Development of extension-AFLP method for conversion of AFLP markers into STS markers in wheat

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Objectives

Amplified fragment length polymorphism (AFLP) analysis has proven to be a powerful tool for tagging genes or QTLs of interest in plants. However, conversion of AFLP markers into sequence-tagged site (STS) markers is technically challenging in the case of wheat due to the complicated nature of its genome. In this study, we developed an 'extension-AFLP' method to convert AFLP markers into STS markers. When an AFLP marker of interest was detected with an *Eco*RI+3/*Mse*I+4-selective primer combination, the PCR product was used as a template for an additional selective amplification with four primer pairs in which one base (either A, C, G, or T) was added to the 3'-end of one of the two primers. The extended primer-pair that produced the targeted band was further extended by adding each of the four selective bases for the next round of selective amplification (Fig. 1).

Results

By using the extension-AFLP method, we successfully converted two AFLP markers which are located on chromosome 3BS and are associated with FHB resistance into STS markers (Fig. 2). Our results indicated that the extension-AFLP method is an efficient technique for converting AFLP markers into STS markers in wheat. The developed STS markers may be used for marker-assistant selection (MAS) for FHB resistance in wheat breeding programs.

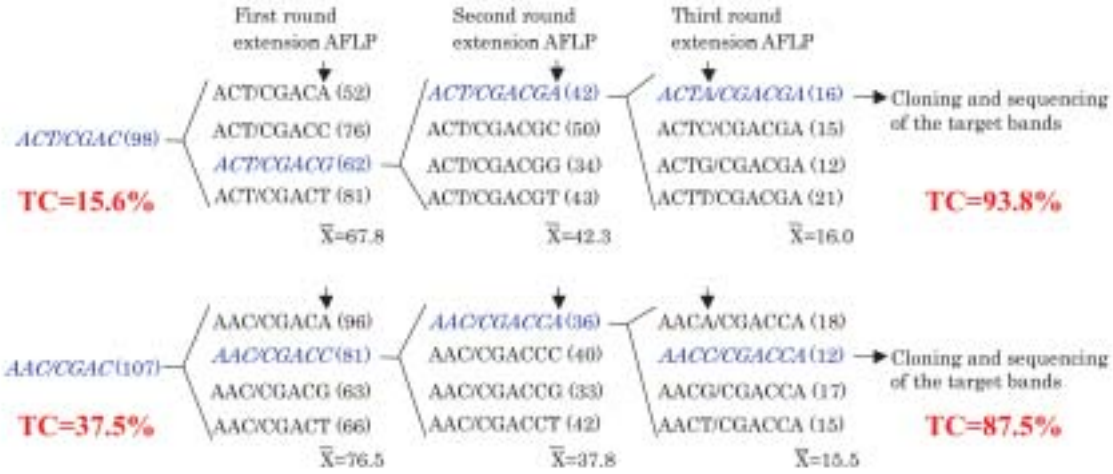

Fig. 1. Flow chart of extension-AFLP for the two AFLP markers, E-ACT/M-CGAC118-120 (upper), and E-AAC/M-CGAC285 (lower). Primer pairs that amplified the target bands are indicated in italics. Numbers in parentheses indicate the total number of AFLP bands produced by each primer combination.

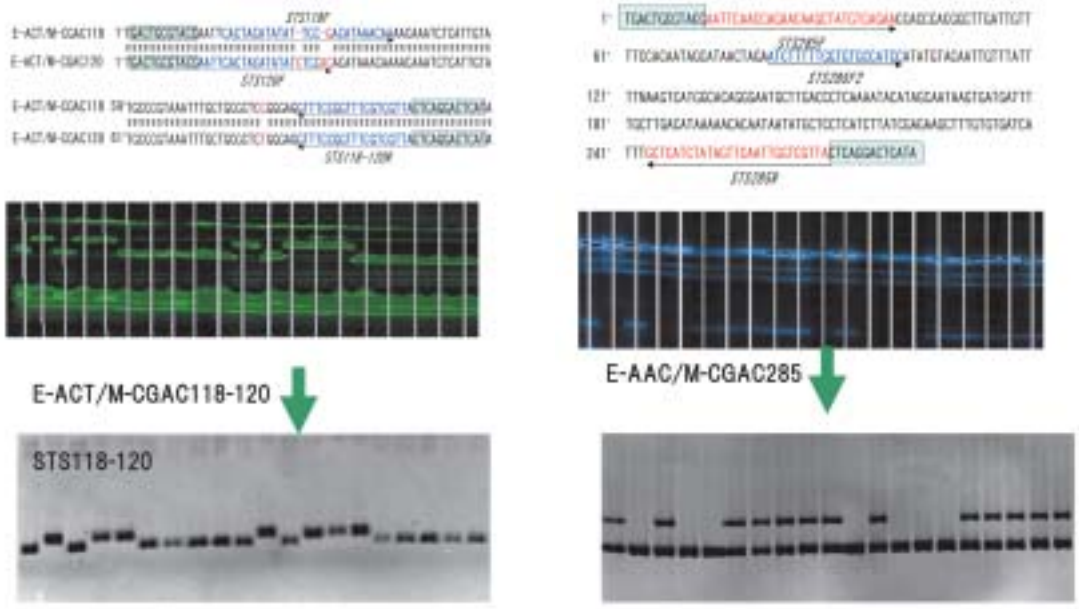

Fig. 2. Sequence alignment between two allelic fragments of AFLP marker E-ACT/M-CGAC118-120. STS primers are indicated with arrows (upper). Adaptor sequences of the two sides are shown in boxes. Band patterns of the two AFLP markers, E-ACT/M-CGAC118-120 and E-AAC/M-CGAC285, and their corresponding STS markers in a deoxycholate hydrogen sulfide lactose (DHL) population derived from 'Sumai 3' and 'Gamenya.' Lane 1, 'Sumai 3'; Lane 2, 'Gamenya'; and Lanes 3-20, DHLs.

References

Xu, D.H. and Ban, T. (2004): Conversion of AFLP markers associated with FHB resistance in wheat into STS markers with an extension-AFLP method. Genome, 47, 660–665.

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