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Genetic molecular markers to accelerate genetic gains in crops

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The advent of molecular marker technology changed methods of plant breeding in a positive direction. Since the boom of the genomic sequencing era, several advancements and innovations originating in the field of molecular markers are enhancing the pace of crop improvement. Over the decades, many reviews on molecular markers have been published, especially pertaining to their application in plant breeding. Here we provide an update on the evolution of marker technologies and their applications for accelerating genetic gains in crops.

Genetic markers can be described as genetic differences between individual organisms or species. We have previously provided an account of the concept, methods and applications for easy understanding by early career researchers [1]. The markers do not represent the target genes themselves essentially but act as ‘signs’ or ‘flags’ and can be classified into three major types: morphological, biochemical and DNA markers. Classification is based on the allelic variation caused by phenotypic traits, isozymes and sites of variation in DNA, respectively [2]. To select an efficient marker system, several criteria are considered. For instance, Gupta *et al.* suggested a set of criteria for an efficient marker system applicable to crop improvement: primarily the markers should be highly polymorphic, evenly distributed, preferably codominant, distinctly allelic, single-copy, cost-efficient and amenable to automation [3].

Different types of molecular marker systems such as restriction fragment length polymorphisms (RFLPs), random-amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), Diversity Arrays Technology (DArT) and simple sequence repeats (SSRs) have been utilized in plant breeding during the last several years. Among these marker systems, SSR markers have been used extensively, primarily due to their high polymorphism rate and the ability to be performed on commonly available lab equipment [4].

The most advanced and commonly used marker systems, however, are single nucleotide polymorphisms (SNPs). Their abundance in the genomes of all organisms and amenability to automation, leading to low-cost, high-throughput genotyping, makes SNPs the most widely adopted marker system for various genomic applications [5]. Technological developments including TaqMan and KASP™ (Kompetitive Allele-Specific Polymerase chain reaction) revolutionized SNP genotyping for individual and multiplexed SNP marker microarray platforms, such as Infinium (Illumina, Inc.) or Affymetrix/Axiom (Thermo Fisher), respectively. Technical optimization and development of breeding specific marker sets brought down the cost of genotyping arrays, making them affordable for utilization in crop improvement [6,7]. Medium-density SNP panels were also developed, such as 1K-Rice Custom Amplicon (1k-RiCA), an amplicon panel of ∼1000 SNPs based on the custom sequencing improvement [8]. The Illumina BeadXpress SNP genotyping platform (Illumina, Inc.) has also been utilized for smaller sets of SNPs for breeding applications; SNP genotyping panels have been developed and utilized in several crops including rice, wheat, chickpea and pigeon pea [6,7,9]. However, in order to develop such SNP genotyping panels, SNP discovery/identification is a prerequisite. To save time and costs in SNP genotyping, genotyping-by-sequencing (GBS) and its various forms have been optimized for simultaneous SNP discovery and genotyping. A detailed comparison of different SNP genotyping technologies has been provided by Mir *et al.* [10].

In the context of using markers for crop improvement, it is important to analyze different components of ‘the breeder’s equation’ ($\Delta G = (\sigma_a)\,(i)\,(r)\,/\,L$) that assesses genetic gain (ΔG). It has the following components: additive genetic variation within the population (σ_a), selection intensity (i), selection accuracy (r) and number of years per cycle (L). In this direction, Cobb *et al.* [11] suggested that optimum contribution selection can be achieved and ΔG can be enhanced in plant breeding by deploying molecular marker technology. Developing a larger population and screening early generations for molecular markers associated with ‘must-have’ traits (forward breeding) can enhance i. Similarly, selection of lines for advancing generation through marker-assisted selection (MAS) can enhance r. Genomics-assisted breeding (GAB) [12] and the ’5 Gs’ breeding approach [13] have also been suggested as ways to use a range of genomics approaches and tools to enhance the precision and efficiency of breeding to deliver higher genetic gains in farmers’ fields [14].

For successful GAB, apart from using genomic tools and approaches, efficient and effective analytical and decision support tools (ADSTs) have been suggested as ‘must haves’ to evaluate and select plants for the next generations in crop breeding; Varshney *et al.* proposed combining ADSTs with several molecular breeding applications such as marker-assisted back crossing (MABC) or MAS, marker-assisted recurrent selection and GS [15]. If the markers for a particular trait are available, they can be used in a MAS/MABC approach and superior lines can be selected effectively by using ADSTs. MARS and GS are two other molecular approaches to crop breeding that should make the best use of ADSTs for the accumulation of superior alleles and for enhancing genetic gains, respectively [15].

Apart from GAB, markers have also been employed successfully in supporting breeding tools such as doubled haploid (DH) technology. DH and MAS technology have been combined in an integrated MAS-DH approach and utilized to increase genetic gain for biotic and abiotic stress tolerance in maize breeding; Xu *et al.* critically reviewed the factors for optimizing the combination of methods to maximize the cost–effectiveness of the breeding programs [16]. These factors include logistics planning and stakeholder engagement. This integrated MAS-DH approach has also been reported for developing superior restoration lines for hybrid rice breeding, indicating its high impact in commercial breeding programs.

The availability of several marker-based platforms for low- to high-throughput genotyping mandates proper decision-making to choose the appropriate marker system, depending on the objectives and field of application. High-density genotyping platforms for discovery studies and linkage mapping experiments, and medium-density genotyping platforms for GS, genetic diversity analyses and background selection have been suggested [17]. Low-density genotyping platforms such as KASP can be well utilized for routine breeding applications like forward breeding through MAS, MABC and quality control analysis [17].

While the integration of genomics in breeding is essential to accelerate genetic gains in developing countries, there are several challenges, especially in terms of generating genotyping data by establishing laboratories with high-end equipment and running them in a cost-effective and sustainable manner. Several articles have suggested outsourcing genotyping as a means of providing the data for lower cost in less time [14,18,19]. However, in this context, it remains essential to develop capacity for data analysis and decision-making to select superior lines. Many genotyping and sequencing centers, for example the Center of Excellence in Genomics & Systems Biology at ICRISAT [20], are providing genotyping services as well as training the next generation of scientists so that molecular markers can be used in breeding applications in developing countries.

Several collaborative projects and platforms such as the High Through-Put Genotyping (HTPG) Project [21], the Genomic Open Breeding Informatics Initiative (GOBii) [22], the Integrated Breeding Platform [23] and the CGIAR Excellence in Breeding (EiB) platform [24] are providing shared genotyping services, analytical tools and decision support systems for modernization of breeding across CGIAR centers as well as assisting several national agricultural research systems in developing countries. For instance, ICRISAT is leading the HTPG project in collaboration with IRRI, CIMMYT and EiB with the financial support of the Bill & Melinda Gates Foundation. The HTPG project facilitates low-cost, high-throughput genotyping for CGIAR, NARS and small-medium private sector organizations by aggregating the genotyping demand, mainly for forward breeding applications. Through a collaborative agreement, Intertek-AgriTech [25] offers the genotyping services for HTPG as an external service provider. The HTPG platform, at present, offers SNP genotyping services for over 100 traits in 18 crops with fully flexible pricing, with an initial set-up cost of US$1.60–1.80 per sample and an incremental cost of US$0.05–0.20 per SNP, including DNA isolation. HTPG services are well utilized by breeders of 8 CGIAR centers and over 30 NARS partners, in addition to the private sector, in 28 countries, mostly in south Asia and sub-Saharan Africa. As a result, the HTPG project has streamlined integration of molecular markers into breeding applications in about 28 countries around the world.

In summary, molecular markers are one among many tools available in the breeders' toolkit. Adopting integrated breeding approaches by carefully selecting the right tools will help in accelerating the rate of genetic gain in breeding programs [14]. These tools should be combined with ADSTs through open-source platforms; this approach, together with the adoption of shared genotyping services like HTPG and data management systems, would facilitate efficiency in GAB approaches. Proper cost analysis is crucial before adopting modern technologies together with the addition of innovative concepts. Careful consideration of economical, logistical and technical factors will help to achieve the full success of integrating different molecular strategies into plant breeding programs [26]. Finally, integrated breeding approaches by combining efficient genotyping, phenotyping and ADSTs will help to accelerate the rate of genetic gain in staple food crops. This will address threats like climate change through delivery of better varieties to farmers, and ensure the security of global food and nutrition.

Author contributions

RK Varshney conceived the idea, R Bohar and A Chitkineni contributed to writing different sections and RK Varshney together with R Bohar and A Chitkineni finalized the manuscript.

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