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Gene Identification and Marker Assisted Selection for Introgression of important traits in barley

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Abstract

Gene identification is the process of identifying genomic DNA regions that encode proteins. It has a wide range of applications in structural genomics, functional genomics, metabolomics, transcriptomics, proteomics, and other genetic-related studies such as genetic disorder detection, treatment and prevention. There are several methods for finding genes, including the ab-initio method (intrinsic) and the sequence similarity search. Crops are typically improved by crossing plants with desired traits, such as high yield or drought tolerance, and selecting the best offspring after multiple generations of testing. It could take 8 to 10 years to develop a new variety. Breeders are very interested in new technologies that can speed up or improve the efficiency of this process. The term "marker-assisted selection" has been used in plant breeding and genetics science since the mid-1990s. The term MAS broadly refers to all forms of selection based on genetic data. MAS is becoming increasingly important in today's world because it aids and improves plant breeding efficiency by monitoring the presence or absence of desirable genes in breeding populations. This is achieved by precisely transferring genomic regions of interest (Foreground Selection) and hastening the recovery of the recurrent parent genome (Background Selection).

Keywords: Background Selection, Conventional plant breeding, Foreground Selection, Gene, Marker-assisted selection.

1. Introduction

Gene finding, defined as the process of identifying genomic DNA regions encoding proteins, is an important scientific research programme with broad applications in structural genomics, functional genomics, metabolomics, transcriptomics, proteomics, genome studies, and other genetic related studies such as genetic disorder detection, treatment, and prevention (Ghorbani et al., 2015). Previously, the process of gene discovery relied on laborious experiments on organisms and living cells, which required a significant investment in both money and time, but these difficulties have recently been alleviated thanks to advances in statistical and bioinformatics tools. Statistical

approaches combined with computational techniques allow for the analysis of homologous recombination rates of various genes, which leads to the determination of their order on a specific chromosome. The information obtained from such experiments aids in the creation of a genetic map, which aids in the specification of the rough locus of known genes related to each other. The availability of powerful computational tools aided gene discovery and demonstrated a significant role in genome studies. It is noteworthy that the identification of fundamental and essential elements of the genome, such as functional genes, introns, exons, splicing sites, regulatory sites, gene encoding known proteins, motifs, EST, ACR, and so on, is the primary basis of the studies, and these functions are employed by the gene prediction or finding process. As a result, the gene discovery process is critical in the study of genome-related programmes. There are several methods for finding genes, including sequence similarity searches and ab initio gene prediction methods (Wang et al., 2004).

2. Sequence similarity search

The search for sequence similarity is a conceptually simple approach that is based on finding similarities in gene sequences between ESTs (expressed sequence tags), proteins, or other genomes and the input genome. This method is based on the assumption that functional regions (exons) are more evolutionary conserved than nonfunctional regions (intergenic or intronic regions). When there is similarity between a specific genomic region and an EST, DNA, or protein, the similarity information can be used to infer gene structure or function. The disadvantage of EST-based sequence similarity is that ESTs only correspond to small portions of the gene sequence, making it difficult to predict the complete gene structure of a given region. Both local alignment and global alignment are based on similarity searches. The BLAST family of programmes, which detects sequence similarity to known genes, proteins, or ESTs, is the most commonly used local alignment tool.

3. *Ab initio* gene prediction methods

The second class of methods for computational gene identification is to use gene structure as a template to detect genes, also known as ab initio prediction. Signal sensors and content sensors are two types of sequence information that are used in ab initio gene predictions. Splice sites, branch points, poly-pyrimidine tracts, start codons, and stop codons are examples of signal sensors. Exon detection must rely on content sensors, which are patterns of codon usage that are unique to a species and allow statistical detection algorithms to distinguish coding sequences from surrounding non-coding sequences. Many algorithms, such as Dynamic Programming, linear discriminant analysis, Linguist methods, Hidden Markov Model, and Neural Network, are used to model gene structure. A large number of ab initio gene prediction programmes have been developed based on these models. Some of the most commonly used ones are GeneParser, Genie, and GRAIL, which combine similarity searches.

Choi et al., 2002 identified the cbf3 gene in barley and obtained F_1 progeny from a cross between Dicktoo (winter barley) and Morex. In F_1 progeny, RNA is extracted and cDNA libraries are created using a barley bacterial artificial chromosome that is sequenced using the di-deoxy chain termination method, followed by mapping of the Hvcbf3 gene on chromosome 5H between markers WG 364b and saflp58.

4. Conventional breeding

Plant breeding is the process of creating improved varieties of plants with desirable characteristics that differ from existing ones. Plant introduction, selection, and hybridization are some of the breeding methods used to develop improved varieties with economically desirable traits. Selection is an important breeding strategy used to improve many economic traits in various plant varieties. Depending on the mode of reproduction, different types of selection procedures are used. For self-pollinated crops, pureline and mass selection methods are used, recurrent selection schemes are used for cross pollinated crops, and clonal selection is used for vegetatively propagated plants.Despite the fact that phenotypic recurrent selection is regarded as an effective strategy for improving polygenic traits by increasing the frequency of desirable genes for various economic characters and aiding in the maintenance of high genetic variability in heterozygous populations, its efficiency and effectiveness are not satisfactory in the majority of cases because the phenotypic selection of genotypes is influenced by the environmental effects and the genotypic selection may take a longer time i.e. at least 2-3 crop seasons for one cycle of selection (Gokidi et al., 2016).

Molecular marker-assisted selection (MAS), on the other hand, is an approach that has been developed to avoid the problems associated with conventional plant breeding by shifting the selection criteria from selection of phenotypes to selection of genes, either directly or indirectly. Molecular markers are clearly not environmentally regulated, are unaffected by the conditions under which the plants are grown, and can be detected at all stages of plant development. With the availability of a wide range of molecular markers and genetic maps, MAS is now possible for traits governed by major genes as well as quantitative trait loci (QTLs).

The usefulness of a molecular marker is determined by its ability to reveal polymorphisms in the nucleotide sequence, which allows discrimination between different molecular marker alleles (Rafalski 2002, Kumawat et al. 2020). Classical markers and DNA/molecular markers are the two broad categories of genetic markers. Classical markers include morphological, cytological, and biochemical markers, as well as DNA markers such as restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), microsatellite or simple sequence length polymorphisms (SSR), random amplified polymorphic sequences (RAPD), cleavable amplified polymorphic sequences (CAPS), single strand conformation polymorphisms (SSCP), singlenucleotide polymorphisms (SNPs) and diversity arrays technology (DArT) markers (Nadeem et al.,2017; Salgotra and Stewart 2020; Bohar et al.,2020Fig.1).

5. Molecular markers

The discovery of molecular markers in the 1980s ushered in a new field of agriculture known as molecular breeding. This was a significant breakthrough in the characterization of traits governed by multiple genes (quantitative traits). There are two types of molecular markers used in genetics and plant breeding: classical markers and DNA markers (Xu, 2010). Morphological markers and biochemical markers are examples of traditional markers. Many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) have evolved (Collard et al., 2005).

Morphological markers:

During the early history of plant breeding, visible traits such as leaf shape, flower colour, pubescence colour, awn type and length, fruit shape, rind (exocarp) colour and stripe, flesh colour, and so on were commonly used as markers. Selection of semi-dwarfism in rice and wheat was one of the critical factors that contributed to the success of high-yielding cultivars during the green revolution. However, the number of available morphological markers is limited, and many of these markers are not associated with important economic traits (e.g. yield and quality).

Biochemical/protein markers:

High molecular weight glutenin subunit (HMW-GS) in wheat is an example of a biochemical marker used in plant breeding.

DNA markers:

A DNA marker is a fragment of DNA that reveals mutations/variations and can be used to detect polymorphism between different genotypes or alleles of a gene in a population or gene pool. These fragments are linked to a specific location in the genome and can be detected using molecular technology.

These DNA markers are further classified into three types based on how they are detected:

1. Hybridization-based molecular markers:The most common hybridization-based molecular marker is RFLP. In 1975, RFLP markers were used for the first time to identify DNA sequence polymorphisms for genetic mapping of a temperature-sensitive mutation of adenovirus serotypes (Grodzicker et al., 1975).
2. PCR-based markers:Depending on the primers used for amplification, the various PCR-based techniques fall into two categories:

i. In the lack of prior sequence knowledge, random or semi-arbitrary primed PCR procedures were created (e.g., AP-PCR, DAF, RAPD, AFLP, ISSR).

ii. Site-targeted PCR techniques based on known DNA sequences (e.g., EST, CAPS, SSR, SCAR, STS).

3. Sequence based markers: Because of the shortcomings of traditional mapping methods, the emphasis shifted from simple genotyping using molecular markers to sequence-based genotyping. Single nucleotide polymorphisms (SNPs) and insertions and deletions

(InDels) were found to be abundant and widely distributed throughout the genomes of variousspecies, including plants (Batley et al., 2003). Because of the prevalence of these polymorphisms in plant genomes, the SNP marker system is an appealing tool for mapping, marker-assisted breeding, and map-based cloning (Gupta et al., 2001; Rafalski, 2002; Batley et al., 2003). DArT is a microarray hybridization-based technique that allows for the simultaneous typing of hundreds of polymorphic loci spread throughout the genome (Jaccoud et al., 2001; Wenzl et al., 2004). Nonetheless, in the case of some crops, such as maize, wheat, groundnut, and soybean, genome-wide SNP calling is frequently hampered by genome-level complexity. As a result, reduced representation-based sequencing approaches such as reduced-representation libraries (RRLs) or complexity reduction of polymorphic sequences (CRoPS), restriction-site-associated DNA sequencing (RAD- seq), and low coverage genotyping have been developed and are now in use.

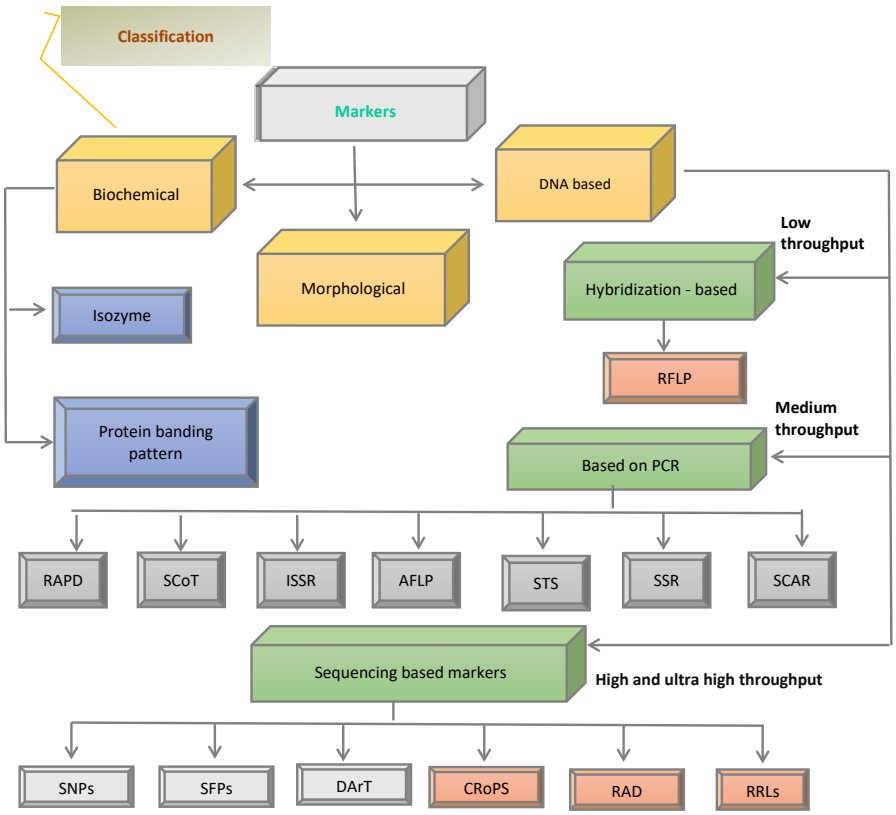

Fig. 1: Classification of markers

Table 1: Advantages and disadvantages of some of the most commonly-used molecular markers.

S.No	Marker	Advantages	Disadvantages
1	RFLP (Restriction Fragment Length Polymorphism)	Highly reproducible,transferrable across population, robust, reliable, locus specific.	High quality and quantity of DNA required Time consuming, laborious, expensive, Limited polymorphism, Not amenable for automation.
2	AFLP (Amplified Fragment Length Polymorphism)	Highly reproducible, highly polymorphic, provides good genome coverage.	High quality and quantity of DNA required
3	RAPD (Rapid Amplified Polymorphic DNA)	Quick and simple,Inexpensive, Small quantity of DNA required.	Non reproducible,non-transferable.

4	SSRs(Simple Sequence Repeats)	Highly reproducible, transferrable across population, robust, reliable, locus specific. Amenable for automation.	High development cost, primer development is time consumingandlaborious.
5	ISSR (Inter Simple Sequence Repeats)	Highly polymorphic, simple,	Non reproducible Generally not transferrable
6	EST (Expressed Sequence Tags)	Highly reproducible, robust, reliable, High degree of sequence conservation, transportable across pedigree and species	Marker development limited to species for which sequence database already exists
7	SNP (Single Nucleotide Polymorphism)	Highly reproducible, reliable, transferrable across population, amenable to automation and high throughput techniques	SNP calling often hindered by complexity at genome levels.
8	SFP (single Feature Polymorphism)	Assays multiple loci within each gene for detecting polymorphisms, High level of specificity	Prior sequence information required Fails to detect polymorphisms due to SNPs
9	DArT (Diversity Array Technology)	Reproducible Cost effective Identifies polymorphism due to both sequence variation and DNA methylation.	Moderate resolution
10	CRoPS (Complexity reduction of polymorphic sequences)	Only subset of genomic region to be sequenced Cost effective and saves time.	Not suitable for large genomes Less suitable for QTL mapping
11	RRLs (Reduced representation libraries)	Does not require every base of the genome to be sequenced Saves cost and time.	Not suitable for large genomes Less suitable for QTL mapping

6. Main types of DNA markers used in MAS

There are five main considerations for the use of DNA markers in MAS: reliability; quantity and quality of DNA required; technical procedure for marker assay; level of polymorphism; and cost (Mohler & Singrun 2004).

Reliability

Markers should be genetically close to target loci, preferably within 5 cM. The use of flanking markers or intragenic markers will \sgreatly increase the reliability of the markers to predict phenotype(Fig. 2).

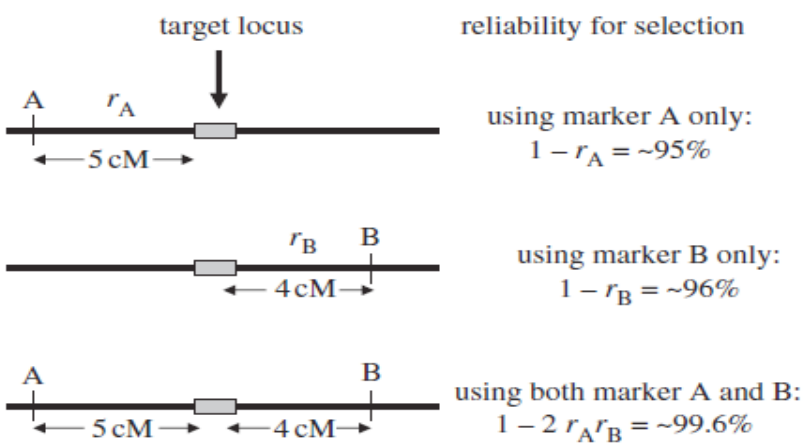

Fig. 2. Single and flanking marker selection reliability (derived from Tanksley (1983), assuming no crossover interference).Recombination between the target locus and marker A occurs around 5% of the time (5 cM).As a result, recombination between the target locus and the marker may occur in about 5% of the progeny.Recombination between the target locus and marker B occurs around 4% of the time (4 cM). Recombination between markers A and B (i.e. twofold crossover) is substantially less likely than recombination between single markers (approx. 0.4 percent). As a result, when flanking markers are employed, the selection reliability is substantially higher (Collard and Mackill 2008)

DNA quantity and quality

Some marker techniques necessitate large amounts of high-quality DNA, which can be difficult to obtain in practise, raising the cost of the procedures.

Technical procedure

The technique's level of simplicity and the time required are critical considerations. Methods with a high throughput that are simple and quick to implement are highly desirable.

Level of polymorphism

Ideally, the marker should be highly polymorphic in breeding material (i.e., it should be able to distinguish between different genotypes), particularly in core breeding material.

Cost

In order for MAS to be practical, the marker assay must be cost-effective.

7. MAS Procedure

Using a single cross as an example, the following is the general procedure:

a. Choose parents and cross them so that at least one (or both) of them has the desired trait's DNA marker allele(s).

b. Plant F_1 population and detect the presence of the marker alleles to eliminate false hybrids.

c. Plant segregating F_2 population, screen individuals for the marker(s), and harvest the individuals carrying the desired marker allele(s).

d. Plant $F_{2:3}$ plant rows, and screen individual plants with the marker(s). A bulk of F_3 individuals within a plant row may be used for the marker screening for further confirmation in case needed if the preceding F_2 plant is homozygous for the markers. Individuals with the requisite marker alleles and other desirable features should be selected and harvested.

e. In the subsequent generations (F_4 and F_5), conduct marker screening and make selection similarly as for $F_{2:3}$s, but more attention is given to superior individuals within homozygous lines/rows of markers.

f. In $F_{5:6}$ or $F_{4:5}$ generations, bulk the best lines according to the phenotypic evaluation of target trait and the performance of other traits, in addition to marker data.

g. Plant yield trials and comprehensively evaluate the selected lines for yield, quality, resistanc eand other characters of interest (Guo-Liang Jiang 2013).

8. Marker Assisted Recurrent Selection (MARS)

MARS is a recurrent selection technique that employs molecular markers to discover and select numerous genomic areas involved in the expression of complex phenotypes in order to construct the best-performing genotype within or across populations (Ribaut et al., 2010). MARS is a scheme that allows for genotypic selection and intercrossing among selected individuals during the same crop season for one selection cycle (Jiang et al., 2007).As a result, MARS could improve the efficiency of recurrent selection and hasten its progress, particularly in integrating multiple favourable genes or QTLs from different sources via recurrent selection based on multi-parent populations (Gazal et al., 2015; Fig. 3).

9. Genomic selection (GS)

Genomic selection (Meuwissen et al., 2001) is a marker-based strategy that incorporates all available molecular marker data covering the entire genome into a model to predict the genetic value of progenies for selection (Lorenz, 2013). In this strategy, each marker is considered a putative QTL, and all genes or QTLs present throughout the genome are in linkage disequilibrium with at least one marker, reducing the possibility of small-effect QTLs being missed (Guo et al., 2012). A computer simulation study conducted by Bernardo and Yu in 2007 revealed that using all molecular markers covering the entire genome provided better prediction and accuracy of breeding values rather of employing subsets of markers that are significantly linked to QTLs

10. Marker-assisted pyramiding

Watson and Singh (1953) pioneered the concept of gene pyramiding. Gene pyramiding is a technique for combining multiple desirable genes from multiple parents into a single genotype for a specific trait. MAS allows multiple genes to be combined into a single genotype. Gene pyramiding is a breeding technique that involves assembling multiple genes that have known effects on target traits. It is primarily used to improve existing elite cultivars for a few unsatisfactory traits, for which genes with significant positive effects have been identified. The

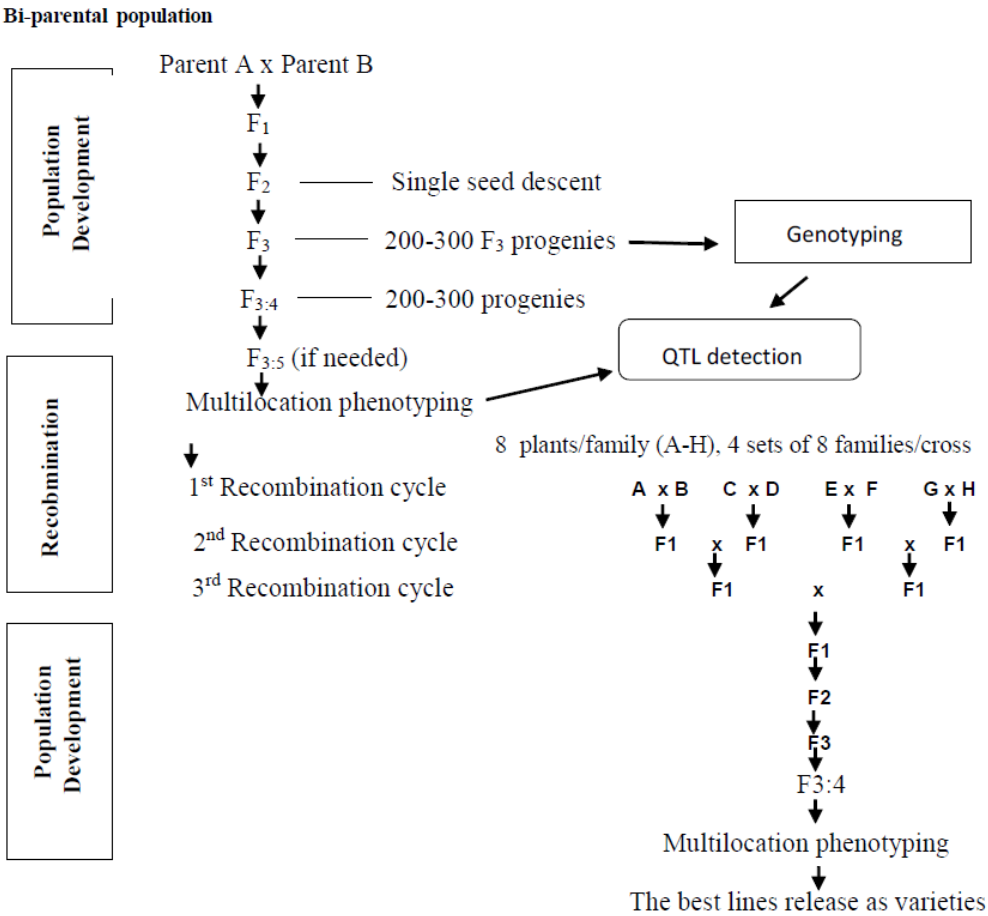

Fig. 3: The mechanism of MARS scheme (Source: Godiki Y. *et al.*, 2016)

most common use of pyramiding has been to combine multiple disease resistance genes (To put it more simply, integrating qualitative resistance genes into a single genotype). Molecular markers, or DNA tags, that have been linked to traits of interest are especially useful for incorporating genes that are highly affected by the environment, genes for disease and pest resistance, and accumulating multiple genes for disease and pest resistance within the same cultivar – a process known as gene pyramiding (Malav et al., 2016).

11. Advantages of MAS

Easier method than phenotypic screening.

- Especially for traits that require time-consuming screening
- You could save time and money.
- Seedling selection is critical for traits such as grain quality.
- Greater dependability.
- There are no environmental consequences.

Applications of marker assisted selection

- It is useful in disease and insect resistance gene pyramiding.
- It is used in backcrossing programmes.
- It is used to transfer male sterility into cultivated genotypes from various sources.
- MAS is being used to improve quality characteristics in various crops, such as protein quality in maize.

12. Marker assisted selection in barley

Raman et al. (2002) improved the efficacy of selection for Al tolerance in barley.The researchers developed hydroponic pulse-recovery screening methods to test tolerance of traits based on root development using F_2 progeny obtained from a single cross between Yambla (moderately tolerant of Al) and WB229 (tolerant of Al).The segregation ratios of tolerant and sensitive genotypes, as well as F_3 progeny tests, indicate that Al tolerance was controlled by a single main gene (Alt).They tested tolerant and sensitive bulks with 30 EcoRI/MseI primer combinations, and 12 of these

allowed sensitive and tolerant bulks to be distinguished. The Alt gene was found on barley chromosome 4H, according to AFLP study of wheat-barley chromosome addition lines.Four microsatellite markers on chromosome 4H (Bmac310, Bmag353, HVM68, and HVMCABG) were found to be strongly associated to Alt.We were able to use the microsatellite marker Bmag353 for routine marker-assisted selection for Al tolerance because of the substantial allelic variation identified, and 396 plants could be screened on a single gel.

Yu et al. (2018) introduced the leaf rust resistance gene Rph26 from *Hordeum bulbosum* into the barley (*Hordeum vulgare*) cultivar 'Emir.' Rph26 reduced the observed symptoms of leaf rust infection (uredinium number and infection type) while also lengthening the fungal latency period. The donor plant (*Hordeum bulbosum*) is crossed with the recipient plant (*Hordeum vulgare*) Emir to produce the introgressed line 200A12, which is then backcrossed with Emir to produce an F_2 population focusing on interspecific recombination within the introgressed segment. A total of 1368 individuals from this F_2 population were genotyped using flanking markers at either end of the 1HL introgression, yielding 19 genotypes that had undergone interspecific recombination within the original introgression. Rph26 was genetically mapped to the proximal end of the introgressed segment on chromosome 1HL's distal end. Rph26-related molecular markers have been identified, allowing this disease resistance gene to be combined with other sources of quantitative resistance to maximise the effectiveness and durability of leaf rust resistance in barley breeding. Toojinda et al., 1998 used marker-assisted line to introduce stripe rust resistance QTLs into adapted backgrounds. They crossed Steptoe and BSR41 to produce F_1 progeny, which was later backcrossed with Steptoe, the most widely grown six-row feed barley in the Pacific Northwest of the United States. They got BC_1 from F_1, the first backcross generation, and determined the number of first backcrosses (66), after which genotypes at marker loci bordering stripe rust resistance QTLS on chromosomes 4 (4H) and 7 (7H) were used to make subsequent selections (5H). Each BC_1 plant produces 134 double haploids, and the ten most resistant double haploid lines were chosen by genotyping the 134 DH lines with 4 RFLP, 106 AFLP, and 8 RAPD markers, as well as identifying introgressed QTLs on chromosomes 7 (5H) and 4 (4H) from BSR41 into the Steptoe background.

Xu et al., (2018) used molecular marker-assisted backcrossing breeding to transfer a thermostable beta-amylase gene from wild barley into an elite variety. Wild barley has a wide variation for malting quality traits such as alpha and beta-amylase, beta glucanase, limit dextrinase, and other hydrolases. Gairdner is crossed with AB75 to produce F_1, and F_1 is backcrossed with the recurrent parent Gairdner to produce BC_1F_1. Marker assisted selection was used at each step starting with BC_1F_1 to track the transfer of the target allele by the CAPS marker (Paris et al., 2002). For backcrossing to the BC_3F_1 generations, seven BC_1F_1 individuals with the Sd1Sd3 genotype were chosen. The BC_3F_1 individuals were selfed to produce BC_3F_2, and malting quality analysis was performed, with the introgressed line exhibiting increased thermostable beta-amylase levels.

Conclusions

Plant breeding has advanced agricultural improvement tremendously, and it is vital that this trend continues. It appears that contemporary breeding programmes are making success using widely used breeding techniques. Backcrossing of significant genes into elite parents using both foreground and background selection has already shown useful with marker assisted selection (MAS). Although the impact on variety development has been limited, MAS could substantially assist plant breeders in achieving this goal. With the advancement of technologies for marker analysis and identification of candidate genes for economic variables, more widespread use of MAS is predicted. The deployment of MAS will be largely driven by forecasting economics. MAS is an attractive choice for certain features that are expensive or logistically difficult to examine. For the full potential of MAS to be realized, deeper integration with breeding programmes is required, as well as a thorough understanding of current barriers and the development of relevant solutions. Exploiting the benefits of MAS over traditional breeding could have a significant influence on agricultural improvement. In the foreseeable future, the high cost of MAS will continue to be a key barrier to its implementation for some crop species and plant breeding in developing nations. Distinct MAS techniques may be required for specific crops, characteristics, and financial constraints. New marker

technology has the potential to significantly cut the cost of MAS. If the new approaches' efficiency is proven and the necessary equipment is readily available, MAS should become more commonly used in crop breeding programmes.

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Conflict of interest

The authors declare no conflict of interest

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