Optimization of PCR Protocols for SSR Markers Based Molecular Characterization of *Camellia sinensis* (Tea)

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Use of SSR markers in molecular characterization of tea is useful in differentiation among tea collections to distinguish closely related genotypes. Polymerase Chain Reaction (PCR) is used to generate many copies of a selected DNA segment determined by SSR markers. Amplification of reproducible DNA segments is essential and different for different SSR markers. Objective of this study was to optimize PCR protocols for Camsin M1 and Camsin M5 tea SSR markers. PCR protocols were conducted for reaction volume of 30 µl. Concentrations of premix components were changed for 10x buffer from 2 µl to 3 µl, 2 mM dNTPs from 2 µl to 3 µl, forward and reverse primers from 0.4 µl to 3 µl, Taq DNA polymerase from 0.2 µl to 0.4 µl and DNA template from 1.2 µl to 6 µl per reaction. 3 µl of 2 mM $MgSO_4$ was added in premix preparation and number of cycles in PCR program were increased to 42 cycles from 35. For Camsin M1 annealing temperature was increased to 52 ºC while 60 ºC was used for Camsin M5. 2% agarose gel electrophoresis was used to observe PCR products and clear DNA bands were obtained for optimized PCR protocols with 1x buffer, 0.2 m M $MgSO_4$, 0.2 mM dNTPs, 1 µM forward and reverse primers, 0.7 U Taq DNA polymerase and 10 ng DNA template of final concentration for one reaction in premix for both SSR markers and 42 cycles in the PCR program. Annealing temperature was optimized at 52 ºC for Camsin M1 and remained at 60 ºC for Camsin M5. $MgSO_4$ was used as the Mg^{2+} source along with the Mg^{2+} ions included in the 10x PCR buffer. Optimized PCR protocols could be used for further analysis of SSR markers based molecular characterization in *Camellia sinensis* (Tea).

Keywords: PCR protocols, SSR markers, Molecular characterization, *Camellia sinensis*