Viet Nam and 5 samples of non-aromatic rices from France. Results show that both methods allow to distinguish aromatic varieties from non aromatic ones. Furthermore, analysis with SPME/MS allows as well to discriminate the 2 aromatic varieties used in this work. Further works are needed to improve the methods.

INTRODUCTION

Since the middle of the nineties, Viet Nam has been more and more concerned with the production of aromatic rice as it may contribute to support the competitivity of Vietnamese rice on both domestic and international markets, and to increase rice farmers' income due to higher price paid to this rice compare to ordinary rice.

A lot of aromatic varieties are available in Viet Nam. Three types could be distinguished : traditional, improved and exotic (Nghia et al., 2001). Traditional varieties have been neglected for a long time because they were not suitable for intensive production due to their long growth duration and low yield. However, as their grain quality is highly appreciated by Vietnamese people, they are paid more attention, especially Nang Thom Cho Dao (NTCD) in the Mekong Delta and Tam Xoan in the Red River Delta. Improved varieties are modern varieties with short growth duration and high potential yield. The most famous are Jasmine 85, VD20 and OM3536. At last, the exotic type concerns the two well known aromatic varieties grown in the world : Khao Dawk Mali 105 (KDML105) from Thailand and Basmati 370 from India. KDML105 has been successfully introduced and cropped in the Mekong Delta for many years while the adaptation of Basmati 370 hasn't been achieved so far.

Despite this genetic potential, several socio-economic and technical factors hampered the production of aromatic rice in Viet Nam. The most important issue is the variation of grain quality, especially aroma intensity, with the agro-ecological conditions which limits the extension of this production. For instance, outside its narrow native area, NTCD variety loses its aroma and the grain quality doesn't meet the consumer taste. The same trend is observed for KDML105 which performs better in coastal areas than in central ones (Bui Chi Buu, 2000). On the socio-economic point of view, good planning of aromatic rice production and breeding of appropriate varieties matching the demand is limited by the lack of knowledge on consumers' preferences. Basic research works including several disciplines (agronomy, crop physiology, breeding and food technology) are required to tackle these issues.

A common limit to the research works on aroma is the way aromatic level is assessed. Sensory method used by breeders is very rapid but insufficiently sensitive and reproducible. 2-acetyl-1-pyrroline, one of the

[1] French Agricultural Research Center for International Development, CIRAD, France
[2] Cuu Long Rice Research Institute, CLRRI, Vietnam
[3] Institute of Agricultural Science of South Vietnam, IAS, Vietnam

main component of rice aroma, can be assessed after extraction by various solvents, separation by using Gas Chromatography (GC) and quantification by flame ionisation detector or mass spectrometry. These procedures are however very long and tedious and cannot be used routinely. In addition, they are focused on the assessment of one single component of the aroma which is far to represent the diversity encountered in the various aromatic rices. Semi-quantitative methods based on the characterisation of the whole volatile fraction through Mass Spectrometry (MS) or Near Infrared Spectrometry (NIRS) could be useful for a quick and reliable assessment of aroma.

This communication presents a preliminary study on the application of such methods for classifying aromatic rice grown in Viet Nam.

MATERIAL AND METHODS

Rice samples

A set of 53 grain samples have been used. Among those samples, 48 were aromatic rices collected in farmers' or experimental fields in two locations in Viet Nam (Table 1). Two aromatic varieties were collected: Nang Thom Cho Dao and OM3536. The former is a traditional variety characterize by long growth duration (170-175 days), low potential yield (<4 t/ha) and intermediate to strong aroma (Nghia et al., 2001). The latter is a modern variety with short growth duration (90-95 days), high yield potential (>4t/ha) and light to intermediate aroma. The collected paddy were sun-dried then dehulled, polished and sorted out at laboratory before analysis.

The remaining 5 samples were non aromatic milled rices bought in a French supermarket.

Table 1 : Characteristics of the rice samples

Quality type	Variety or rice type	Production area	Sample numbers
Aromatic	Nang Thom Cho Dao	Long An province (Viet Nam)	1-28 41-50
	OM3536	Can Tho province (Viet Nam)	31-40
Non aromatic	Market samples	Europe and South America	51-55

Sensory analysis of aroma

Aroma intensity of each sample was assessed by sensory test in Viet Nam prior instrumental analysis in France. 20 samples were analysed at the Cuu Long Rice Research Institute (CLRRI) and the 28 other samples at the Institute of Agricultural Science of South Viet Nam (IAS). In both institute, sensory tests were performed with a procedure based on that of Sood and Siddiq (1978).

Solid Phase Micro Extraction/Mass Spectroscopy (SPME/GC-MS).

Extraction of volatile fraction of rice was performed by using a Supelco® DVB/ Carboxen/ PDMS (divinylbenzène/ Carboxen/ polydiméthylsiloxane) fiber. 4.5 g of milled rice was placed in a 10 ml vial that was sealed. It was equilibrated at 80°C for 5 minutes then the fiber was introduced in the headspace surrounding rice at the same temperature for 15 minutes. After adsorption, the fiber was directly introduced in the GC/MS injector operating in splitless mode for 4 minutes at 250°C. A Agilent 6980 gas chromatograph equipped with a DB-WAX fused silica capillary column (60 m × 0.25 mm d.i.; film thickness = 0.25 μm) coupled with a Agilent 5973N mass spectrometer was used for the GC/MS analysis. The transfer line and the injector temperature were respectively maintained at 260°C and 250°C. He at 2 ml/mn was the carrier gas. The column was maintained at 220°C for 15 mn. Source temperature was 150 °C and the mass spectra were scanned at 70 eV in the m/z range from 40 to 200 at 8,17 scans/seconde. The global signal registered between 2.8 and 10 minutes was transformed by using the Pirouette® software.

Near Infrared Spectroscopy (NIRS).

NIRS was performed using a Foss-Perstorp 6500 device operating in reflectance mode and using a spinning module with small ring cups. Spectra were registered between 400 to 2,500 nm with a 2 nm step. Two spectra were registered for each sample with two subsamples of 6 g of whole milled rice. Average spectrum was thus calculated using NIRS 2 software (4.11 release, InfraSoft International).

Statistical interpretation.

SPME/MS and NIRS datas were interpreted using Principal Component Analysis module of Pirouette® software.

RESULTS AND DISCUSSION

Sensorial analysis.

Nang Thom Cho Dao (NTCD) was rated between 0 and 2 on a 0 (non aromatic) to 3 (very aromatic) scale. The mean value calculated for the 38 NTCD samples was of 1.3. It was lower than the mean value calculated for the ten samples of OM 3536 (1.8). This was surprising as traditional aromatic varieties such as NTCD are generally considered to have higher aromatic potential than improved cultivars (OM 3536).

Typology based on the volatile fraction.

The volatile fraction was extracted from each sample using SPME and directly injected in the GC/MS system. A fingerprint Mass Spectra was obtained by the transformation of MS data with Pirouette® Software. A principal component analysis (PCA) was performed on the 53 fingerprint Mass Spectra of the milled rices. Six samples were considered as outliers (with Mahalanobis distance superior to 3) and were excluded for the calculation of the axes. The six first PCs explained 99.3 % of the total variance of the database (82.5, 13.3, 1.7, 0.8, 0.5 and 0.5 % for PC 1 to 6 respectively). Excluded samples could be clearly displayed on the plan built by the 2nd and 3th axis of the PCA (Figure 1). They comprised the 5 non aromatic rices (numbers 51 to 55) and one aromatic rice (number 50). It should be noticed that the latter was one of the two NTCD samples that exhibited a sensorial aromatic score of 0 (thus appearing as non aromatic to the panellist). This tends to indicate that the methodology used is a promising tool for discriminating aromatic rices from non aromatic ones.

In addition, the two aromatic cultivars could be clearly distinguished on the 6th axis of the PCA (Figure 2) that though represented only 0.5 % of the total variance. NTCD samples were plotted on the top of the plan built with 2nd and 6th axes whereas OM 3536 samples were all (except one, # 36) on the bottom of the graph. This methodology could hence be used to build a typology of aromatic cultivars that not only differ about their amount of 2-acetyl-1-pyrroline but also about many other contributing aromatic compounds (Petrov *et al.*, 1996, Jezussek *et al.*, 2002;).

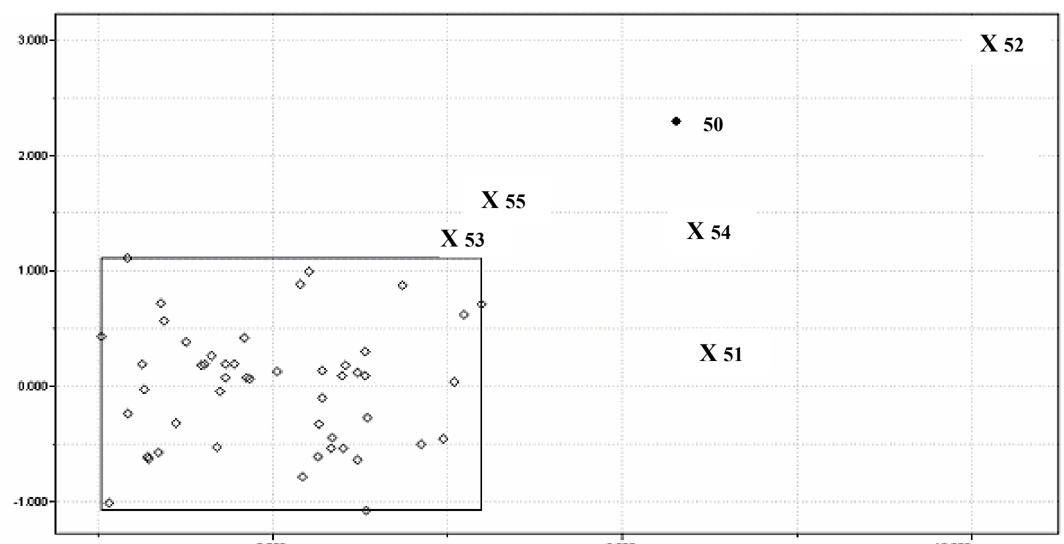

Figure 1. Scatter plot of sample score of aromatic rices (◇) on 2nd and 3rd axes of the principal component analysis. Non aromatic rices (x) and the aromatic outlier (◆) were placed speaking as additional samples

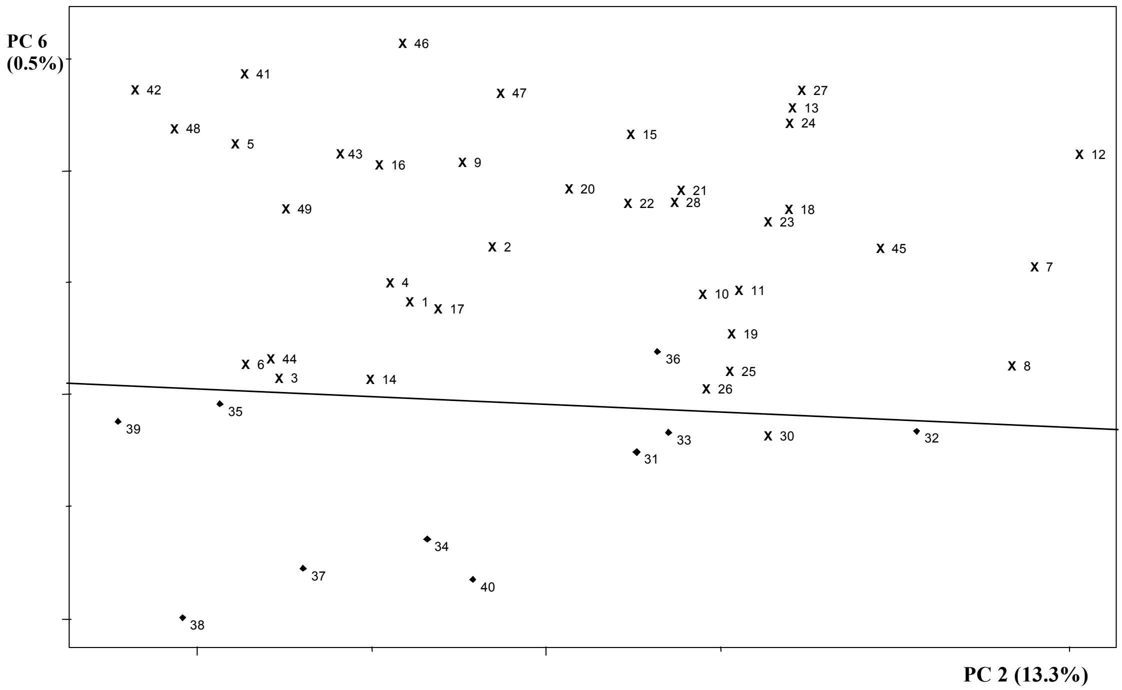

Figure 2. Scatter plot of sample score of aromatic rices on 2nd and 6th axes of the principal component analysis: Nang Thon Cho Dao (X) and OM 3536 (♦)

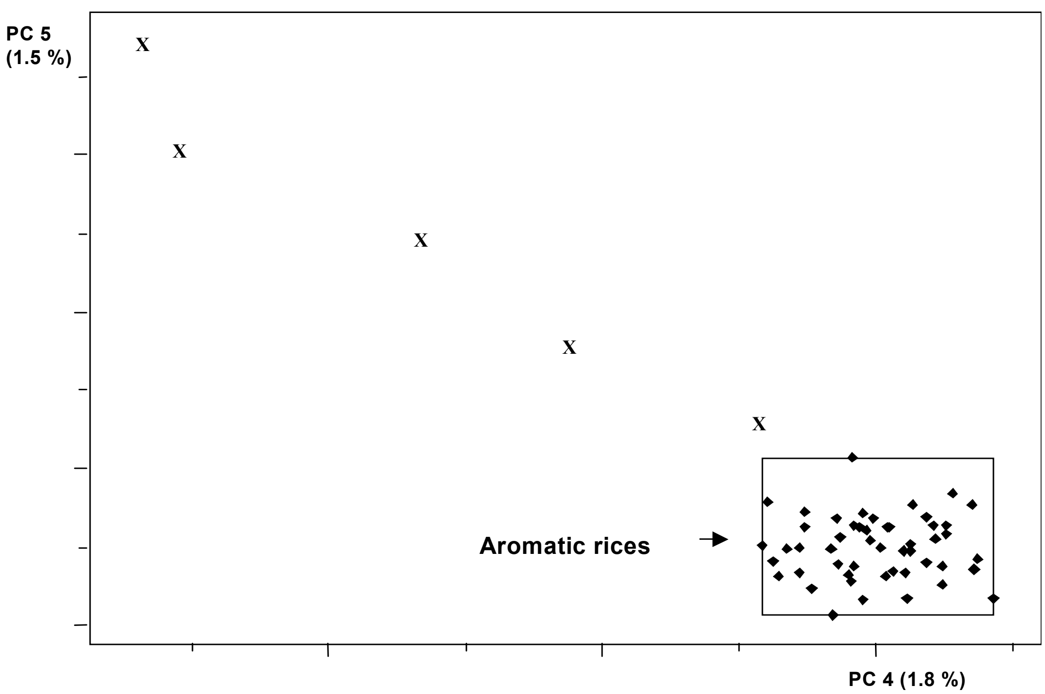

Figure 3. Scatter plot of sample score of aromatic rices (◆) on 4th and 5th axes of the principal component analysis. Non aromatic rices (X) were placed speaking as additional samples

Typology based on whole rice characteristics analysed by NIRS.

The mean reflectance spectra in visible and near infrared domain were collected for the 53 whole milled rices. As for the volatile fingerprint, the 5 non aromatic rices were excluded for the PCA analysis of NIR spectra. The six first CP explained 97.9 % of the total variance of the database (69.0, 17.9, 7.3, 1.8, 1.5 and 0.48 % for PC 1 to 6 respectively). Non aromatic rices could be clearly distinguished when plotted, as additional samples, on the 4th and 5th axis of the PCA (Figure 3). This plan accounted for 2.3 % of the total variance. This indicated that the distinction between aromatic and non aromatic rices was not based on principal variation observed in the spectral NIRS data.

In addition, a separation between the two aromatic cultivars could be evidenced on the first axis of the PCA. This distinction was thus based on the main variation in grain composition (representing 69 % of the variability) on contrary to what was observed for their volatile fraction.

CONCLUSIONS

The results of this preliminary study on the use of semi-quantitative methods to compare rice varieties on the aroma intensity of the grain are promising. Both methods allow to distinguish aromatic varieties from non aromatic ones. Furthermore, analysis of the volatile fraction allows as well to discriminate the 2 aromatic varieties used in this work. We cannot say if this discrimination is linked to genetic make-up of these varieties. However, it is interesting to note that these varieties have very different agronomic behaviour. Further works are needed to enrich the database of fingerprint mass spectra obtained from various aromatic rices and to investigate the respective role of agro-climatic conditions and genotype on the discrimination.

REFERENCES

Bui Chi Buu. 2000. Aromatic rices of Viet Nam. *In Aromatic Rices.* Singh, R.K., Singh, U.S., Khush GS eds. pp188-190.

Jezussek M, BO Juliano, P Schieberle. 2002. Comparison of key aroma compounds in cooked brown rice varieties based on aroma extract dilution analyses. *J. Agric. Food Chem*. **20**, 1101-1105.

Nghia NH, Bui Chi Buu, Luu Ngoc Trinh, Le Vinh Thao. 2001. Improvement of aromatic rice in Viet Nam. *In Speciality Rices of the World.* FAO ed. pp. 191-200.

Petrov M, M Danzart, P Giampaoli, J Faure, H Richard. 1996. Rice aroma analysis: discrimination between a scented and a non-scented rice. *Sc. Alim*. **16**, 347-360.

Sood BC, EA Siddiq. 1978. A rapid technique for scent determination in rice. *Ind. J. Genet. Plant Breed*. **38** (2), 268-271.