Authentication of Basmati rice using SSR-PCR and the QIAxcel® Advanced system

Renaud Cassier[1], Mirjana Kozulic[2]

[1] ADGENE Laboratoire, Thury Harcourt, France

[2] QIAGEN Instruments AG, Hombrechtikon, Switzerland

Introduction

Basmati rice represents approximately 40% of the dry rice market worldwide. Its import to Europe has to comply with various regulations: Council Regulation (EC) Nos 1785/2003 and 1234/2007, and Commision Regulation (EC) No 972/2006. Standard identification methods such as grain dimension, amylose content, elongation upon cooking, and aroma, are time-consuming or unreliable.

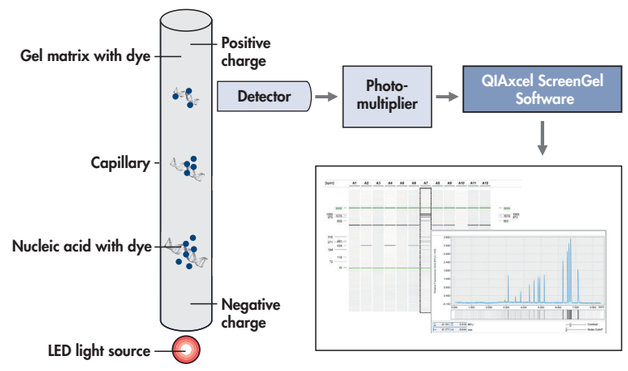
Simple sequence repeats (SSRs) are genetic markers that can be used for plant species identification but the interpretation of results for Basmati rice can be difficult, especially for samples containing more than 3 varieties. Accurate DNA fragment sizing, optimally within 2 bp, is difficult to achieve with classical electrophoresis, which is also very time consuming, especially when performing simplex analyses.

Varieties of basmati rice that can use the description 'Basmati' (1)

Eligible for zero import duty under Regulation (EC) 1549/2004	
Basmati 217	Ranbir basmati (IET 11348)
Basmati 370	Super basmati
Basmati 386	Taraori basmati (HBC-19, Karnal Local)
Kernel basmati (Basmati Pakistan)	Type – 3 (Dehradun)
Pusa basmati (IET 10364)	
Other varieties approved in India and Pakistan	
Basmati 198	Kasturi (IET 8580)
Basmati 385	Mahi Suganda
Haryana Basmati	Punjab Basmati (Bauni Basmati)
(HKR 228/IET 10367)	

Materials and methods

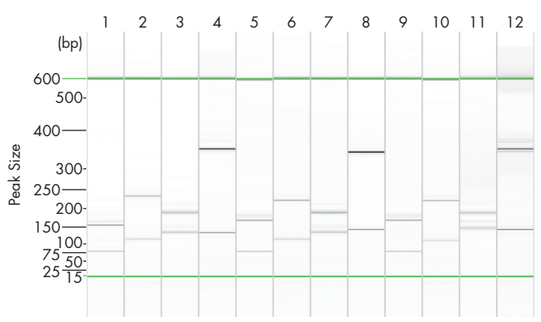
We developed a QIAxcel-based SSR-PCR protocol for Basmati rice routine analysis that allows rice varieties to be identified and quantified, and the percentage of DNA from each rice variety in a mixture can be determined. Genomic DNA was purified from homogenized and ground grains using the QIAsymphony® DSP DNA Mini Kit. Amplification was performed as duplex PCR with 8 SSR markers: RM1+RM72, RM44+RM55, RM241+RM202, and RM348+RM171. Capillary electrophoresis was performed using the QIAxcel Advanced System with the QIAxcel High Resolution DNA Kit, which facilitates resolutions from 3–5 bp up to 500 bp. We used our programmed Excel sheets for fragment identification and quantification and QIAxcel ScreenGel® software to estimate the size and concentration of the analyzed samples.



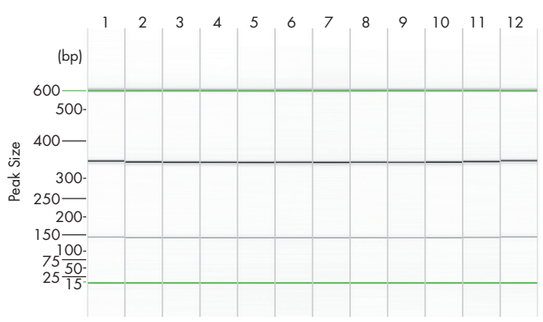
The principle of QIAxcel Advanced capillary electrophoresis.

Identification of Basmati rice

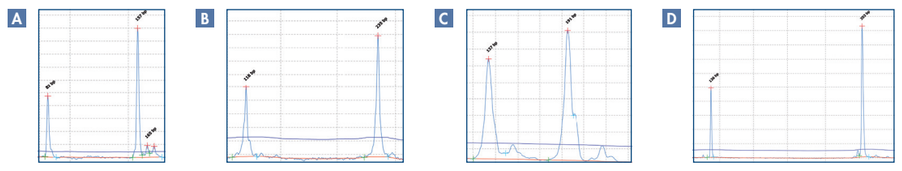
The 8 SSR markers gave high discriminatory power. We tested 13 Basmati and non-Basmati rice samples. The results show characteristic, stable, reproducible DNA fragment profiles for each rice variety. Reproducibility and stability were monitored by repeating the analysis 12 times with a Taraori variety Basmati rice sample. This result allowed us to validate existing, available rice SSR data (2, 3).



Capillary electrophoresis of 3 Basmati rice varieties. Lanes 1–4: Pusa. Lanes 5–8: Taraori. Lanes 9–12: Super Basmati. The SSR markers were RM1+72, RM44+55, RM202+241, RM171+348.



SSR profiles for Basmati rice are reproducible. Taraori variety was analyzed with the SSR markers RM1 + RM72.

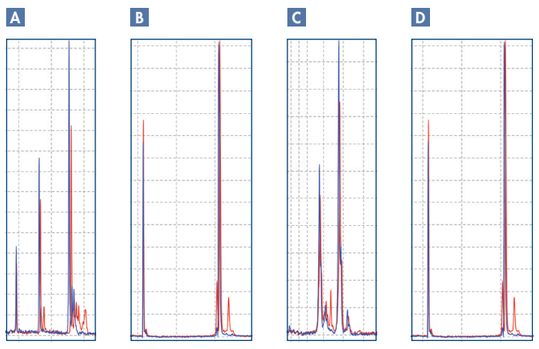


Electropherogram of SSR markers for the Pusa Basmati rice variety. A RM1+72 B RM44+55 C RM202+241 D RM171+348.

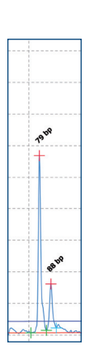
Quantification of Basmati rice adulteration

When non-basmati rice is detected in a sample, the quantity of Basmati rice is presented as a in percentage. The method is based on the comparison of the allele profiles of the known grain and the unknown, tested sample. If a new peak is detected above the threshold level, all possible profiles on all loci need to be identified. For every marker, including those for Basmati rice, the area under the peaks is calculated as a percentage. The average of all values is considered as the quantity of Basmati rice in the tested sample.

The overall uncertainty of the analysis was 5.2%. The uncertainty at more than 95% Basmati rice in the mixture (EU regulation value) was 1.9%. The quantification has an accuracy of 0.2% and a dispersion of 2.0% based on 133 measurements made on 37 different mixtures of different Basmati and non-Basmati varieties.



Comparison of Taraori grain and a sample containing 75% Taraori 75% (blue peak) and 25% adulterant (red peak). A RM1+72 B RM44+55 C RM202+241 D RM171+348.



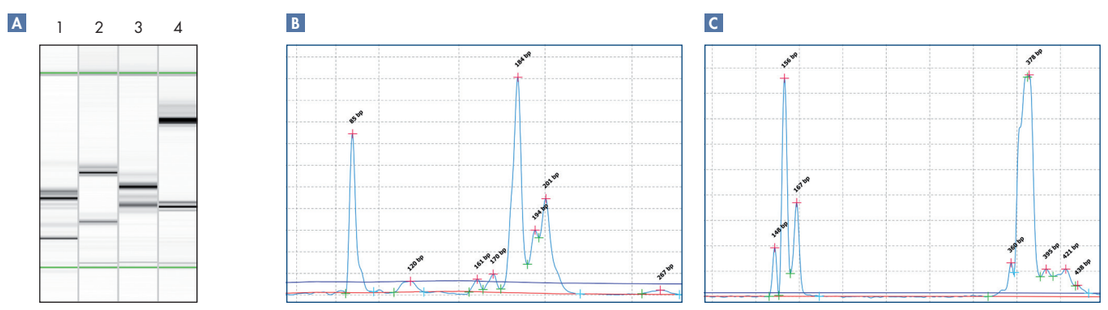
#	Size (bp)	NA	NA%
1	15	0.0018099	n/a
2	82	0.0026379	75.86
3	92	0.0008393	24.10
4	600	0.0020555	n/a

Quantification ot RM1 marker in s sample of 75% Taraori Basmati and 25% non-Basmati rice. The 82-bp peak comes from Basmati and the 92-bp peak from non-Basmati rice. The normalized area percentage (NA%) represents the quantity of the Basmati rice and adulterant.

Analysis limits

To increase the reliability of rice identification, we developed 2 additional markers (data not shown).

When a sample consists of 3 or more different rice varieties, the allele patterns become very complex, making complete sample identification and quantification impossible. However, in most cases identification and quantification can be done by analyzing all the physically different grains independently (based on grain profiles). This is time consuming and more expensive, with an uncertainty between 6.5% (>95% Basmati rice content) and 22.2% (various % Basmati rice content), with an average of 14.1%.



Analysis of a sample containing more than 3 rice varieties. A Lane 1: RM1+RM72. Lane 2: RM44+RM55. Lane 3: RM202+RM241. Lane 4: RM171+RM348. B Electropherogram of RM1 and RM72 markers. C Electropherogram of RM171 and RM348 markers.

Conclusion

- The SSR-PCR protocol for the QIAxcel Advanced System proved to be accurate, reliable, and fast. Accurate identification is dependent on a rice SSR marker allele database.
- Identification and quantification of Basmati rice and its adulterants is possible with the QIAxcel High Resolution DNA Kit, which determines the size of the SSRs with an accuracy of 2–3 bp
- The method is best suited for samples containing 1 or 2 different rice varieties. Analysis of samples with 3 or more different rice types is more complex and requires additional testing.
- The method detects adulterant contents as low as 0.1%, with quantification of up to 99.0% Basmati rice. The overall uncertainty is 5.2%, and only 1.9% for quantities of Basmati rice higher than 95%.

References

1. British Retail Consortium (2005) Code of Practice on Basmati Rice. http://www.brc.org.uk/Downloads/Basmati_Code.pdf
2. Archak, S., Lakshminarayanareddy, V., and Nagaraju, J. (2007) High-throughput multiplex microsatellite marker assay for detection and quantification of adulteration in Basmati rice (*Oryza sativa*). Electrophoresis **28(14)**, 2396.
3. Vemireddy, L.R., Archak, S., and Nagaraju, J. (2007) Capillary electrophoresis is essential for microsatellite marker-based detection and quantification of adulteration of Basmati rice (*Oryza sativa*). J. Agric. Food Chem. **55(20)**, 8112.

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