Genotyping by Sequencing: A Powerful Tool for MAS

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Abstract

The ability to withstand and overcome challenges from living organisms or environmental factors is crucial for the survival and growth of plants. The latest development in genotyping-by-sequencing (GBS) has revolutionized the field of marker-assisted selection (MAS), leading to faster advancements in plant breeding and crop enhancement.

Introduction

Utilizing molecular markers, specifically single nucleotide polymorphisms (SNPs), marker-assisted selection (MAS) is a technique in crop improvement that aids in the identification of favourable traits. The advancement of next-generation sequencing (NGS) technologies has greatly enhanced whole genome sequencing, allowing for the analysis of large amounts of genetic information that has transformed plant genotyping and breeding methods. To overcome the challenges posed by complex crop genomes like maize and wheat, genotyping-by-sequencing (GBS) has emerged as an extension of NGS applications. GBS is a cost-effective and robust MAS tool, widely used in various plant breeding programs for activities such as genome-wide association studies (GWAS), assessment of genomic diversity, genetic linkage analysis, discovery of molecular markers, and large-scale genomic selection. MAS entails utilizing molecular markers to indirectly select desired traits in crops (Zhao et al., 2014).

Need

Plant breeding can be achieved through classical breeding and molecular breeding. Classical breeding involves interbreeding closely related individuals to create new cultivars with desired traits, but it is limited in addressing global food security (Tester and Langridge, 2010). Molecular breeding includes marker-assisted selection (MAS) and genetic transformation to develop new cultivars (Moose and Mumm, 2008). although genetic transformation is hindered by concerns over food safety and environmental impacts (Nicolia et al., 2014). MAS, which uses molecular markers for indirect selection, has been widely used to enhance crop yield, quality, and tolerance to stresses. Genotyping-by-sequencing (GBS) is a powerful MAS tool is using for accelerating plant breeding and crop improvement.

Advances in NGS have made GBS (genotyping-by-sequencing) feasible for high diversity and large genome species (Elshire et al., 2011). GBS developed in Buckler lab, is a simple and cost-effective system for constructing reduced representation libraries for genetic analysis and genotyping (Elshire et al., 2011). It has several advantages like reduced sample handling, fewer PCR and purification steps, low cost, no size fractionation, no reference sequence limits, that make it efficient and easy to scale up (Davey et al., 2011) that makes it important for genomics-assisted breeding in various plant species.

Procedure

The process of genotyping-by-sequencing (GBS) involves several steps, which can be summarized as follows. Firstly, the genomic DNA is quantified using a fluorescence-based method. Next, the genomic DNA is normalized in a fresh plate to ensure that all samples are equally represented and that the molarity of the DNA and adapters is consistent. Then, a master mix containing restriction enzyme(s) and buffer is added to the plate and incubated. After that, the DNA barcoded adapters are introduced, along with ligase and ligation buffers. The samples are then pooled and cleaned. Following this, the GBS library is amplified through polymerase chain reaction (PCR). The amplified library is subsequently cleaned and evaluated using a capillary sizing system. Finally, the libraries are sequenced. In terms of data analysis, the raw data from the sequencing run, stored in FASTQ files, is used to assign sequencing reads to their respective samples by utilizing the DNA barcode sequence. Once the reads are matched to individual samples, they are aligned to a reference genome. In cases where the species lacks a complete reference genomic sequence, the reads are internally aligned, and single nucleotide polymorphisms (SNPs) are identified based on a 1 or 2 bp sequence mismatch.

Errors in sequencing can lead to the presence of single nucleotide polymorphisms (SNPs) (Zhao et al., 2014).

Applications

Genomic selection (GS), a quick and affordable method require to genotype large populations of potential selection candidates. In 2001, Meuwissen et al. introduced genomic selection as a means to incorporate all small effect loci in genomic prediction models. This approach utilizes dense genome-wide molecular markers, fitting effects to all markers simultaneously, and avoids statistical testing. By employing GS models, breeders can forecast the performance of new experimental lines early on and make informed decisions regarding crosses and selections based on these predicted results (Jannink et al., 2010). When combined with quick generational turnover, selection based on predicted breeding values determined by GBS marker data has the potential to significantly enhance gains in plant breeding programs (Meuwissen et al., 2001; Jannink et al., 2010).

Poland et al. (2012b) specifically focused on the complex wheat genome and demonstrated the applicability of GBS markers in developing GS models. Remarkably, these markers exhibited high prediction accuracies for yield and other agronomic traits, making them ideal for breeding purposes.

High-density markers derived from Next-Generation Sequencing (NGS) are becoming essential in genomics. Genotyping-by-sequencing (GBS) is a cost-effective and efficient tool for genetic mapping, breeding, and diversity studies. GBS is particularly useful for researchers working with obscure or foreign species. In plant breeding, GBS has great potential for implementing genomic selection on a large scale. Ongoing advancements in sequencing output will lower costs even further. GBS is also valuable for genomics-assisted breeding in orphan and commercial crops. Despite challenges in data management, genomic selection through GBS holds promise for enhancing traditional crop development. Sequence-based genotyping will continue to be important for diversity and genomic studies in various fields (Allendorf et al., 2010).

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