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Marker Assisted Selection: Biotechnology Tool for Rice Molecular Breeding

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Abstract

The biotechnology tool of MAS has irreversibly changed the disciplines of conventional rice breeding. Molecular markers are indispensable tools for measuring the diversity of rice varieties and rice breeding. However, MAS is not always advantageous, so careful analysis of the costs, convenience, ease of assay development and automation are important factors to be considered when choosing a technology relative to the conventional breeding programs. This review focuses on possibilities for the application of marker-assisted selection in the genetic improvement of rice breeding.

Keywords: Rice; Biotechnology; Molecular breeding; Hybridization

Introduction

Rice is a dietary staple food for at least 62.8% of the world population. In Asia it accounts for 29.3% [1]. Global rice consumption is projected to increase from 450 million tons in 2011 to about 490 million tons in 2020 and to about 650 million tons by 2050 [2].The main challenge encountered by scientists involved in rice research and production in the world is to find appropriate solutions for major issues such as the impacts of climate change viz. temperate, water use efficiency and availability of pollution will play a key role in determining food security in large parts of the world. Also the environmental crisis and plant diseases and pests have been the factors that decrease rice production in many countries all around the world [3].At this time when the world's population is increasing rapidly and the demand for food is high, these problems have threatened food security and people health worldwide. In order to meet these growing problems in future ahead, it is necessary to use rice varieties with higher yield potential, durable resistance to diseases and insects and tolerance to abiotic stresses.

Yield potential of rice can be improved with the help of various strategies; conventional hybridization and selection procedures, ideotype breeding, heterosis breeding, wide hybridization and molecular breeding [4]. There are two strategies in biotechnological application in molecular rice breeding; one is by Marker-Assisted Selection (MAS), also called marker-assisted breeding (MAB) and the other one is by developing the Genetically Modified crops. A genetic marker is any visible character or otherwise assayable phenotype, for which alleles at individual loci segregate in a Mendelian manner. MAS is a technique that does not replace traditional breeding, but can help to make it more efficient. It does not include the transfer of isolated gene sequences such as genetic engineering, but offers tools for targeted selection of the existing plant material for further breeding.

The genetic markers covered include (1) morphological markers (2) biochemical markers (alloenzymes and other protein markers) and (3) molecular markers (based on DNA-DNA hybridization).

DNA-based molecular markers

DNA marker is a small region of DNA sequence showing polymorphism between different individuals. They arise from different classes of DNA mutations such as substitution mutations (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA [5]. These markers are selectively neutral because they are usually located in non-coding regions of DNA. DNA markers are the most widely used type of marker predominantly due to their abundance. Unlike morphological and biochemical markers, DNA markers are practically unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant [6].

Properties which desirable for ideal DNA markers include highly polymorphic nature, codominant inheritance (determination of homozygous and heterozygous states of diploid organisms), frequent occurrence in the genome, selective neutral behavior (the DNA sequences of any organism are neutral to environmental conditions or management practices), easy access (availability), easy and fast assay, high reproducibility, and easy exchange of data between laboratories [7]. Also should follow Mendelian inheritance, genetically linked to trait in question and not affected by pleiotropism and epistatic interactions [8].

There are two basic methods to detect the polymorphism: Southern blotting, a nuclear acid hybridization technique and polymerase chain reaction (PCR) technique. Using PCR and/or molecular hybridization followed by electrophoresis (e.g. Polyacrylamide gel electrophoresis, Agarose gel electrophoresis, Capillary electrophoresis), the variation in DNA samples or polymorphism for a specific region of DNA sequence can be identified based on the product features, such as band size and mobility [9].

Among the techniques that have been extensively used on plant breeding, are the Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified

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Polymorphic DNA (RAPD), Microsatellites Or Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Expressed Sequence Tag (EST), Cleaved Amplified Polymorphic Sequence (CAPS), Diversity Arrays Technology (DArT), Sequence Characterized Regions (SCARs), Sequence Tag Sites (STSs) and Single Nucleotide Polymorphism (SNP) [10,11].According to a causal similarity of SNPs with some of these marker systems and fundamental difference with several other marker systems, the molecular markers can also be classified into SNPs (due to sequence variation, e.g. RFLP) and non-SNPs (due to length variation, e.g. SSR) [10]. RFLP is the most widely used hybridization-based molecular marker [12]. The various PCR-based techniques are of two types depending on the primers used for amplification: 1) Arbitrary or semi-arbitrary primed PCR techniques that developed without prior sequence information (e.g., AP-PCR, DAF, RAPD, AFLP, ISSR). 2) Site targeted PCR techniques that developed from known DNA sequences (e.g., EST, CAPS, SSR, SCAR, STS, SNP)[13,14].

PCR-based markers are more attractive for MAS, due to the small amount of template required and more efficient handling of large population sizes. AFLP, RAPD and Sequence tagged site (STS) are dominant markers, which limits its application for differentiation of homozygous and heterozygous individuals in segregating progenies. Among the DNA markers, the most widely used markers in major cereal crops are SSRs or microsatellites [14,15].

Marker assisted selection

Marker assisted evaluation of breeding materials involves cultivar identity, assessment of purity and genetic diversity, parental selection, study of heterosis and identification of genomic regions under selection [16]. MAS refer to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening. These DNA markers should reliably predict phenotype. By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype. A marker can either be located within the gene of interest or be linked to a gene determining a trait of interest, which is the most common case. Thus MAS can be defined as selection for a trait based on genotype using associated markers rather than the phenotype of the trait [17].

Molecular markers can be used in many steps of a rice breeding program, e.g. germplasm characterization, pedigree and evolution studies, parental selection for crossing, test for F1 hybrid confirmation, test for genetic purity of seeds, cultivar protection, breeding strategies establishment, link- age map construction, and mapping of genes and QTLs associated with biological processes.

Application of DNA markers in MAS indicated in five main considerations viz. i.) Reliability: Molecular markers should co-segregate or tightly linked to traits of interest, preferably less than 5 cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype. ii.) DNA quantity and quality: Some marker techniques require large amounts and high quality DNA, which may sometimes be difficult to obtain in practice and this, adds to the cost of the procedures. iii.) Technical procedure: Molecular markers should have high reproducibility across laboratories and transferability between researchers. The level of simplicity and time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable. iv.) Level of polymorphism: Ideally, the marker should be highly polymorphic in breeding material and it should be co-dominant for differentiation of homozygous and heterozygous individuals in segregating progenies. v.) Cost: Molecular markers should be user-friendly, cheap and easy to use for efficient screening of large populations. The marker assay must be cost-effective in order for MAS to be feasible [18].

Applications of MAS

With respect to important MAS strategies, three main uses of molecular markers in rice breeding can be emphasized: Marker assisted evaluation of breeding material, Marker assisted introgression and Marker-assisted pyramiding.

Marker assisted evaluation of breeding material

To improve early generation selection, markers should decrease the number of plants retained due to their early generation performance, and at the same time they should ensure a high probability of retaining superior lines [19]. Markers are also frequently used to select parents with desirable genes and gene combinations, and Marker-assisted recurrent selection (MARS) involve several successive generations of crossing individuals based on their genotypes. This type of evaluation has the potential to make parental selection more efficient, to expand the gene pool of modern cultivars and to speed up the development of new varieties [20].

Marker assisted introgression

Introgression is the procedure of the transfer of genetic information from one species to another as a result of hybridization between them and repeated backcrossing. The process, where a gene or a QTL from a population A is introduced to a population B by crossing A and B and then repeatedly backcrossing to B, is called introgression [21]. Here, molecular markers can be used to control the presence of the target gene or QTL and to accelerate the return of background genome to recipient type. Marker-assisted introgression is very effective for introgressing genes or QTLs from landraces and related wild species, because is reduces both the time needed to produce commercial cultivars and the risk of undesirable linkage drag with unwanted traits of the landrace or wild [22].

Marker-assisted pyramiding

Marker assisted pyramiding is the process of combining several genes together into one genotype and using DNA markers for selection [23].Gene pyramiding is a useful approach to the durability or level of pest and disease resistances, or to increase the level of abiotic stress tolerance. Genes controlling resistance to different races or biotypes of a pest or pathogen and genes contributing to agronomic or seed quality traits can be pyramided together to maximize the benefit of MAS through simultaneous improvement of several traits in an improved genetic background [22].

Discussion

Adequate genotyping and phenotyping are both important for the success of rice breeding with MAS. MAS can be used in any breeding method (e.g. backcross marker assisted method) for any single gene transfer procedure if reliable markers exist and the indirect selection is more advantageous than the direct selection of the trait.

Many agronomically important traits/genes of rice have been mapped with linked markers [Table 1]. When the selected trait is expressed late in plant development, like seeds and flower features or adult characters in crop with a juvenile period, faster selection process because an individual's phenotype can be predicted at a very early stage since screen can follow at the seedling stage or even as seeds rather

Table 1: Some of the success stories related to application of MAS in rice [28-37].

No.	Target trait Gene(s)/ QTL(s)	Type/name of marker(s) used	Remarks
1.	eating, cooking and sensory quality : Wx gene	microsatellite	MAS applied for Marker-assisted backcross breeding
2.	Os2AP or badh2 gene Wx gene	microsatellite	Marker Assisted Backcrossing
3.	Sub1, Wx gene, Osbadh2, SSIIa loci,	microsatellite	Ideotype breeding
4.	Bacterial blight (BB) resistance And Blast resistance xa5, xa13, Xa21 &Pi25	CAPS, STS	MAS applied for pyramiding multiple genes
5.	Blast resistance Pi-9	STS	Introgressed the broad-spectrum blast resistant gene Pi-9(t)
6.	Genetic Diversity Analysis	RAPD and SSR	SSR markers for the accurate determination of relationships between accessions
7.	Bacterial blight (BB) resistance xa13 and Xa21	CAPS , STS	Improved the two traditional BB-susceptible Basmati varieties
8.	Bacterial blight (BB) resistance And stem borer Xa21, Bt & Chitinase	STS	MAS applied for pyramiding of target traits. Bt gene and Chitinase gene originally
9.	BPH resistance Bph1 Bph2	STS	MAS applied for gene pyramiding
10.	Submergence tolerance Sub1QTL	SSR	MAS applied for backcross breeding

than having to wait for the individual to develop to a stage where the adult phenotype is apparent [24].With the fast and constant advance of molecular technologies, it is plausible to predict that the main constraint in the near future will be the ability of the breeder to make a high quality phetotyping. The total number of lines that need to be tested can be reduced. Since many lines can be discarded after MAS early in a breeding scheme, this permits more efficient use of glasshouse.

MAS using co-dominance markers (e.g. SSR and SNP) can allow effective selection of recessive alleles of desired traits in the heterozygous status. No selfing or test crossing is needed to detect the traits controlled by recessive alleles, thus saving time and accelerating breeding progress[25].

With comparison to transgenesis, MAS can be considering that there are not major issues of biosafety and intellectual property rights. MAS respect species barriers and is accepted by the public. Genetic engineering is not the most effective tool to develop crops with complex traits such as drought and salinity tolerance, nor is it necessary.

Startup expenses and labor costs are higher in many cases. Therefore, as other new methods of rice breeding like transgenic breeding or genetic manipulation do, MAS cannot replace conventional breeding but is and only is a supplementary addition to conventional breeding. High costs and technical or equipment demands of MAS will continue to be a major obstacle for its large-scale use in the near future, especially in the developing countries [26,27]. For marker assisted backcrossing, the initial cost of using markers would be more expensive compared to conventional breeding in the short term however time savings could lead to an accelerated variety release which could translate into greater profits in the medium to long term.

Conclusion

Adoption of a completely new variety by farmers could take considerable time, whereas chances of acceptability of converted popular varieties are relatively higher. Improvement of these traits viz. yield, grain quality, select efficiency, disease and pest resistance, and stress tolerance, illustrates the superiority of using marker assisted selection in crop improvement compared to conventional breeding. The integration of these techniques towards producing improved varieties should solve farmers demands while contributing to the protection of national food and environmental security. Therefore, integration of MAS into conventional breeding programs will be an optimistic strategy for crop improvement in the future.

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