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Editorial

Application of Molecular Markers in Crop Improvement and Beyond

Dilip R. Panthee

Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, 455 Research Dr., Mills River, NC 28759, USA; dilip_panthee@ncsu.edu

The application of molecular markers in crop improvement first started in the 1980s. Initially, this was in the form of hybridization-based molecular markers, which have since become less popular. The moment PCR-based molecular markers became available, they exploded in popularity due to being easily applicable to several crop plants and easy to screen. Since then, several plant breeding programs and biological research programs have optimized the use of molecular markers associated with various traits, including disease resistance, quality, and abiotic stress tolerance. However, there are several traits that need to be optimized, and in this context, research is ongoing in several parts of the world. With the availability of genome sequences and single-nucleotide polymorphism (SNP) markers developed from the use of such sequences, high-density molecular linkage maps can be developed, and molecular markers associated with the traits of interest can be more precisely identified. Furthermore, the mapping and fine mapping of quantitative trait loci (QTL) can be achieved, and eventually, such research will help us to identify the genes associated with the traits of interest. We invited researchers from around the world to submit manuscripts in this field of research for publication in this Special Issue of the MDPI journal, *Agronomy*. In total, we received 16 manuscripts, 7 of which were accepted for publication. We briefly summarize the contributions of each of these papers below.

Evaluating genetic diversity is one of the first steps of a plant breeding program. This can be carried out based on morphological as well as molecular characterization. Bhardwaj et al. (2023) [1] conducted a genetic diversity analysis of potatoes using 25 single-sequence-repeat (SSR) molecular markers. They used a total of 353 accessions consisting of 256 Solanum tuberosum sub-sp tuberosum, 49 S. tuberosum sub-spp andigena, and 48 Indian potato varieties. They found a high level of allelic polymorphism using these molecular markers for diversity analysis. Genetic diversity can be presented in several forms including clusters, principal components, or Nei's coefficient [2–4]. They performed the cluster analysis, classified the total genotypes into five clusters, and concluded that the SSR molecular markers are suitable for the analysis of genetic diversity in potatoes. They also found six markers associated with late blight resistance, which eventually could be used for marker-assisted selection in potatoes. They performed an association analysis for late blight resistance and found a major QTL on chromosomes 1, 5, 6, 7, and 12 [1]. They concluded that SSR markers are ideal for the analysis of molecular markers in potatoes due to their desirable characteristics, including simplicity, abundance, co-dominance in nature, an extensive coverage on the genome, high reproducibility, and polymorphisms.

Zhang et al. [5] demonstrated the suitability of 18 SSR and other mitochondria-based molecular markers for genetic diversity analysis in Brassica juncea using a panel of 99 accessions consisting of 84 mustard and 15 other Brassicaceae accessions. They found three major clusters. They concluded that there was a genetic diversity in Brassica juncea from China at both nuclear and cytoplasmic levels, which could be exploited in further breeding programs.

Devi et al. [6] reported the white rust resistance-conferring gene BjuWRR1 in 30 genotypes of Indian mustard. They developed a locus-specific molecular marker and screened Indian

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mustard genotypes with molecular markers. They verified a set of ten resistant genotypes by inoculating them with a pathogen. While only five of the lines matched with the molecular markers and disease resistance, the authors concluded that the remaining five resistant lines may have novel genes conferring resistance to white rust (Devi et al., 2022) [6].

Hou et al. [7] evaluated 185 soybean accessions for low light tolerance by growing them under normal and low-light conditions. They evaluated multiple physiological traits, including plant height, stem diameter, cotyledon height, and number of beans per pod. They used a combination of 98 SSR and 639 SNP molecular markers for genotyping and identified 75 molecular markers for low-light tolerance. Furthermore, they identified eight accessions that were tolerant to low-light intensity. This indicated that low light intensity is a genetic trait, based the molecular markers associated with this trait identified by Hou et al. (Hou et al., 2022) [7].

Liu et al. [8] performed a comprehensive study of GWAS and transcriptome analysis in sweet potatoes and identified the candidate key gene, g55964, as a cause of purple coloration in sweet potato roots. They used 300 accessions of sweet potatoes, including 76 landraces and 224 modern cultivars. A total of 567,828 SNP molecular markers were used for genotyping. Phenotypic data on flesh color were collected based on visual rating. RNA-seq data (available online) were analyzed and five unique SNPs were identified. These SNPs were associated with five genes, which were verified by qRT-PCR. From all three analyses, they verified a single gene, g55964, associated with purple root color, based on which molecular markers could be developed and employed for MAS [8].

The pyramiding of the resistance allele is easier to achieve with the use of molecular markers, as shown in yellow rust resistance in wheat [9]. These authors grouped 14 genotypes of wheat into three groups, including susceptible, moderate, and resistant, based on the relative resistance index, screening these genotypes using 19 polymorphic SSR markers. They were able to identify resistant genes, including Yr5, Yr10, Yr15, and Yr26 (all-stage resistance genes); Yr18 and Yr62 (adult plant) resistance genes; and pyramids using molecular markers [9]. In a sexually propagated pathogen such as Puccinia stritiformis, the causal agent of leaf rust, it is essential to pyramid the available resistant alleles and explore new alleles to combat new types of pathogens.

Genome editing (GE) is a relatively novel tool in crop improvement in which DNA fragments can be copied and pasted within the genome of the same species. The prerequisite for GE is the availability of genome sequence and transformation technology. By using this technology, some of the issues related to genetically modified organism (GMO) crops are eliminated since there is no involvement of foreign DNA in the transformed crops. There are still regulatory issues that may take genome-edited crops off the market. Krishna et al. [10] demonstrated that it is possible to overcome this issue using transgene-free genome editing techniques. A well-established PCR-based assay and transgene-killer CRISPR system can rapidly identify transgene-free genome-edited crops. In the transgene killer system, a cassette contains the barnase gene or the CMS2 gene under the control of REG2 or CAMV35 promoter, respectively, in the CRISPR/Cas9 construct. This causes male sterility upon seed maturity. However, in the case of vegetatively propagated crops, genes such as mCherry, GFP, or DsRED cause visible coloration that will help to screen a GE callus using a visual method [10]. These genes have been cleared of any controversy and will be available on the market in due course. Krishna et al. [10] describe possible approaches to crop improvement in sugarcane, including virus-induced-genome editing, particle bombardment, agrobacterium-mediated transformation, and protoplast transformation.

In summary, the use of molecular markers was reported in all aspects of crop improvement, including diversity analysis, novel molecular marker and QTL analysis, marker-assisted selection, GWAS, gene pyramiding, and even genome editing. The articles presented in this Special Issue are excellent contributions from the scientific community to the fields of plant breeding and crop improvement.

Conflicts of Interest: The authors declare no conflict of interest.

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