Feasibility Assessment of Using *CafLess-TCS1* Marker to Identify Low-caffeine Tea Hybrids

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Tea (*Camellia sinensis* (L.) O. Kuntze) is the second most consumed beverage in the world. Caffeine is a purine alkaloid present in tea with known health benefits. However, excessive intake causes negative effects such as palpitations, insomnia, and allergies. Alternatively, low-caffeine tea and decaffeinated tea carry less adverse effects. Decaffeinated tea produced using mechanical methods are low in quality and have poor consumer acceptance, leading to reduced market value. Hence, attempts are made to breed low-caffeine tea cultivars. The current study assessed the feasibility of using the *CafLess-TCS1* marker to identify low-caffeine tea hybrids. Floral assessment was performed to differentiate 36 backcross progenies (BC_1) derived from crosses between the high-caffeine cultivar, TRI3055 and accessions PBGT41, PBGT48, PBGT49, into China (15), Cambod (16) and Assam (5) types. Using a one-way ANOVA and Tukey's mean separation, a selection of 20 hybrid progeny and their parents were evaluated for the caffeine content. Caffeine contents of accession PBGT49, F_1 progeny of TRI3055×PBGT49, and two BC_1 progenies each of the crosses F_1 of (TRI3055×PBGT49) × PBGT49, F_1 of (TRI3055×PBGT48) × PBGT48 and F_1 of (TRI3055×PBGT41) × PBGT41 were not significantly ($P>0.05$) different to the high-caffeine cultivar TRI3055. A PCR fragment of approximately 527 bp was amplified using the *CafLess-TCS1* marker for TRI3055 and selected low-caffeine accessions. The PCR products were resolved on a 8% polyacrylamide gel stained with silver nitrate. The resulted marker profile revealed a unique banding pattern for TRI3055, distinctive from its progeny. However, a clear correlation between the caffeine content and the *CafLess-TCS1* marker could not be derived for the low-caffeine tea accessions used. Hence, it is recommended to adopt a sequence-based approach to identify the variations in these accessions at the *CafLess-TCS1* marker locus and to see if variations observed are specific to any tea type or caffeine level.

Keywords: Caffeine, *CafLess-TCS1, Camellia sinensis*, Low-caffeine

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