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Plant Genomics 2019: The development of a novel SNP genotyping assay to differentiate cacao clones - Jocelyn De Wever - Ghent University

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Plant genetic diversity studies are of high importance for efficient plant conversation and resource strategies (eg. tackling mislabeling, conserving valuable genetic material, parentage analysis, and genetic diversity studies) as they contribute to an increased knowledge on the genetic background and diversity of specific plants. These studies are most commonly analyzed through simple and effective genotyping methods making use of genetic markers, such as SSRs, however SNPs are gaining more interest. Recently, a cost-effective qPCRbased 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 mislabeling 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 analyzed 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 markerassisted 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 GenAIEx 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 GenAIEx 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 markerassisted selection for breeding purposes.

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