

## The Role of Molecular Marker in Barley (*Hordeum vulgare* L.) Improvement

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### Introduction

Barley is ranked fourth in worldwide cereal production, and is used for a variety of end uses, including animal feed, human consumption and malting. The economic importance of barley has meant that marker-assisted breeding approaches are of considerable interest. Deployment of genetic markers that predict the phenotypic trait of interest with 100 % accuracy ('perfect markers') allows efficient tracking of favorable genetic variants through the breeding process, without the need for phenotypic evaluation. Several modern molecular techniques are now being applied together with morphological studies to investigate genetic diversity and relatedness in crops. Various different molecular markers have been used for genetic diversity studies and molecular breeding in barley. These were particularly true for the marker assays such as RAPD (random amplification of polymorphic DNA), AFLP (amplified fragment length polymorphism), restriction fragment length polymorphisms (RFLPs), and STS (sequence tagged site) are the most known molecular markers used for barley breeding.

### Description

Barley is ranked fourth in worldwide cereal production, and is used for a variety of end uses, including animal feed, human consumption and malting. The economic importance of barley has meant that marker-assisted breeding approaches are of considerable interest. Deployment of genetic markers that predict the phenotypic trait of interest with 100 % accuracy ('perfect markers') allows efficient tracking of favorable genetic variants through the breeding process, without the need for phenotypic evaluation [1]. However, development of such markers has been slow, partly due to the large size of the barley genome (5,500 Mbp). Accordingly, many of the molecular markers developed for marker assisted selection (MAS) are actually 'diagnostic markers', which predict phenotype with varying degrees of accuracy. Nevertheless, the deployment of genetic markers within breeding programmes can be of considerable economic benefit. Barley has several advantages over related temperate cereal crops for the investigation of the genetic basis of phenotypic diversity [2].

### Molecular markers used in Barley breeding

With the advances of molecular markers, population genetics of crop plant has become an important tool for conserving and maintaining germplasm collections. Studies of population genetics of crops offer a unique opportunity to identify footprints or signature of selection which can give valuable insight which help to identify new genes [3].

### Restriction fragment length polymorphism/RFLP

RFLP was the first molecular marker technique and the only marker system based on hybridization. Individuals of same species exhibit polymorphism as a result of insertion/deletions (known as InDels), point mutations, translocations, duplications and inversions. Isolation of pure DNA is the first step in the RFLP methodology. This DNA is mixed with restriction enzymes which are isolated from bacteria and these enzymes are used to cut DNA at particular loci (known as recognition sites). This results in a huge number of fragments with different length. Agarose or polyacrylamide gel electrophoresis (PAGE) is applied for the separation of these fragments by producing

a series of bands [4-6].

### Random Amplified Polymorphism DNAs (RAPDs)

The assay was developed independently by two laboratories (Welsh and McClelland, 1990; Williams et al., 1990). The RAPD assay is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence. This procedure detects nucleotide sequence polymorphisms in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA template. If these priming sites are within an amplifiable distance of each other, a discrete DNA product is produced through thermo cyclic amplification. The presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and the oligonucleotide primer, at each end of the amplified product. These molecular markers had been used in barley for detecting genetic diversity and genotype identification [7-9].

### Microsatellite /SSRs

Microsatellites, also called Simple Sequence Repeats (SSRs), are among the advanced genetic markers currently developed (Struss and Plieske, 1998). The SSR assay is increasingly being applied to plant mapping projects due to its relative advantages. First, SSRs are highly polymorphic and thus, highly informative in plants. Second, SSRs can be analyzed by a rapid and technically simple PCR-based assay.

### SNPs

SNPs (single nucleotide polymorphisms) are bi-allelic markers and represent the smallest units of genetic variation in genomes (Rafalski, 2002). However, the extraordinary abundance of SNPs in the genome largely offsets the disadvantage of their being biallelic and makes them the most attractive molecular marker system developed so far. Although the development of SNP markers is still underway in crop plant species, these markers have already been successfully used for genetic diversity studies (Kota et al. 2001, Kanazin et al. 2002, Bundock and Henry 2004, Bundock et al. 2006). The allelic frequencies of a given SNP may vary in different populations. Kanazin et al. (2002) evaluated the prevalence of SNP polymorphism at 54 loci across five genotypes and found 38 SNP loci, which revealed the occurrence of one SNP per 189 bases.

Russell et al. (2004) studied the frequency and distribution of

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nucleotide diversity within 23 genes in three germplasm groups representing European cultivars, landraces and wild accessions and identified one SNP in every 78 bp and insertion deletion one in every 680bp. The integrated map showed good agreement with the published maps in terms of marker order, and known abiotic stress QTL mapped in relevant crosses [10].

### SCoT

A new molecular marker system called Start Codon Targeted Polymorphism (SCoT) was described by Collard and Mackill (2009), based on the observation that the short conserved regions of plant genes are flanked by the ATG translation start codon. The technique uses single primers designed to anneal the surrounding regions of the ATG initiation codon on both DNA strands. The generated amplicons are possibly distributed within gene regions which contain geneson both plus and minus DNA strands.

### Conclusion

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Insertion, deletion, point mutations duplication and translocation are basis of these polymorphisms; however, they do not necessarily affect the activity of genes. An ideal DNA marker should be co-dominant, evenly distributed throughout genome, highly reproducible and having ability to detect higher level of polymorphism. Various different molecular markers such as AFLP, RFLP, RAPD, Microsatellite, SNPs and SCoT have been used for genetic diversity studies and molecular breeding in barley.

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### Conflict of interest

The author declares there is no conflict of interest in publishing this article.

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