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DNA Barcoding Uncovers the True Toll of Wind Turbines on Bats

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Image credit: Amanda Hale

DNA Mini-barcodes for Saffron Identification

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Crocus sativus L. is the most important crop plant of the Iridaceae family. Saffron, the most expensive spice in the world, consists of the dried stigmas of *C. sativus*, and they are greatly appreciated in many dishes for their unique color, taste, and aroma. Owing to its high market value, saffron has been associated with an unrivaled degree of adulteration.

Adulterations of both ground and whole stigma samples have made use of diverse materials and strategies including colorants, mineral compounds, spices, and parts of other plants, such as flowers of *Carthamus tinctorius* or stigmas of other *Crocus* spp., with the aim of increasing the volume and weight of commercial lots. Accurate analytical methods for the detection of potential saffron adulterants are therefore crucial for the evaluation of the product's value, ensuring consumer protection against fraudulent practices.

Different methods have been developed to evaluate the quality and authenticity of saffron based on standardized methods for assessing chemical and sensorial parameters. Saffron authentication relies mostly on the microscopic observation of morphological traits, which is time-consuming, requires experienced personnel, and is susceptible to subjective interpretation.

Among DNA markers, the use of barcodes has recently been proposed as a powerful pharmacognostic tool for the identification of medicinal plants and their adulterants at the species level. However, DNA barcoding entails the amplification and sequencing of relatively large DNA fragments (400-800 bp), which represents a major drawback for the analysis of processed/complex matrices.

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When analyzing samples with degraded DNA, such as processed plants, poorly preserved plants, and/or those stored for a long period of time, DNA barcodes are probably not the best target sequences. In such cases, species-specific primers based on barcodes but targeting small-size amplicons (e.g. DNA mini-barcodes) have been successfully applied for species identification. The recent approach of high resolution melting (HRM) analysis coupled with specific barcodes represents a potential cost-effective tool to detect small nucleotide differences without requiring further sequencing (Costa *et al.* 2016, DOI: 10.1016/j.foodcont.2015.09.035).

Our study focused on the identification and selection of DNA mini-barcodes to further combine with HRM analysis to identify *C. sativus* and differentiate it from closely related species, thereby assessing the authenticity of saffron. As candidate markers, we chose ITS and the chloroplast *matK* gene. New primers were specifically designed targeting three DNA mini-barcode loci, namely ITS1, ITS2, and *matK*.

Successful amplification of ITS2 was only achieved for *C. sativus* and *Crocus cartwrightianus*, a fact explained by the genetic proximity of these two species. *C. sativus* is believed to have derived from a common ancestor of the current *C. cartwrightianus*. For ITS1 and *matK*, all samples from the *Crocus* genus were successfully amplified. From the tested loci, primers targeting the ITS1 barcode region were selected for real-time PCR coupled with HRM analysis to determine their suitability for differentiating *C. sativus* from other allied species.

"With the application of HRM analysis, it was possible to distinguish C. sativus from closely related species..."

With the application of HRM analysis, it was possible to distinguish *C. sativus* from closely related species, namely wild saffron, *Crocus kosaninii*, *Crocus kotschyanus*, *Crocus speciosus*, *Crocus olivieri balansae*, and even *C. cartwrightianus* with high levels of confidence (>99.5%), which was also confirmed by sequencing. This study highlights the successful use of DNA mini-barcodes combined with HRM analysis for saffron authentication.

For more information about this research, see DOI: 10.1016/j.foodcont.2016.01.008

Images by Isabel Mafra.