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Posted Date: 21 April 2025

doi: 10.20944/preprints202504.1646.v1

Keywords: Single Nucleotide Polymorphisms (SNPs); Molecular Markers; Genotyping Platforms; Marker-Assisted Selection (MAS); Next-Generation Sequencing (NGS); Plant Breeding

 doi:10.20944/preprints202504.1646.v1

Review

The Role of Single Nucleotide Polymorphisms (SNPs) in Modern Plant Breeding: From Discovery to Application

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Abstract: The integration of molecular markers has significantly advanced the accuracy and speed of genetic analysis in plants, thereby accelerating the adoption of molecular breeding in field crops and forestry species. Among these, single nucleotide polymorphisms (SNPs) have emerged as the most widely utilized markers due to their high abundance across genomes and compatibility with high-throughput genotyping platforms. These functional markers hold great promises for enhancing the efficiency of marker-assisted selection (MAS) in crop improvement programs. Next-generation sequencing (NGS) technologies continue to play a transformative role in SNP genotyping, particularly for large-scale discovery applications. A key factor in scaling up SNP genotyping lies in minimizing the number of biochemical reactions required per assay. Therefore, the development of robust, multiplexed genotyping methods capable of amplifying numerous genomic loci simultaneously has become essential. Despite these advancements, there remains a gap in the availability of ultra-high-throughput and cost-efficient genotyping platforms suitable for routine use in applied breeding. This review highlights and evaluates four contemporary SNP genotyping assays and platforms, illustrating their roles in achieving scalable and accurate genotyping. Additionally, the paper provides an overview of current progress in SNP marker discovery, validation, and application in quantitative trait locus (QTL) analysis and modern plant breeding strategies.

Keywords: Single Nucleotide Polymorphisms (SNPs); molecular markers; genotyping platforms; Marker-Assisted Selection (MAS); Next-Generation Sequencing (NGS); plant breeding

1.0. Introduction

The advent of molecular markers has transformed plant genetic analysis, significantly enhanced both the pace and precision of research and enabled the widespread employment of molecular breeding in crops and trees. Over the past three decades, marker systems and their detection platforms have undergone remarkable evolution. Molecular markers facilitate the detection of allelic variation within the genome of a species, including differences due to simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and insertions or deletions (InDels) (Wang et al., 1998; Ferguson et al., 2011). These variations have been developed into reliable marker systems for detecting genetic polymorphisms (Benardo, 2008; Jiang, 2013).

Molecular markers are broadly categorized based on their detection method, throughput, and cost into: (1) low-throughput, hybridization-based markers (e.g., RFLPs); (2) medium-throughput, PCR-based markers (e.g., RAPDs, AFLPs, SSRs); and (3) high-throughput, sequence-based markers (e.g., SNPs) (Wang et al., 1998; Benardo, 2008; Ferri et al., 2010). Prior to the advent of PCR technology,

 doi:10.20944/preprints202504.1646.v1

RFLPs were widely used due to their reproducibility and co-dominant inheritance. However, their labor-intensive, time-consuming, and costly detection process limited automation and large-scale application.

The introduction of PCR revolutionized marker development, enabling the emergence of more efficient systems such as RAPDs, AFLPs, and SSRs. While RAPDs can detect multiple loci, their low reproducibility due to random primer binding has limited their use. In contrast, AFLPs offer higher reproducibility and sensitivity using longer selective primers and discriminatory nucleotides at the 3′ ends, making them suitable for organisms with limited genomic information. Nevertheless, the laborious detection steps and lack of automation hindered their widespread application in breeding programs (Zhang et al., 2011).

SSRs became the marker of choice in the 1990s due to their hyper-variability, co-dominant nature, reproducibility, locus specificity, and genome-wide distribution. SSRs offer additional advantages such as multiplexing capability, compatibility with high-throughput genotyping, and simple PCR-based detection via gel electrophoresis at relatively low cost (Jiang, 2013; Vieira, 2016). These features allowed SSRs to overcome many limitations of earlier marker technologies and dominate plant molecular genetics and breeding into the early 21st century (Powell et al., 1996; Mammadov et al., 2012a).

However, in the last two decades, SSRs have been largely supplanted by SNP markers. SNPs, first extensively characterized in the human genome, are now recognized as the most abundant and widely distributed form of genetic variation in plant genomes (Rasheed et al., 2017; Shahabzadeh et al., 2022). Despite being bi-allelic and therefore less polymorphic than SSRs, SNPs offer key advantages, including genome-wide abundance, uniform distribution, and suitability for high-throughput and ultra-high-throughput genotyping platforms (Yan et al., 2009; Allen et al., 2011). These advantages have made SNPs the preferred marker type in plant genetics and breeding (Karim et al., 2020; Korsa & Feyissa, 2022).

SNP discovery has increasingly relied on computational approaches fueled by the rapid growth of publicly available genomic data. However, the complexity of plant genomes poses challenges in identifying informative SNPs, necessitating alternative discovery strategies for certain crops. The cost of genotyping remains a key factor influencing marker adoption, particularly in breeding programs that require genotyping of large segregating populations (Hiremath et al., 2012; Raj et al., 2022).

The development of high-throughput genotyping platforms and chemistries has facilitated the generation of dense genetic linkage maps, enhancing the appeal of SNPs for marker-assisted breeding (Collard & Mackill, 2008; You et al., 2018). Advances in next-generation sequencing (NGS) have further improved the speed, accuracy, and affordability of SNP discovery and genotyping. NGS-based approaches have proven invaluable for identifying, validating, and deploying genome-wide SNP markers and for developing array-based genotyping systems in a wide range of plant species (Davey et al., 2011; Manivannan et al., 2021).

This review provides an overview of SNP discovery, validation, and application in crop improvement. It also highlights key developments in modern genotyping platforms and assays, emphasizing their roles in advancing genomic-assisted plant breeding in the 21st century.

2.0. Methodology

This review employed a comprehensive and systematic literature analysis to synthesize current knowledge on the discovery, validation, and application of single nucleotide polymorphisms (SNPs) in modern plant breeding. Relevant peer-reviewed journal articles, technical reports, and books published between 2000 and 2024 were retrieved using scientific databases such as PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect. The search employed keywords and Boolean operators such as “SNP markers AND plant breeding,” “genotyping platforms,” “marker-assisted selection,” “next-generation sequencing AND crops,” and “SNP validation AND QTL mapping.” The selection criteria for inclusion were based on relevance to SNP development, technological advancements in genotyping, their integration in breeding programs, and evidence of practical

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applications in crop improvement. Emphasis was placed on studies that addressed the scalability, cost-effectiveness, throughput, and flexibility of genotyping platforms. Articles focusing on major and underutilized crops, particularly those discussing high-resolution applications like genome-wide association studies (GWAS), marker-assisted backcrossing (MABC), and genomic selection, were prioritized.

Data extracted from the selected studies were thematically organized into five core areas: (1) historical overview and importance of molecular markers in breeding; (2) strategies for SNP discovery, especially in complex genomes; (3) SNP validation and genotyping chemistry; (4) comparative analysis of major SNP genotyping platforms; and (5) practical applications of SNPs in trait mapping, germplasm characterization, and modern breeding strategies.

Critical evaluation of genotyping technologies was informed by benchmarking throughput (samples × markers), cost per data point, platform flexibility, and suitability for polyploid crops. This approach enabled a holistic assessment of how SNP-based tools are transforming the landscape of precision breeding and crop improvement across diverse agricultural systems.

3.0. Strategies for SNP Discovery in Complex Plant Genomes

Uncovering SNPs in crops characterized by low genomic complexity, such as *Arabidopsis thaliana* and *Nicotiana tabacum*, is relatively straightforward. In contrast, identifying SNPs in crops with complex genomes presents significant challenges due to the high prevalence of repetitive sequences. The development of SNP markers for genome-wide screening typically begins with identifying a broad pool of candidate SNPs. This is commonly achieved through non-targeted methods like resequencing or high-resolution melting (HRM) analysis in a diverse set of genotypes. These approaches enhance the likelihood of discovering informative polymorphisms that are suitable for downstream applications in breeding programs.

Earlier efforts, such as the resequencing of unigene-derived amplicons via Sanger sequencing and mining SNPs from EST databases followed by PCR validation, enabled researchers to target gene-rich regions while avoiding repetitive sequences. Although these strategies yielded gene-based SNPs (Ganal et al., 2009), they were limited by low polymorphism rates in conserved coding sequences and were ineffective in capturing SNPs located in non-coding regulatory or intergenic regions. Moreover, these methods were labor-intensive and costly.

The advent of next-generation sequencing (NGS) technologies, including platforms like Roche 454, SOLiD, Ion Torrent, and Illumina HiSeq, revolutionized SNP discovery by enabling high-throughput sequencing of targeted exonic regions through sequence capture and enrichment strategies (Hodges et al., 2007; Mammadov et al., 2012a). However, the design of probe-based sequence capture requires prior reference genomes, limiting their use to well-characterized crops. While transcriptome (EST) and exon resequencing remain valuable for detecting SNPs in coding regions and causal mutations (Okou et al., 2007), they fail to capture regulatory SNPs often found in non-coding regions (Dean, 2006; Emberton et al., 2005).

To address genome complexity, several reduction techniques have been developed (Costa et al., 2017; Kim et al., 2024). Methods such as methylation filtering, High-Cot selection, and microarray-based genomic selection reduce repetitive content but cannot eliminate all duplicated sequences, leading to false positives (Van Orsouw et al., 2007; Akbari et al., 2021). Alternative strategies like DOP-PCR, combined with in silico analysis, have also been proposed to simplify genome-wide SNP genotyping (Jordan et al., 2002; Lopes et al., 2022; Panahi et al., 2024).

Genome-wide sampling sequencing (GWSS) has further advanced SNP discovery. Despite its promise, GWSS is often hampered by data inconsistency, missing reads, and variable site coverage (Jiang et al., 2016). Optimizing library preparation to ensure even genome coverage remains essential.

SNP detection in polyploid crops is particularly difficult (Clevenger et al., 2015). Homoeologous and paralogous sequences can introduce false-positive SNPs. Unlike diploids, where minor allele frequencies suffice, distinguishing true SNPs in polyploids may require incorporating haplotype information (Chutimanitsakun et al., 2011; Bus et al., 2012; Yu et al., 2008). Accurate SNP validation

 doi:10.20944/preprints202504.1646.v1

in allopolyploids hinges on differentiating these sequence variation classes (Yu et al., 2011). Computational pipelines and genome complexity reduction methods are thus indispensable for reliable SNP detection in polyploid species (Narechania et al., 2009; Mammadov et al., 2012b).

Genotyping-by-sequencing (GBS) has emerged as a cost-effective, scalable method for SNP discovery and genotyping (Pootakam, 2023). It enables the construction of high-density linkage maps, facilitating genome-wide association studies (GWAS) (Elshire et al., 2011; Nelson et al., 2011). GBS leverages restriction enzymes like ApeKI to reduce genome complexity and has been successfully used in species like maize and sorghum (Mammadov et al., 2009; Wang et al., 2020). Although GBS does not require a reference genome, its output contains significant missing data, necessitating imputation using tools such as BEAGLE or IMPUTE2 (Howie et al., 2009; Marchini & Howie, 2010; Mora-Márquez et al., 2024).

As sequencing technologies improve, GBS continues to gain popularity despite challenges such as PCR amplification bias, missing data, and the need for robust bioinformatics pipelines (Hindorff et al., 2009; Davey et al., 2013; Panahi et al., 2024). These innovations have driven the discovery of SNPs associated with economically important traits. For instance, GWAS has successfully identified markers for dry matter and carotenoid content in cassava (Esuma et al., 2016; Rabbi et al., 2017), and disease resistance loci in potato and cassava (Wolfe et al., 2016; Kansup et al., 2020; Okada et al., 2019). These advances underscore the growing impact of SNP-based technologies in modern plant breeding

4.0. SNP Validation and modern genotyping platforms and chemistries

SNP marker development for genome screening generally involves three key stages: detection, validation, and final selection (Figure 1). However, the identification of SNPs, even with the support of reference sequences and advanced software, does not guarantee their suitability as reliable genetic markers. To confirm the Mendelian nature of a discovered SNP, ongoing validation in different or independent populations is essential (Sun et al., 2010; Andelkovic et al., 2020).

Marker validation involves designing a genotyping assay based on the polymorphism and then testing it across a diverse panel of germplasm and segregating populations (Mammadov et al., 2012b). For effective genome-wide SNP assay development, it is crucial to ensure adequate genome coverage by the selected SNP set. Once SNPs are selected, screening can be performed using high-throughput techniques on segregating germplasm sets, tailored to the breeding goal or research objective (Myles et al., 2010).

Using segregating populations for marker validation is generally more informative than unrelated lines, as it enables the evaluation of segregation patterns and the marker's discriminative power. This also helps distinguish true Mendelian loci from repetitive or duplicated sequences that may escape detection by bioinformatics pipelines (Velazco et al., 2019). Given the cost constraints of validating every discovered SNP, most programs validate a representative subset, using it to define parameter thresholds for selecting SNPs in final assays (Senthilvel et al., 2019).

Recent advances in multiplexing and automation have greatly improved SNP genotyping efficiency (Zhang et al., 2020). Various high-throughput platforms are now capable of genotyping from a single SNP to up to a million in parallel. Some of the widely adopted platforms include Illumina's BeadArray-based technologies (GoldenGate® and Infinium®), Affymetrix arrays, Life Technologies' TaqMan OpenArray® system, and KASP (Kompetitive Allele-Specific PCR) offered by KBiosciences through the SNP Line™ platform (Fan et al., 2003; Steemers et al., 2007; Mammadov et al., 2012b; Fernando et al., 2015; Lui et al., 2022).

These platforms vary in chemistry, throughput capacity, and cost. Selecting a platform depends on factors such as the total number of SNPs required, the SNP context sequence length, and available budget, as genotyping remains a cost-intensive aspect of molecular breeding (Kumpatla et al., 2012; Mammadov et al., 2012a). A more detailed comparison of these genotyping platforms will be discussed later in this review.

Although most platforms perform well across various crops, SNP analysis in polyploid species remains challenging due to complex allelic combinations.

 doi:10.20944/preprints202504.1646.v1

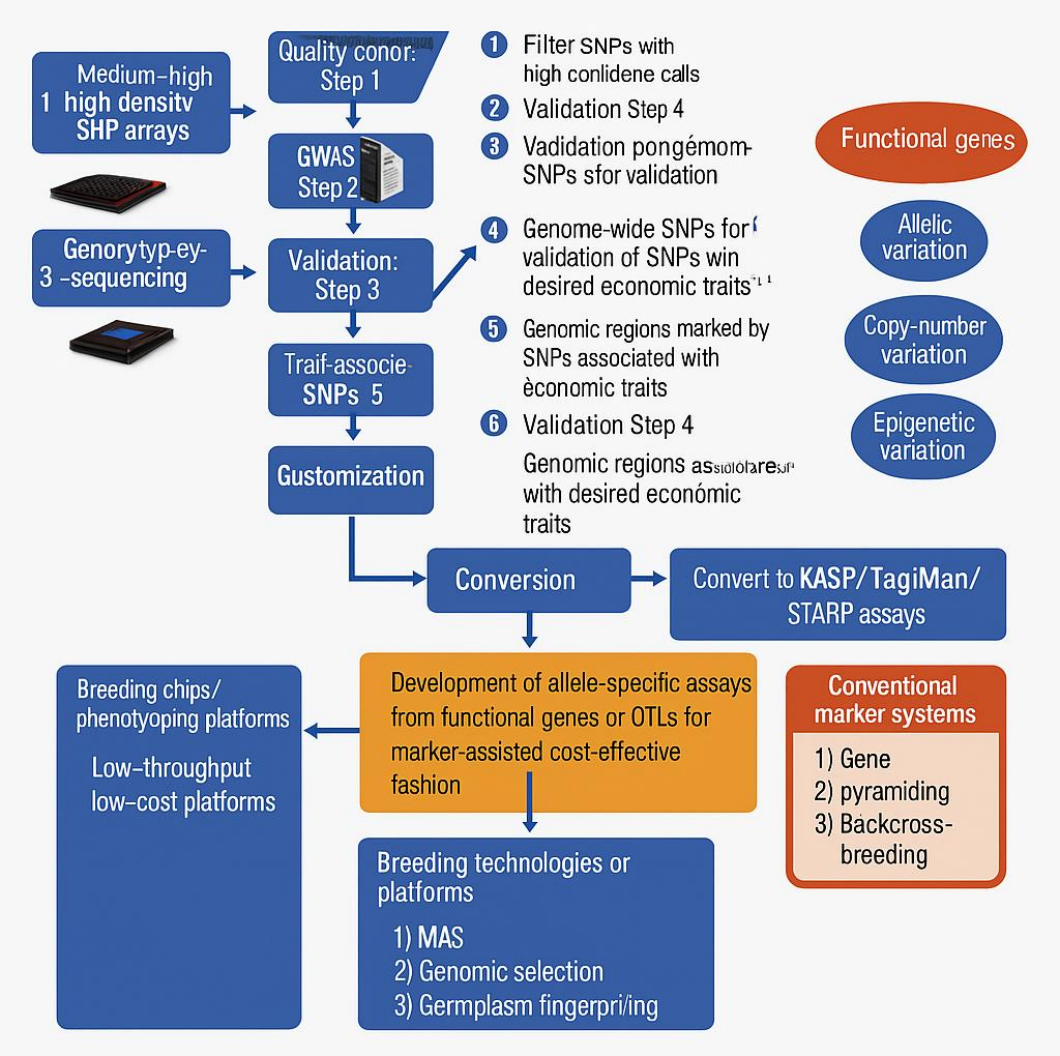

Figure 1. A workflow system to develop practical Breeding Chips with Possible Genetic information from Linkage Mapping, GWAS, and Functional Genes.

In such species, SNPs can be categorized into **simple SNPs**, **hemi-SNPs**, and **homoeo-SNPs**. Mammadov et al. (2012a) classified these using examples from diploid and tetraploid cotton species. The genome of tetraploid cotton, **Gossypium hirsutum** (AD1) and **G. barbadense** (AD2)—includes two subgenomes: A (from progenitors such as *G. herbaceum* and *G. arboreum*) and D (from *G. raimondii*).

- **Simple SNPs** identify allelic differences at corresponding loci within a single subgenome, leading to distinct genotype groupings.
- **Hemi-SNPs** reveal genetic variations that appear as homozygous in one organism but heterozygous in another.
- **Homoeo-SNPs**, on the other hand, identify variation at homoeologous or paralogous loci across A and D subgenomes but are often monomorphic in tetraploid species (Mammadov et al., 2012a; Abdelraheem et al., 2017; Akter et al., 2019).

Simple and hemi-SNPs are considered valuable for genetic mapping and diversity analysis. Simple SNPs generally segregate like diploid markers and represent about 10–30% of polymorphic loci in polyploid crops, while hemi-SNPs can account for 30–60% and are particularly useful in F2, RIL, and DH populations. In contrast, homoeo-SNPs are less suitable for mapping due to their

 doi:10.20944/preprints202504.1646.v1

inconsistent genotype patterns resulting from polymorphism among homoeologous sequences or duplicated loci (Mammadov et al., 2012a; Kumpatla et al., 2012).

Numerous high-throughput genotyping platforms, both multiplex and single-plex, have been proposed for marker-assisted selection (MAS) in crops such as wheat, rice, maize, and legumes. However, large-scale adoption in practical breeding is limited by high costs, lower customization efficiency, and expensive equipment requirements (Aurélie et al., 2009; Masouleh et al., 2009; Varshney et al., 2016).

The development of ultra-high-throughput, cost-efficient genotyping systems tailored for applied breeding remains a crucial goal for enhancing genetic gain. This review explores such platforms currently utilized in SNP genotyping and discusses their implications for accelerating crop improvement. We anticipate that the perspectives provided here will foster further discourse on optimizing genotyping technologies for future breeding applications.

5.0. Comparative analysis of four genotyping assays and platforms

5.1. The OpenArray technology (TaqMan system)

OpenArray technology, developed by Life Technologies, utilizes endpoint TaqMan chemistry to perform high-throughput SNP genotyping (Brenan & Morrison, 2005). It employs a miniaturized OpenArray plate, approximately one-eighth the size of a standard 384-well plate, yet capable of holding up to 3,072 individual assays. This is achieved through a design comprising 48 subarrays, each containing 64 through-holes. These holes are coated with hydrophobic and hydrophilic surfaces, allowing reagents to remain suspended via capillary action (Schleinitz et al., 2011; Mammadov et al., 2012a).

OpenArray offers high flexibility, enabling varied SNP-to-sample combinations. The plates typically come preloaded with assay reagents, with users only needing to add DNA samples. To further increase throughput, Kumpatla et al. (2012) suggested integrating slide towers with PACR thermocyclers; using two such systems, up to 2,048 samples across 128 SNPs can be processed daily in a 128 x 24 format. While high-throughput systems like Affymetrix GeneChip and Illumina BeadChip are preferable for genome-wide projects with numerous SNPs and limited sample volumes, OpenArray is more suitable for scenarios involving fewer SNPs and larger sample populations (Thomson, 2014; Dagnall et al., 2018).

OpenArray is particularly beneficial in marker-assisted selection (MAS), where a limited number of SNPs are used to track specific QTLs across many samples. For such targeted applications, single-plex genotyping methods like KASP and TaqMan may also be ideal (Neelam et al., 2013; Fernando et al., 2015). For instance, Rienzo et al. (2018) employed TaqMan assays in tomato to detect resistance-breaking isolates of Tomato Spotted Wilt Virus with high sensitivity, outperforming high-resolution melting (HRM) techniques. The protocol developed was not only accurate but also streamlined, utilizing crude leaf RNA extractions (Patil et al., 2017; Rienzo et al., 2018).

5.2. Illumina's BeadArray platform

Illumina's BeadArray (microarray) technology uses silica beads embedded in microwells and coated with oligonucleotide probes specific to genomic loci (Adler et al., 2013). It supports ultra-high-throughput SNP genotyping and structural variation analysis. Technologies like GoldenGate (GG) and Infinium assays fall under this platform, which can genotype up to 3,072 and 1.1 million SNPs, respectively (Fan et al., 2003; Rasheed et al., 2017).

BeadArray analysis utilizes the iScan confocal scanner and GenomeStudio software for data acquisition and interpretation. GG assays use universal primers that prevent primer interactions, allowing the amplification of all loci in a single tube. In Infinium, the assays are immobilized on a chip, while GG uses a suspension format. Both use distinct bead types based on SNP structure: Infinium I (for A/G and T/C SNPs) require two bead types, while Infinium II requires one.

Processing throughput is constrained to three 96-well plates per day due to TECAN robot limitations. Despite this, the platform remains a preferred choice for validating numerous SNPs in

 doi:10.20944/preprints202504.1646.v1

fewer samples, with a minimum of 24 and a maximum of 288 samples processed across one to twelve BeadChips.

5.3. High-Resolution Melting (HRM)

HRM is a post-PCR genotyping technique that uses intercalating dyes to detect DNA melting transitions with high sensitivity and specificity (Reed et al., 2007). Fluorescence decreases as double-stranded DNA melts, and the unique melting curve profiles can distinguish even single-nucleotide differences (Er & Chang, 2012).

This method is especially valuable for identifying SNPs in exploratory studies or routine diagnostics (Krypuy et al., 2006; Singh et al., 2012; Song et al., 2015). Homozygous samples yield single-peak curves, while heterozygous ones show broader, dual-peak profiles due to imperfect duplex formation (Mader et al., 2008). HRM is economical and minimizes contamination risk through its closed-tube format (Ebili & Ilyas, 2015; Slomka et al., 2017).

Nonetheless, challenges remain. Pipetting inconsistencies and environmental variations can compromise accuracy (Tucker & Huynh, 2014). Additionally, certain SNP types (e.g., A>T or T>A transitions) may produce indistinct profiles on some instruments (Vossen et al., 2009; Slomka et al., 2017).

5.4. Kompetitive Allele-Specific PCR (KASP)

KASP is a single-plex SNP genotyping method renowned for its cost-effectiveness, high sensitivity, and adaptability across different plate formats (Semagn et al., 2014). It uses a PCR mix containing allele-specific primers and a common primer, along with a fluorescence-based reporting system (Alvarez-Fernandez et al., 2021).

The assay employs Förster resonance energy transfer (FRET) for detection, using quenched fluorescent oligonucleotides that emit a signal upon binding to their targets. KASP is compatible with standard lab equipment and has been used widely in crop breeding programs, such as in chickpeas, wheat (Yu et al., 2017), and cassava (Udoh et al., 2017).

KASP assays are also used by major research centers like CIMMYT for high-volume genotyping (Rabbi et al., 2014; Semagn et al., 2015). SNPs linked to traits like heat stress tolerance in maize have been validated through KASP using RNA-seq data (Jagpot et al., 2020; Schaarschmidt et al., 2020).

In comparison to KASP, the cost and design lead time for TaqMan, GG, and Infinium assays vary. TaqMan assays can be synthesized within two weeks, while GG and Infinium take six and nine weeks, respectively (Ahn et al., 2018; Ayalew et al., 2019). Additionally, platforms like Illumina enforce minimum sample requirements per batch (480 for GG and 1,152 for Infinium), which may limit their use for smaller validation studies (Kumpatla et al., 2012).

Selecting a genotyping platform involves considering several key factors, including, SNP number, sequence context length, project scale, budget, and urgency (Kwok, 2002; Bui et al., 2017; Ruff et al., 2020). For example, Illumina HiSeq reads (~70 nt) are compatible with GG and Infinium assays that require 50 nt on either side of the SNP (Benardo et al., 2015), but may not suit TaqMan designs, which require longer sequences (~100 nt) to accommodate probe and primer binding (Jatayev et al., 2017; Yang et al., 2020). Thus, no one platform is universally ideal; rather, the decision must align with the project's specific needs.

6.0. Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) platforms have emerged as transformative tools for cost-effective genotyping, particularly in underutilized and orphan crops. These technologies enable simultaneous SNP discovery and genotyping with minimal bias across diverse genetic backgrounds (Davey et al., 2013). NGS techniques have proven invaluable for the discovery, validation, and assessment of genetic markers, thereby accelerating the development of genome-wide SNP arrays and high-throughput genotyping chemistries (Thomson, 2014; Rasheed et al., 2017).

 doi:10.20944/preprints202504.1646.v1

To optimize SNP discovery in complex genomes, it is crucial to apply genome complexity reduction strategies in tandem with NGS. These strategies help avoid repetitive and duplicated DNA regions, improving the efficiency and specificity of sequencing efforts (Yuan et al., 2003; Benardo et al., 2015). Historically, Sanger sequencing dominated genomic studies; however, unlike Sanger's dependence on capillary electrophoresis, NGS leverages massively parallel sequencing, advanced imaging technologies, and computational algorithms to decode sequence data at a much faster rate.

NGS platforms offer broad applications, including microRNA sequencing, transcriptome profiling, target sequencing, whole-genome resequencing, de novo assembly, and chromatin immunoprecipitation sequencing. Based on sequencing read length, NGS methods are generally classified into short-read and long-read technologies (Schilbert et al., 2020; Satam et al., 2023).

The term "genotyping-by-sequencing" (GBS) serves as an umbrella description for sequencing-based genotyping approaches (Rasheed et al., 2017). Scheben et al. (2016) identified thirteen distinct GBS methodologies used in crop plants, each offering unique features. These include classical GBS (Elshire et al., 2011; Poland et al., 2012; Kim & Tai, 2013), diversity arrays technology sequencing (DArT-seq) (Li et al., 2015), sequence-based genotyping (SBG) (Van Poecke et al., 2013), restriction fragment sequencing (REST-seq) (Stolle & Moritz, 2013), and restriction enzyme site comparative analysis (RESCAN) (Kim & Tai, 2013).

Despite its strengths, GBS is not without limitations. Common issues include genotyping errors due to low sequencing coverage, particularly misclassification of heterozygotes as homozygotes. These errors are especially pronounced in outcrossing species and early-generation mapping populations. The absence of a reference genome and the presence of polyploidy further complicate SNP identification, as paralogous sequences may be mistakenly interpreted as identical reads. Increasing sequencing depth, using rare-cutting restriction enzymes, and reducing the number of multiplexed samples during library preparation are effective strategies to mitigate these challenges (Davey et al., 2013).

Additional drawbacks include amplification bias favoring shorter fragments with higher GC content, labor-intensive library preparation protocols, high levels of missing data, limited bioinformatics tools for accurate imputation, and challenges in data storage and analysis (Hindorff et al., 2009). Nevertheless, the rapid advancements in sequencing chemistries, long-read platforms, and enhanced reference genomes have fueled the growing popularity of GBS in crop genetics and breeding.

NGS has dramatically accelerated genomic research across plants, animals, and microbes due to its high-throughput, cost efficiency, and precision. Table 1 presents a comparative summary of array-based and NGS-based genotyping platforms, detailing differences in cost, throughput, flexibility, and application. For more in-depth insights into NGS capabilities, readers are referred to reviews by Rasheed et al. (2017) and Scheben et al. (2016).

 doi:10.20944/preprints202504.1646.v1

Table 1. Features of Modern Genotyping assays and platforms available for gene Discovery and Modern Molecular Breeding in Crop Species.

Platform	Technology	Provider	Cost Per Sample	Cost Per Data Point	Analysis of Complexity	Prior Genomic Knowledge	Throughput	Flexibility	Application
Array-based	GoldenGate	Illumina	High	Moderate	Moderate	Yes	172 × 1.5K	No	Tier 1
	Infinium XT	Illumina	Moderate	Low	Moderate	Yes	96 × 50K	No	Tier 1
	Infinium HD/HTS	Illumina	High	Low	Moderate	Yes	24 × 90K/ 24 × 700K	No	Tier 1
	Axiom	Affymetrix	Moderate to high	low	Moderate	Yes	96 × 1000K Or 384 ×55K	No	Tier 1
NGS based	GBS	Non-commercial	Moderate	Low	Difficult	No	depend on sample multiples	Low	Tier 1
	RAD-seq	Non-commercial	Moderate	Low	Difficult	No	-do-	Low	Tier 1
	SLAF-seq	Biomarker Tech	High	Low	Difficult	No	-do-	Low	Tier 1
	Exome capture	Agilent/Nim-bleGen	High	Low	Difficult	Yes	-do-	Low to moderate	Tier 1
	DArT-seq	DiveraityArray	Moderate	Low	Commercial support available	No	96 × 50-100K	Low	
	rAmseq	Non-commercial	Very low	Low	Difficult	Yes	Multiplex	Low	Tier 1
Targeted GBS/low density array	fluidgm	fluidgm	Moderate	moderate	moderate	yes	96 × 96/ 24 × 192/ 48 × 48	moderate	Tier 2
	Sequenom MassARRAY	Agena Bioscience	Moderate	Moderate	Moderate	yes	96 × 48	Low	Tier 2
	Eureka	Affymetrix	Moderate	Moderate	Moderate	Yes	At least 5K × 3K	Low	Tier 2
	AmpliSeq	Thermo Fisher	Moderate	Moderate	Moderate	Yes	customizable	Moderate	Tier 2
Single Markers	KASP	LGC Group	Depend on reaction volume and assay number	High	Easy	Yes	Single-plex (up to ~150K data points/day)	Scalable	Tier 2
	TaqMan	Roche Molecular System	-do-	High	Easy	Yes	-do-	-do-	Tier 2
	STARP	Non-commercial	-do-	Moderate	Easy	yes	-do-	-do-	

 doi:10.20944/preprints202504.1646.v1

7.0. Application of SNPs in Crop Breeding

Molecular markers derived from modern genomics tools, as illustrated in Figure 1, possess high density, scalability, and cost-effectiveness. They have proven especially useful in genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, and gene discovery. The information extracted through these approaches enables the precise manipulation of trait variation for diverse breeding objectives, thereby improving crop productivity and resilience (Varshney et al., 2016; Rasheed et al., 2016).

Despite their potential, the integration of marker-assisted and genomic selection into plant breeding programs has not yet reached its full potential. This underutilization may stem from infrastructural limitations, lack of technical expertise, or insufficient investment in capacity-building. Yet, with the advent of commercial platforms, most genotyping tasks can now be outsourced at affordable rates, democratizing access to molecular tools.

Once molecular markers such as SNPs are validated in independent populations, they become reliable assets for a wide array of genetic applications, including gene and QTL mapping, linkage disequilibrium-based association mapping, map-based cloning, germplasm characterization, genetic diagnostics, event detection, and marker-assisted trait introgression (Berdugo-Cely et al., 2017; Xu et al., 2019). Such validation efforts are essential for ensuring robustness and reproducibility across genetically diverse backgrounds.

Constructing recombination-based genetic linkage maps is a prerequisite for understanding marker order along chromosomes. These maps are typically generated using segregating populations such as backcrosses, F2s, recombinant inbred lines (RILs), or doubled haploids (DHs). While major crops now possess dense SNP-based genetic maps, many minor or underutilized crops rely on mixed marker systems, combining SNPs with older markers like RFLPs or SSRs (Cheema & Dicks, 2009; Poland et al., 2012). Notably, SNP genotyping lends itself well to automation, facilitating high-throughput breeding applications (Jones et al., 2007) (Figure 2).

Rafalski (2002) highlighted that the availability of efficient SNP genotyping platforms enables the genetic dissection of economically important traits. Their widespread deployment accelerates both marker-assisted and genomic selection, increasing genetic gain per unit time. SNPs are ideal for fine-mapping QTLs due to their abundance and genome-wide distribution, often yielding the highest possible map resolution.Throughput refers to the total data output from a single sequencing run, typically described as 'number of samples × number of markers' (where K = 1000). Although all next-generation sequencing (NGS) technologies offer high-throughput capabilities, the actual throughput depends largely on how many samples can be multiplexed in a single run. Flexibility describes the ability to freely select the number of samples and markers based on research needs. NGS-based approaches are generally more adaptable in this regard, allowing greater customization in sample and marker selection. Applications can be broadly categorized into two tiers: Tier 1: Includes high-resolution genetic studies such as genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, genomic selection, and assessments of genetic diversity. Tier 2: Encompasses targeted applications like gene tagging, marker-assisted backcrossing (MABC), and background selection. Several inherent features make SNPs highly suitable for molecular breeding: their ubiquitous distribution across genomes, bi-allelic nature (which facilitates straightforward data analysis), frequent location within gene-rich regions, and relative stability due to low mutation rates (Gore et al., 2009; Tian et al., 2020). These properties render SNPs powerful tools not only for high-resolution mapping but also for genome-wide predictions in genomic selection frameworks.

In cassava, SNP markers have been instrumental in assessing genetic diversity and parental selection. Markers have been linked to traits such as cassava mosaic disease (CMD) resistance (Wolfe et al., 2016; Rabbi et al., 2020), provitamin A content, dry matter content (Esuma et al., 2016; Rabbi et al., 2017; Udoh et al., 2017), and starch composition (de Oliveira et al., 2014). Recent studies confirm the complementary value of combining agro-morphological and molecular data to effectively distinguish genotypes, thus maximizing selection accuracy in breeding pipelines (Zhang et al., 2018).

 doi:10.20944/preprints202504.1646.v1

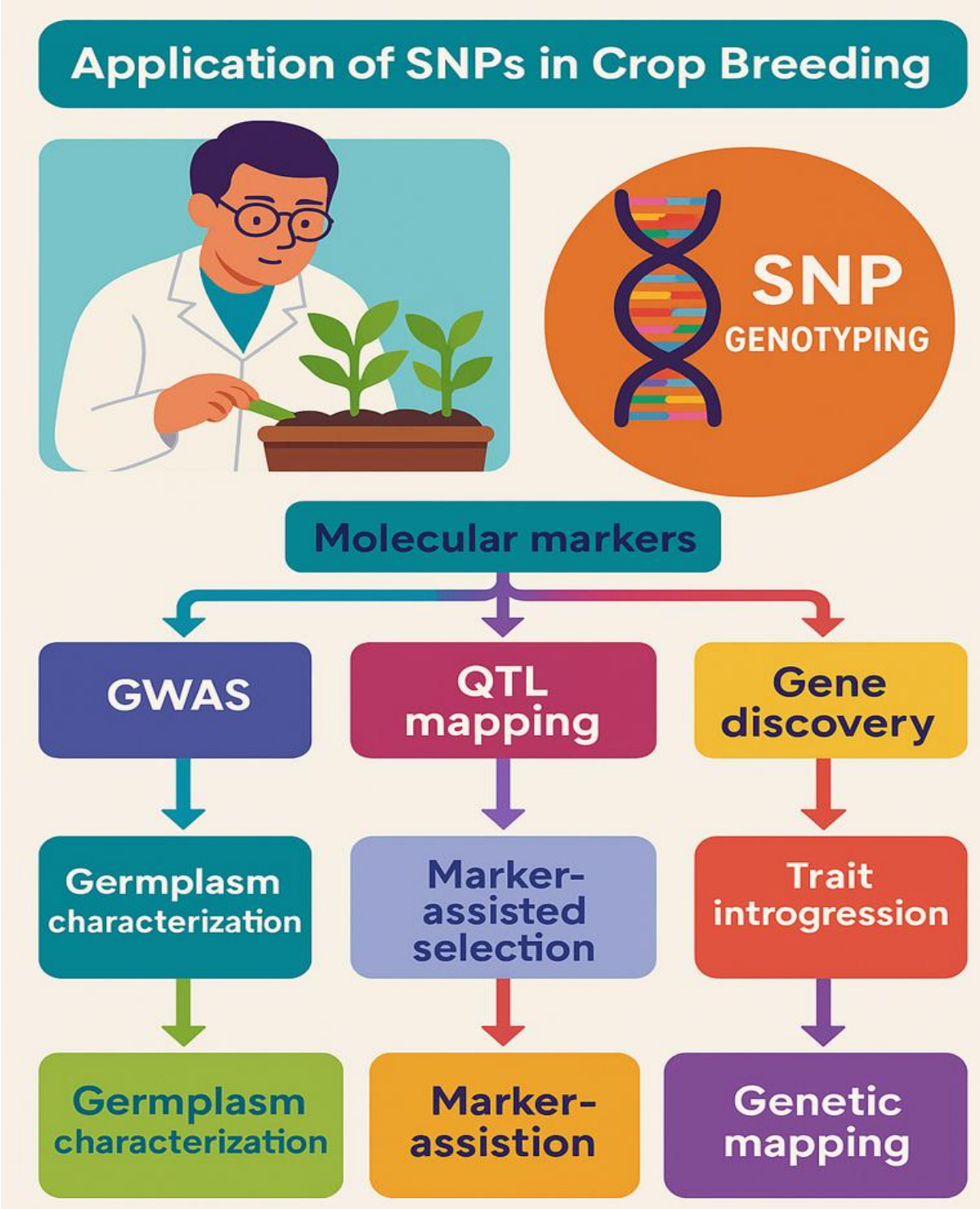

Figure 2. Schematic representation of the application of SNP markers in modern plant breeding. The diagram outlines the process from marker discovery and validation to their use in gene/QTL mapping, genomic selection, germplasm characterization, and marker-assisted selection (MAS), highlighting the integration of SNPs into high-throughput and precision breeding pipelines.

SNP development and validation have also been advanced in maize. The NSF-funded project on maize genomic diversity (Zhao et al., 2012) contributed significantly by identifying SNPs in over 3,000 genes and mapping over 1,100 SNPs in Nested Association Mapping (NAM) populations (Yu et al., 2008). Although validation can be hindered by unknown polymorphisms in uncharacterized

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germplasm (Jones et al., 2009), this is mitigated using high-quality sequence alignments and validated marker databases across diverse genetic backgrounds.

Given the demand for high-throughput marker technologies in maize improvement, multiple SNP arrays have been developed (Ganal et al., 2011; Unterseer et al., 2014; Xu et al., 2017). Buckler et al. (2009) used NAM populations to dissect flowering time in maize, revealing its polygenic nature with 29 QTLs identified through 1.6 million SNPs. Similarly, SNP markers have been linked to resistance genes in soybean, such as Rag2 for aphid resistance and markers linked to root-knot nematode resistance (Kim et al., 2009; Monteros et al., 2010; Kim et al., 2010).

Ultimately, the strategic identification of tightly linked SNPs enhances the precision of MAS, reduces breeding cycle time, and provides cost-effective tools for trait introgression and genome-wide selection across multiple crops.

8.0. Conclusions

Plant breeding plays a pivotal role in achieving global food security amid challenges such as land scarcity, a growing population, and climatic variability. The synergy between genotypic and phenotypic data is critical for unraveling the genetic basis of complex traits, enabling the implementation of precise and efficient breeding strategies.

The explosion of sequence data has catalyzed the development of single nucleotide polymorphism (SNP) markers across a wide spectrum of crop species. Once validated across diverse populations, these markers become invaluable tools for genetic mapping, trait introgression, and accelerated gene discovery. Their integration into high-throughput genotyping platforms has led to the construction of dense genetic linkage maps, laying the foundation for marker-assisted and genomic selection in both major and underutilized crops.

Unlike earlier marker systems, SNPs enable the generation of saturated genetic maps, facilitating genome-wide tracking, fine mapping of target loci, and rapid identification and cloning of key genes or QTLs. The development of robust, large-scale SNP genotyping protocols is a cornerstone of modern breeding pipelines, supporting scalable trait dissection and selection.

Nonetheless, several challenges persist. While SNP chips exist for many crops, their design is often based on limited reference panels, which can reduce their applicability to genetically distant populations. This limitation underscores the need for customizable and economically viable genotyping solutions that accommodate broader genetic diversity. In this context, next-generation sequencing (NGS)-based platforms offer a promising alternative, enabling concurrent SNP discovery and genotyping with less bias toward specific genetic backgrounds.

Genotyping-by-sequencing (GBS) offers a low-cost, high-resolution means of identifying SNPs across numerous samples. However, the successful application of GBS still hinges on adequate computational infrastructure, including reliable imputation algorithms and sufficient data processing capacity. The bioinformatics demands of these technologies remain a bottleneck for many breeding programs in low-resource settings.

Despite these hurdles, the combination of SNP markers, genomics tools, and NGS technologies presents a powerful avenue for accelerating genetic gains in crop breeding. Their application not only enhances selection efficiency but also supports the rapid deployment of improved varieties that are resilient, high-yielding, and tailored to future agricultural challenges.

As we look ahead, the continued development of flexible, scalable, and accessible SNP-based genotyping platforms, paired with investments in data analysis capabilities, will be essential for realizing the full promise of genomics-assisted plant breeding

Acknowledgements: The authors express their gratitude to Dr. Olasanmi Bunmi (PhD) for his invaluable supervision and guidance throughout this work. We also acknowledge the Pan African University for providing institutional support that made this study possible.

Conflict of Interest: All authors contributed equally to the preparation of this manuscript.

 doi:10.20944/preprints202504.1646.v1

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