

Developing Next generation sequencing-based methods for efficient genetic analysis and applying their methods in breeding

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Abstract

For our sustainable lives, the breeding of climate-resilient, high-yielding and pest and disease resistant/tolerant crop varieties should be accelerated. One of the most efficient methods in crop breeding is marker-assisted selection (MAS). In MAS, DNA markers tightly linked to genes controlling favorable traits are used to select the progeny inheriting the desirable traits without the need for phenotyping, which is a time-consuming and laborious step in conventional breeding. However, applying MAS requires identifying genes or genomic regions underlying target traits as a prerequisite, suggesting that this step is still a rate-limiting step in plant breeding.

Recent approaches combining next generation sequencing (NGS) and bulked-segregant analysis (BSA), such as MutMap series and QTL-seq developed by our group, have enabled rapid gene identifications¹⁾. However, there still were the cases in which these methods cannot be directly applied for mapping and identifying a gene in highly heterozygous plants and polyploidy plants. For expanding the application of these NGS-based BSA to various plants, we have improved the original QTL-seq protocol²⁾. Additionally, we developed Sat-BSA for isolating structural variations (SVs) associated with favorable trait in the progeny from a cross between cultivars³⁾. Many of NGS-based BSA relying on re-sequencing technique cannot identify the gene locating at the genomic regions displaying SVs between the cultivar for developing the segregating progeny and the cultivar for constructing the reference genome. Sat-BSA address this limitation of NGS-based BSA by combining local *de novo* assembly and analyzing the association between phenotype, SVs and gene expression patterns in multiple cultivars.

I believe that the NGS-based methods developed by our group will open enormous opportunity for rapidly and efficiently isolating gene controlling agronomically important traits and contribute to accelerate the breeding in various crops.

1) Takagi *et al.* New Phytol. 200: 276–283 (2013).

2) Itoh *et al.* Theor. Appl. Genet. 132: 2913-2925 (2019).

3) Segawa *et al.* Breed. Sci. doi: 10.1270/jsbbs.20148 (2021).