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The development of a novel SNP genotyping assay to differentiate cacao clones

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Abstract (300-word limit)

Plant genetic diversity studies are of high importance for efficient plant conversation and resource strategies (eg. tackling mislabeling, conserving valuable genetic material, parentage analysis, genetic diversity studies) as they contribute to an increased knowledge on the genetic background and diversity of specific plants. These studies are most commonly analysed through simple and effective genotyping methods making use of genetic markers, such as SSRs, however SNPs are gaining more interest. Recently, a cost-effective qPCR-based method has been proposed for SNP genotyping purposes, coined double-mismatch allele-specific (DMAS) qPCR as cheap alternative to other methods. It's an accurate and fast multi-sample and multi-locus method, based on straightforward readout of DNA-binding dye based qPCR technology. Its design, optimization, validation and application on *Theobroma cacao* L., an important cash crop involved in the chocolate production, has shown successful. It offered valuable knowledge on the background of cocoa which is often plagued by mislabelling and inefficient and limited management resources. The method, optimized here, showed 98.05% efficient in calling the right cacao genotype and identified 15.38% off-types and two duplicates in an internationally recognized cacao population (n=65), using a limited amount of markers (n=42). Furthermore, only 13 markers were needed to differentiate all analysed accessions. Notably, the described method can easily be optimized and implemented in any molecular biology lab for a wide range of objectives and organisms e.g. mutation detection and to facilitate gene mapping and marker-assisted selection for breeding purposes.

Methodology and Theoretical Orientation:

In this study, first the need for more genotyping in plant specific studies with focus on cocoa is elucidated, focused on the available markers and methods, together with their advantages and disadvantages. DMAS-qPCR SNP genotyping seems a cheap and reliable alternative for such analysis and has been analysed. In this study, the design (PrimerXl), optimization and validation (sequencing and database dependent) of the DMAS qPCR SNP genotyping method is pointed out specifically for cocoa. In addition, several genotyping models have been optimised, of which two can be automated, to translate the retrieved Cq values from DMAS qPCR assay to allele calls and finally a genotype. Thereafter, its applicability on cocoa genetic diversity and mislabelling studies, using GenAlEx v6.5, has been analysed and confirmed on a Vietnamese cocoa population.

Findings:

Cocoa DMAS-qPCR based SNP genotyping method has been optimized and consist of 42 SNP markers, which showed 98.05% as efficient in calling correct genotypes. In addition 15.38% off-types and two duplicates have been identified in an internationally recognized cacao population (n=65). Furthermore, three genotyping models have been proposed, of which two could be used in an automated set-up starting from the qPCR data retrieved. Thereafter, key descriptive analysis of the markers, representing the applicability of this method in cocoa genetic diversity studies, using GenAlEx v6.5 has been described in more detail. From this analysis it has been concluded only 13 SNP markers from the DMAS-qPCR assay were needed to differentiate all accessions individually.

Conclusion and Significance:

In conclusion, we have developed a robust and accurate method for cacao genotype identification using a limited set of SNPs. The ease of use and cost-efficiency of the method – without the need of specialized instruments – can contribute to the adoption of routine-based genotyping to prevent mislabeling in germplasm collections and select optimal breeding parents in cacao and other organisms. The described method can easily be implemented in any molecular biology lab in the context of genotyping, genetic diversity studies, parentage analysis, mutation detection and to facilitate gene mapping and marker-assisted selection for breeding purposes.

Image:

Recent Publications (minimum 5)

1. Helena Everaert, Hayley Rottiers, Phuoc Hong Duc Pham, Lam Thi Viet Ha, Thi Phuong Dung Nguyen, Phuong Diem Tran, Jocelyn De Wever, Kevin Maebe, Guy Smagghe, Koen Dewettinck, Kathy Messens (2017). Molecular characterization of Vietnamese cocoa genotypes (Theobroma cacao L.) using microsatellite markers. Tree Genetics & genomes. 13(5): 99 DOI: 10.1007/s11295-017-1180-6
2. Hayley Rottiers, Daylan Amelia Tzompa Sosa, Liesbet Van de Vyver, Michael Hinneh, Helena Everaert, Jocelyn De Wever, Kathy Messens, Koen Dewettinck (2018). Discrimination of Cocoa Liquors Based on Their Odor Fingerprint: a Fast GC Electronic Nose Suitability Study. Food Analytical Methods. 12(2): 475-488 DOI: 10.1007/s12161-018-1379-7
3. Hayley Rottiers, Helena Everaert, Pascal Boeckx, Gaston Limba, Geert Baert, Jocelyn De Wever, Kevin Maebe, Guy Smagghe, Koen Dewettinck, Kathy Messens (2018). Unraveling the genetic background of the Yangambi Research Center cacao germplasm collection, DR Congo. Tree Genetics & Genomes. 14:68 DOI: 10.1007/s11295-018-1285-6
4. Jocelyn De Wever, Helena Everaert, Frauke Coppieters, Hayley Rottiers, Koen Dewettinck, Steve Lefever, Kathy Messens (2019). The development of a novel SNP genotyping assay to differentiate cacao clones. Nature scientific reports. Submitted
5. Helena Everaert, Jocelyn De Wever, Steve Lefever, Kim Hong Tang, Lam An Vu, Hayley Rottiers, Koen Dewettinck, Kathy Messens (2019). The individual and synergetic strength of SSR and SNP markers in understanding the genetic background of Vietnamese cocoa cultivars. Theoretical and applied genetics. In preparation



Biography (150-word limit-mandatory)

Jocelyn De Wever is a, soon finishing, PhD student with synergetically combined technical (lab technician) and theoretical (bio-engineering) background on applied genetics. She developed a passion in trying to understand the encrypted secrets of flavour in the cocoa its genetics. Thereafter she gained several on-field experiences concerning the cocoa cultivar selection and post-harvesting treatment. Furthermore, she is the main actor on the optimized hands-on DNA and RNA extractions from the difficult and highly contaminated cocoa tissues, and aided in the development of several cocoa genotype and transcriptome based studies.

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Notes/Comments

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