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Effective DNA extraction method for coffee leaves and other high phenolic contaminant plant tissues

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Introduction

Isolation of DNA from plant tissues which have high phenolic content is often difficult. The extraction of DNA from coffee leaf sample by general CTAB DNA extraction method is often resulted in low DNA quality with high phenolic contaminants that interfere with the subsequent manipulation. This study aimed to combined the CTAB method[1] with Tai and Tanksley method[2] as called the Combined method.

Materials/Methods

The fresh coffee leave of CM80, Typica and Robusta varieties were extracted by the combinations of two extraction buffers containing CTAB, SDS and NaCl1, as main ingredients. DNA concentration and purity were compared between the Tai and Tanksley + CTAB method and CTAB method. DNA quantity from PCR process was performed using two SSR markers and checked DNA banding on 1 % agarose gel.

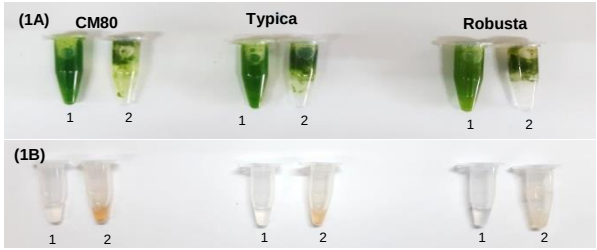

Figure 1: Comparison between Tai and Tanksley + CTAB method (as labeled by 1) and CTAB method (as labeled by 2). (1A) Extraction buffer mixed with mashed coffee leave and (1B) DAN solution

Table 1: DNA concentration and purity

Varieties	Combined Tai and Tanksley + CTAB method	DNA conc. (ng/µl)	CTAB method	DNA conc. (ng/µl)
CM80	1.85	413.1	1.55	430
Typica	1.82	395.4	1.43	452.2
Robusta	1.79	402	1.79	871.6

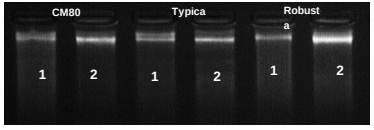

Figure 2: Checking genomic DAN banding on 1 % agarose gel **Note:** number 1 and 2 represented Tai and Tanksley + CTAB method and CTAB method, respectively.

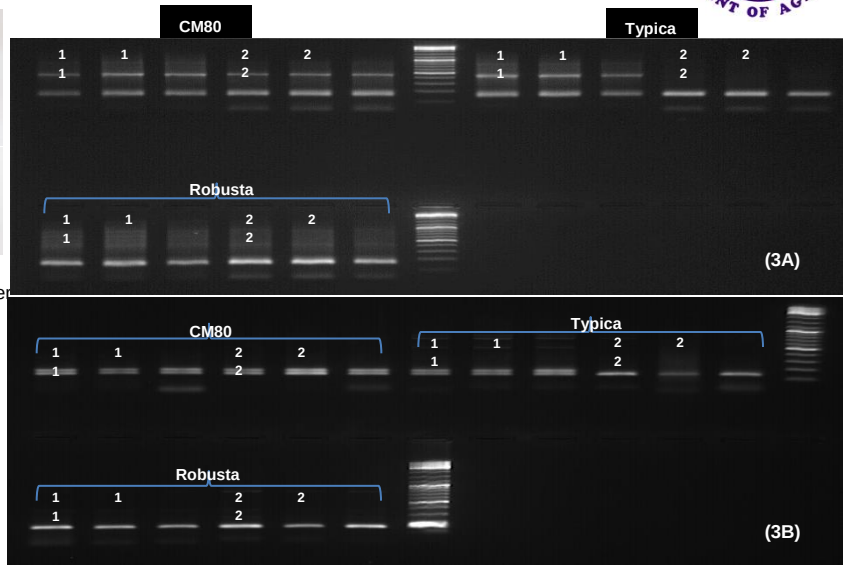

Figure 3: PCR products were amplified by two SSR primers which are specific for Coffee. Each variety was replicated three times. **Note:** (3A) SSR45 primer[3] (3B) SSR50 primer[3] ; number 1 and 2 represented Tai and Tanksley + CTAB method and CTAB method, respectively.

Results/Discussion: The high quality and amplifiable DNA from the fully expanded leaf tissue of coffee (*Coffea Arabica* L.) were successfully obtained. The quality of the resulting DNAs detected by A260/280 ratio was in the range of 1.79 to 1.86 indicating low protein and ethanol contamination. The DNA yields were ranged from 400 to 2000 ng per 1 µl from the 0.2 g fresh leaf extract. Agarose gel electrophoresis showed clear intact genomic DNA. The PCR reaction performed by SSR primer showed clear and fully amplifiable products indicting low interference from possible contaminations.

Conclusion/Perspectives: This extraction protocol is suitable for DNA extraction from coffee leaf sample. Moreover, this extraction technique can also be applied for DNA extraction of other problematic leaf sample containing high phenolic compounds.

References:
[1]Doyle JJ and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 11-15.
[2]Tai TH and Tanksley SD. 1990. A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol. Biol. Rep. 8: 297-303.
[3] Poncet et al. 2008. DOI: 10.1139/G07-073