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

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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) No 1785/2003 and 1234/2007, and Commission 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.

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 purified from homogenized and ground grains using the QIAxcelphy® 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 to 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.

Identification of Basmati rice

The 8 SSR markers go 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 of the results 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).

Quantification of Basmati rice adulteration

When non-Basmati rice is detected in a sample, the quantity of Basmati rice is presented as a 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 lof rice 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% based on 133 measurements made on 37 different mixtures of different Basmati and non-Basmati varieties.

Materials and methods

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. 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.

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

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