SNP based haplotyping towards development of salt tolerant rice

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Abstract

Traditional rice varieties harbour a large repertoire of genetic diversity. Sequencing and re-sequencing of multiple rice genomes are presenting opportunities to identify natural allelic diversity that is tracked as molecular markers to identify desired traits. Exploring the genomes of rice germplasm carrying favourable alleles brings tremendous achievements in crop improvement. With the advent of SNP (Single Nucleotide Polymorphisms) based markers, tagging of agriculturally important genes have been greatly facilitated due to their genome-wide abundance and amenability for high throughput detection platforms. In this review, we discuss the application of SNP markers in the selection of haplotype and designing the experimental strategies towards the development of salt tolerant rice.

Key words

Candidate gene, haplotyping, polyamines, rice, salt tolerance, SNP,

Introduction

Natural variants of crops, either wild relatives or landraces, which have the inherent adaptability to various extreme environmental conditions, are considered as 'reserve currency' for any crop improvement programme to cater the food demands of the ever increasing human population. The advent of high-throughput 'Next-Gen' sequencing technology now enables rapid and accurate resequencing of a vast number of crop genomes to detect the genetic basis of phenotypic variations in the hitherto unexplored gene pool of crop plants as well as their wild relatives.

Polymorphisms are the changes in DNA sequences, results for the existence of different forms of alleles of the same gene. These differences are tracked as molecular markers to identify desired traits. As a marker must be found close enough to the gene of interest with variations (alleles), both the marker and the gene are to be inherited together. Therefore, a genetic marker provides information about allelic variation at a given locus. The presence of one or more Single Nucleotide Polymorphisms (SNP) contributes to allelic variations of a particular gene/trait.

A haplotype stands for a set of linked SNPs on the same chromosome. SNPs have become the preferred markers for genome-wide association studies because of their high abundance. The detection of a significant association between SNP (or haplotype), biochemical quantitation and phenotyping lead to understanding the maximum probability of a desirable trait that in turn greatly accelerate studies on crop designs via genomics-assisted breeding.

Soil salinity is one of the major limiting factors for worldwide agricultural productivity. For better management and efficient soil amelioration programmes in salt-affected areas, genetic tailoring of the crop plants is considered to be more practical and economically viable with tremendous potential. In this regard, selection of germplasm with the inheritance of physiological traits for salinity tolerance is considered as the best starting material for the successful breeding programme. Molecular markers such as the Single Nucleotide Polymorphisms (SNPs) in combination with genomics and other next-generation technologies, has the power and efficiency of genotyping of the desired traits for categorising haplotypes, which in turn will accelerate the Marker-Assisted Breeding (MAB) programme for economically improved crops.

Evolution of Molecular Markers: From early generation to 'Next – Gen."

Development of molecular marker is of immense importance to study a complex trait's underlying gene network. An ideal molecular marker should have the following criteria: (i) cost effectiveness, (ii) polymorphism and even distribution throughout the genome, (ii) provision of adequate resolution of genetic difference, (iii) co-dominance and (v) linkage to distinct phenotypes.

To a plant breeder, DNA marker is an efficient selection tool for Marker Assisted Breeding programme for the development of improved crop variety. DNA markers are DNA profiles that give information about the

genotypes which provides advantages over the phenotypic and biochemical markers by giving direct insight the genetic makeup of the whole genome including coding and non-coding regions[1]. During the last three decades, there have been tremendous advances in the evolution of molecular marker systems primarily driven by the two practical and technical factors: (i) Cost effectiveness and (ii) the level of reproducibility.

One can classify DNA markers under three types. (a) Hybridization-based (such as Restriction Fragment Length Polymorphisms - RFLP); (b) PCR-based (such as Random Amplification of Polymorphic DNA - RAPD and Simple Sequence Repeat - SSR) or (c) markers that combine both the principles of restriction digestion and PCR technique (Amplified Fragment Length Polymorphism - AFLP)[1].

Table-1: DNA Markers

FIRST GENERATION DNA MARKERS			
Year	**Acronym**	**Nomenclature**	**Reference**
1974	RFLP	Restrition Fragment Length Polymorphism	Grodzicker *et al.* (1974) [2]
1985	VNTR	Variable Number Tandem Repeats	Jeffreys *et al.* (1985) [3]
1989	STS	Sequence Tagged Site	Olsen *et al.* (1989) [4]
SECOND GENERATION DNA MARKERS			
1990	RAPD	Randomly Amplified Polymorphic DNA	Williams *et al.* (1990) [5]
1992	CAPS	Cleaved Amplified Polymorphic Sequence	Akopyanz *et al.* (1992) [6]
1992	SSR	Simple Sequence Repeats	Akkaya *et al.* (1992) [7]
1993	SCAR	Sequence Characterized Amplified Region	Paran and Michelmore (1993) [8]
NEW GENERATION DNA MARKERS			
1994	ISSR	Inter Simple Sequence Repeats	Zietkiewicz *et al* (1994) [9]
1994	SNP	Single Nucleotide Polymorphisms	Jordan and Humphries (1994) [10]
1995	AFLP (SRFA)	Amplified Fragment Length Polymorphism (Selective Restriction Fragment Amplification)	Vos *et al.* (1995) [11]

RFLP markers detect the single base substitutions in the recognition sequence of a restriction enzyme of DNA sample isolated from various sources resulting in the changed pattern of restriction fragments. Although it was discovered in the 1960s, however, till late eighties, RFLPs were the most popular molecular markers widely used in plant molecular genetics. The requirement of a high volume of starting materials i.e. DNA, labour, time and extensive uses of radioactive probes make this process a bit inconvenient.

The invention of PCR technology and the application of this method for the rapid detection of polymorphisms have generated PCR-based markers at the beginning of the 1990s. Randomly Amplified Polymorphic DNA (RAPD) can detect polymorphic loci in various regions of a genome. RAPD is a low-cost marker but has some limitations such as RAPD bands of same molecular weight need not have same nucleotide sequence.

The combined principles of RFLP and PCR generate AFLP (Amplified Fragment Length Polymorphism) marker. AFLP marker involves selective amplification of double digested restriction products using adaptors linked to restriction fragment end acting as specific primer binding site for PCR amplification. For clearer amplification one to three extra nucleotides (arbitrarily chosen) are added to adapter sequence. The AFLP technique generates fingerprints of any DNA regardless of its source, and without any prior knowledge of DNA sequence. Due to lengthy, costly, laborious detection method as well as its dominant nature, AFLP markers have not found widespread application in molecular breeding.

Simple Sequence Repeat (SSR) or microsatellite markers are one of the most advanced molecular marker techniques and considered as the "markers of choice". Simple Sequences Repeat (SSR) are the short tandem repeat motifs of 1-6 bp that are ubiquitously present in the genome. Variation in the number of tandem repeated units impart polymorphism to a particular locus of the gene of interest. This polymorphism allows detecting many different alleles for a single marker. SSR markers are highly reproducible, polymorphic, and amenable to automation. DNA fragments have different lengths due to the number of SSR repeats, which can be separated and detected by Gel Electrophoresis. Furthermore, being co-dominant in nature it is capable of allelic discrimination as shown in the figure below.

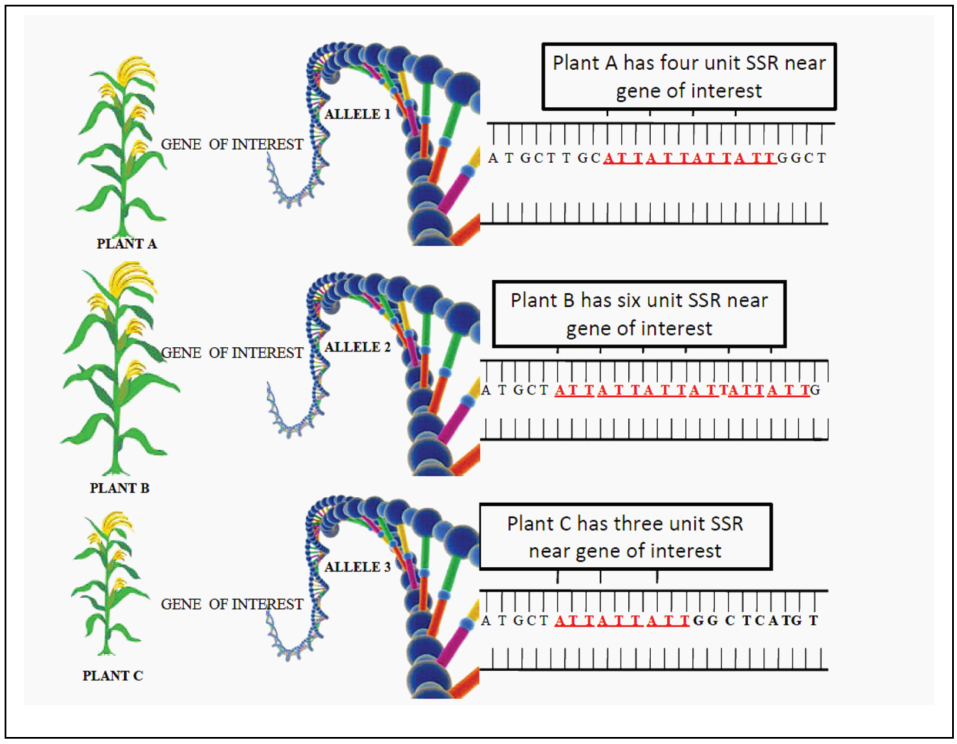

Figure 1: Diagrammatic representation of Single Sequence Repeats Markers or Microsatellites

In recent years, Single Nucleotide Polymorphisms (SNPs) within DNA sequences (of candidate genes) are being used for their unique merits and, therefore, preferred over the other classes of markers. SNPs are the single base pair sequence alternatives (alleles) present in individuals in the same population and are the most common source of genetic polymorphisms resulting from DNA replication errors and damage. An SNP ("snip") is a single base mutation in DNA and are 'conserved' across the genome, their high abundance in the genome makes them informative. Presence in the coding region attributes for phenotypic change that pinpoints functional polymorphism.

Salt tolerance vis-à-vis the role of Polyamines

One of the major environmental factors that reduce rice productivity globally is soil salinity. Salinity creates the particular problem of ion toxicity because a higher concentration of sodium is detrimental to all cells. Polyamines (PAs) (putrescine, spermidine and spermine) are a group of polycationic aliphatic amine universally present in almost all living entities including plants. Though polyamines play a significant role in cell growth and development, evidence has showed their direct involvement in various stress tolerance in plants including salinity [12-15]. The higher polyamines specifically well known for the contribution in stress tolerance are the triamine spermidine (Spd^{3+}) and the tetraamine spermine (Spm^{4+}). Studies have suggested that the super cationic nature of higher polyamines i.e. triamine spermidine and (Spd^{3+}) and the tetraamine spermine ($Spm4^{+}$) interact with the negatively charged head group of phospholipid membrane components, thereby stabilizing the plasma membrane and reduce the salt stress induced deleterious effect [16-18].

Extensive analyses of polyamine levels have revealed that the salt tolerant rice cultivars accumulate high levels of higher polyamines (Spd and Spm) in root plasma membrane during salinity stress in comparison to the salt sensitive cultivars [19-21]. The addition of polyamines along with sodium chloride reduces membrane damage, lipid peroxidation, senescence induced protease and RNAse activities as well as chlorophyll loss.

Genetic transformation with polyamine biosynthetic genes encoding arginine decarboxylase (ADC), ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (SAMDC) or spermidine synthase (SPDS) showed diverse stress tolerance including salinity, drought, low and high temperature in various plant species [22-25].

The spermidine and spermine are synthesized by spermidine synthase (SPDS) and spermine synthase (SPMS) respectively through the addition to diamine putrescine of an aminopropyl moiety donated by decarboxylated-S-adenosylmethionine (dcSAM) (Figure 2). The decarboxylated-S-adenosylmethionine i.e. dcSAM is formed by S-adenosylmethionine decarboxylase enzyme (SAMDC). S-adenosylmethionine decarboxylase is a key factor involved in the biosynthesis of higher polyamines viz. spermidine and spermine by the synthesis of dcSAM.

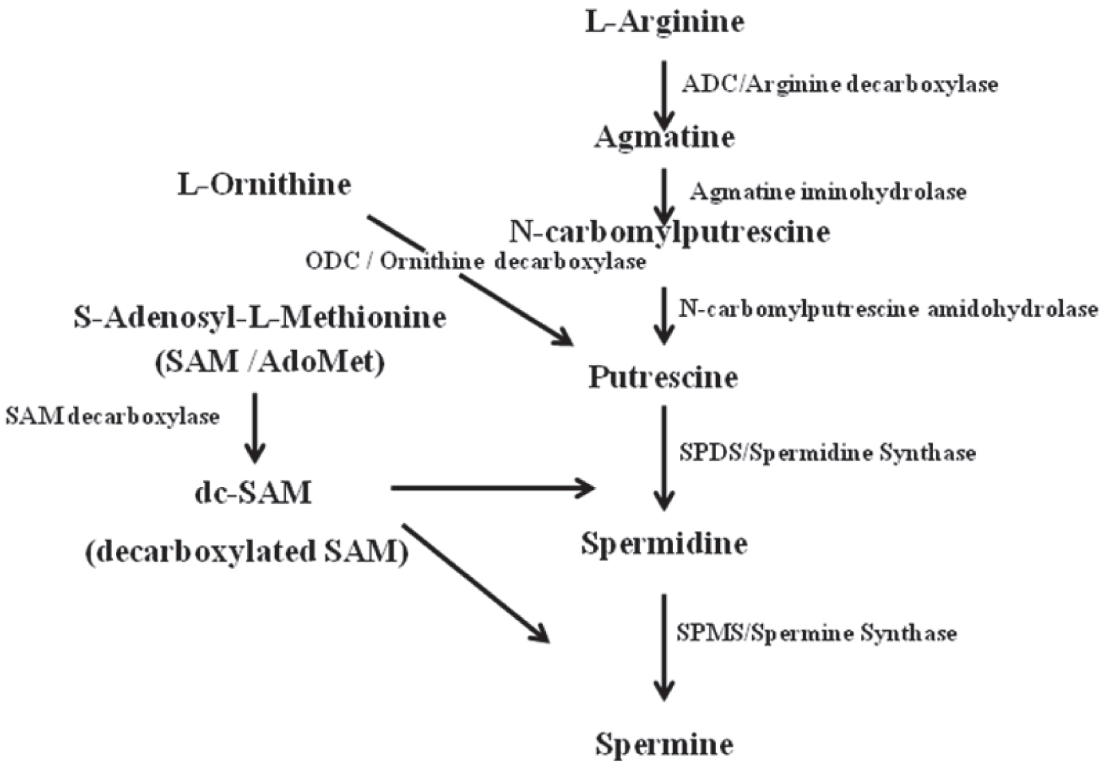

Figure 2: A brief outline of Polyamine biosynthetic pathway

Candidate genes and Haplotyping

The candidate gene approach to conducting genetic association studies focuses on associations between genetic variation within pre-specified genes of interest and phenotypes. This approach is in contrast to Genome Wide Association Studies (GWAS), which scan the entire genome for common genetic variation (Figure 3). In the present case, the aforesaid three essential genes of polyamine biosynthetic pathway can be considered as candidate genes. SNPs within the full gene sequences of these genes in rice landraces will pave the way for the illusive allele imparting salt tolerance in rice by Marker Assisted Breeding.

The 'Haplotype' of an individual describes particular alleles at some of linked SNP loci associated with each chromosome of a homologous pair. When the chromosome contains more than two or even up to a dozen SNPs, those are considered together for haplotyping. Haplotypes are 'blocks' of associated SNPs, a set of closely linked genetic markers present on one chromosome which tends to be inherited together (not easily separable by recombination).

Rice landraces have in-built adaptability to various extreme environmental conditions. Exploration of the non-cultivated or locally cultivated germplasm can be a real source of candidate genes of polyamine biosynthetic pathway responsible for the salt tolerance property. Single Nucleotide Polymorphism (SNP) markers can be used to large-scale genotyping. They are required to maximize the use of DNA sequence variation and determine the functional relevance of those candidate genes for complex stress tolerance traits through haplotyping. Haplotypes that are DNA sequences containing identical allelic variants at all identified polymorphic sites at a locus, but derived from separate individuals, are identified visually or by alphabetical sorting of the list of sequence variants at a locus. Identification of SNP haplotypes within the landraces of rice provides preliminary insights into the genotype with desired traits. The identified haplotypes which associate with the desired genotypes of the candidate gene follows biochemical testing. This accentuates Marker Assisted Breeding programme for improved germplasm.

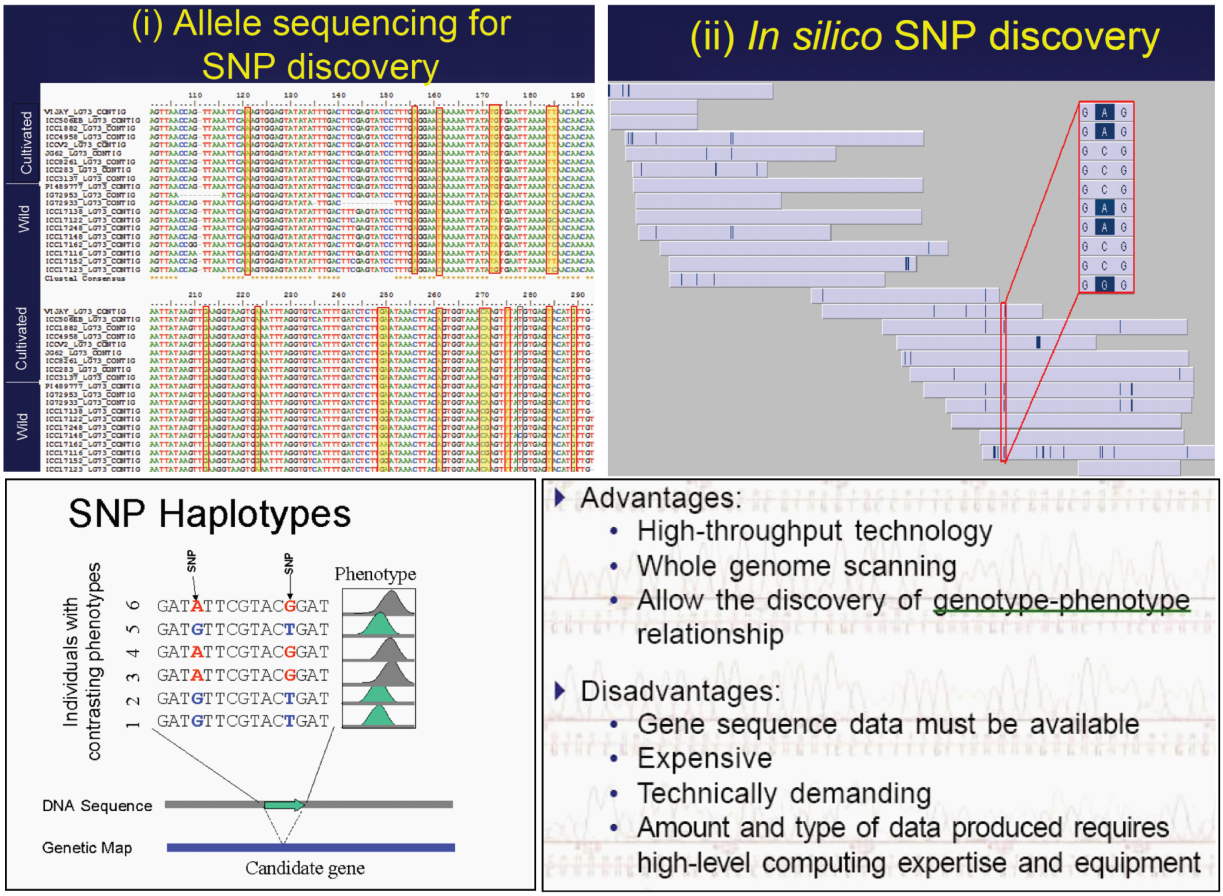

Figure 3: Two approaches to SNP discovery.

Conclusions

Enormous genomic and cDNA sequence data that are accumulating in the databases are extremely useful for discovery of new SNPs. The hypothesis is the SNPs vis-à-vis allelic variation of each polyamine biosynthetic candidate gene like spermidine / spermine synthase is behind differential Salt stress response in different genotypes. Development of SNP-based molecular markers associated with tolerance to salinity and related abiotic stresses will accelerate breeding progress. This endeavour will help breeding efforts aiming to enhance rice productivity in salt Prone regions.

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