

Connecting Rice Germplasm to Plant Breeding: Backcrossing for Allele Mining and Recurrent Selection for Allele Pyramiding Through Molecular Marker Technology

Jian-Long Xu^{1,2*} and Jauhar Ali³

¹Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

²Shenzhen Institute of Breeding & Innovation, Chinese Academy of Agricultural Sciences, Shenzhen, China

³Plant Breeding, Genetics and Biotechnology, International Rice Research Institute, Metro Manila, Philippines

*Corresponding author: Jian-Long Xu, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China, Tel: 010-82105854; E-mail: xujlcaas@126.com

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Green Revolution

'Green revolution' in 1960s and successful utility of heterosis in 1970s have resulted in two successive leaps in rice productivity [1,2]. Although extensive breeding efforts were made for high yield in past decades, the yield potential of modern rice varieties has remained stagnant for many years. However, the world's rice production need to double again by the year 2030 to keep up with the demands of a growing population [3] and much of this increase will mainly depend on improved rice cultivars. Therefore, further improving yield potential has been the top priority in almost all rice breeding programs worldwide.

QTL Mapping and Allele Mining by Advanced Backcross Method

The rich genetic diversity in the worldwide rice germplasm collections contains genes capable of improving almost all traits of modern varieties. Favorable genes affecting yield and other agronomic traits can be identified by molecular genetic maps [4]. Recently, a wide range of segregating populations derived from bi-parental crosses, including recombinant inbred lines (RILs), doubled haploid lines (DH), F₂ and its derived populations, and BC or testcross populations, have been used for mapping QTL [5]. Huge numbers of genes/QTL have been identified and mapped on the 12 rice chromosomes (<http://www.grammene.org/>). However, two factors may be contributing to the less-than-expected impact of marker-based QTL analysis on the development of varieties with enhanced quantitative traits:

(1) Allele diversity can't be dissected and analyzed due to segregation of only two alleles at each locus in the bi-parental derived populations, resulting in failure to mine favorable or novel alleles from germplasm. The phenotypic effects of different alleles in rice germplasm at those identified QTL and their values in rice breeding remain largely unknown, making it difficult to use them in breeding.

(2) Separation of QTL mapping from breeding practice. Because QTL expression largely depends on genetic background and environment, QTL detected in the mapping populations can't be directly applied in the breeding population.

To integrate QTL mapping with improvement of rice variety, Tanksley and Nelson [4] proposed the method of advanced backcross QTL (AB-QTL) analysis as a way to control background genetic variation in QTL mapping (Figure 1). By simultaneously identifying and transferring favorable QTL alleles from unadapted to cultivated

germplasm, AB-QTL analysis has since become a popular way of genetic dissection of quantitative trait variation in many crops such as rice [6,7] maize [8], wheat [9] and barley [10]. The advantages of advanced populations over balanced populations (e.g., F₂, BC1, RILs) are: (1) Accuracy in measurement of yield and other traits in similar background with the elite variety, thus ensuring QTL mapping results more meaningful. (2) Less epistasis detected due to the overall lower frequency of donor alleles in advanced generations, making a better prediction of the identified QTL when it is transferred into the cultivated background. (3) High efficient integration of QTL discovery with variety improvement in the same population. However, AB-QTL analysis also faces the challenge of incapability of mining allele diversity and high cost of genotyping probably resulting from the large BC mapping population derived from bi-parents. Subsequently, a strategy of genome-wide trait-specific introgression lines (ILs) was proposed that had got widely used in molecular dissection of complex phenotypes and pyramiding of favorable alleles for enhanced quantitative traits in rice [10-13], which is consisted of three steps (Figure 1):

(1) Development of advanced backcross populations. Series of introgressed bulk populations can be developed in elite variety's backgrounds using diverse germplasm accessions as donor parents by backcross breeding procedure.

(2) Trait screening and progeny test. BC bulk populations will be screened and progeny-tested for target traits such as high yield, resistances or tolerances to different abiotic and biotic stresses, resulting in multiple sets of trait-specific introgression lines (ILs), which much outperform the recurrent parents for the target traits.

(3) Gene/QTL detection and allelic mining. Gene/QTL detection can be conducted by tracking and characterizing genome-wide responses of donor segments to selection. Deviation (excess or deficiency) of donor allele frequency at single loci in ILs from the expected level estimated in the specific random BC populations can be detected by X² tests [12]. Favorable alleles for the target traits will be identified by comparisons of gene effects of different alleles from diverse donors in the same elite background. Based on phenotypic performance and distribution of favorable alleles of ILs, it is easy to pyramid different favorable alleles in the same elite background by marker-assisted selection (MAS).

Although analogous to the AB-QTL analysis approach, the trait-specific ILs mapping strategy was more time-saving and cost-effective than the conventional QTL mapping approach in at least three aspects:

(1) Selecting extreme phenotypes visually was much easier than phenotyping the whole population practiced in typical QTL mapping experiments, thus minimizing errors in QTL mapping resulting from phenotyping fluctuation.

(2) A small number of ILs with extreme phenotypes selected from each BC population greatly reduces the genotyping and phenotyping cost.

(3) Trait-specific ILs are actually target-trait improved materials in the elite background with many trait-enhanced alleles from diverse donors, providing an ideal platform of materials for rice molecular breeding.

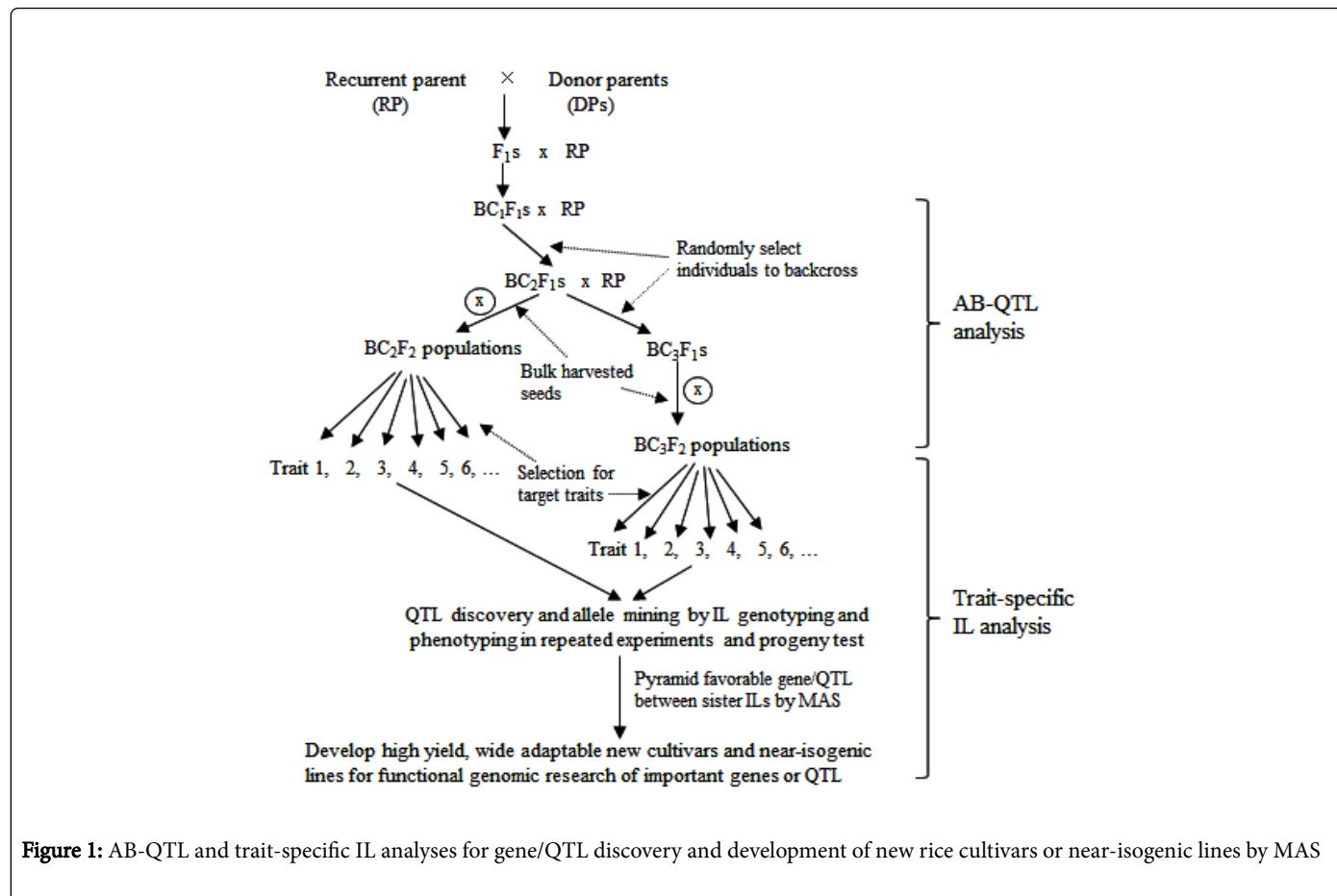


Figure 1: AB-QTL and trait-specific IL analyses for gene/QTL discovery and development of new rice cultivars or near-isogenic lines by MAS

Favorable allele pyramiding by recurrent selection

Chronic crossing with high pressure of selection within populations derived from elite varieties has been a mainstream method of modern breeding in crops. The pedigree method usually involves a limited number of elite progenitors-frequently related to each other – that are crossed. The long-term risk with pedigree selection is a reduced genetic base and small genetic gains. Owing to unfavorable linkage drag from exotic germplasm, many main-effect or major QTL identified from wild rice haven't been successfully utilized in rice breeding programs. This is a reason why inbred variety hasn't made breakthrough in yield over few past decades. Developing populations with broad genetic bases and using improvement methods that permit the continuous accumulation of favorable alleles can overcome these disadvantages. Recurrent selection (RS) can fit population improvement by high

efficient pyramiding of different favorable alleles from diverse donors. Recently, multi-parents advanced generation inter-cross (MAGIC) population has been introduced to plant community [14]. MAGIC population is of characteristics of diversities both in alleles and phenotypes which are from multiple parents. In addition, relatively large recombination events within the population ensure resolution of QTL mapping. Based on QTL mapping information and phenotypic performance, ideal individuals will be selected by pyramiding of multiple favorable alleles in the consecutive recurrent selection populations derived from intercrossing among the top plants in each round of RS (Figure 2). MAGIC populations have been developed for the purpose of integration of QTL mapping with breeding in rice [15] and wheat [16] crops.

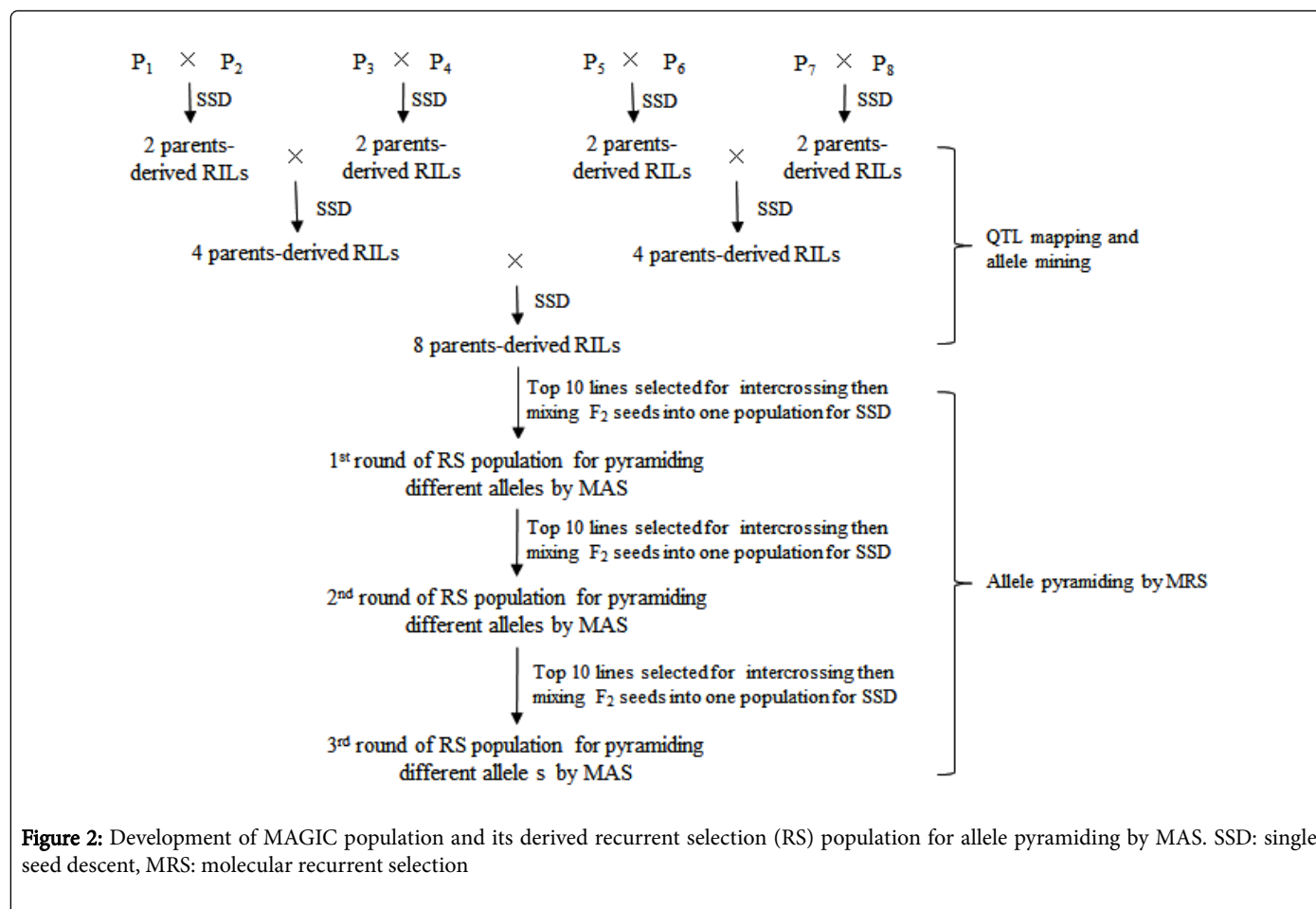


Figure 2: Development of MAGIC population and its derived recurrent selection (RS) population for allele pyramiding by MAS. SSD: single seed descent, MRS: molecular recurrent selection

Discovery and introgression of a dominant male nuclear sterile (DMNS) gene [17] into elite backgrounds make easy recombination of different alleles, producing half sterile individuals and another half fertile individuals in each round RS population. Human-aided outcrossing between DMNS sterile plants and fertile plants greatly facilitates recombination of alleles at all loci among individuals in the progeny of the RS population. The strategy of molecular marker combined with RS, referred to as molecular recurrent selection (MRS), probably provides a very promising way to pyramid different favorable alleles (Figure 3). It should allow more effective to identify recombinant of favorable alleles at all target loci and more effective breakdown of genetic drags in the MRS population through DMNS facilitated random mating. Furthermore, genomic selection (GS) can be entirely integrated with RS. Bulk-harvested seeds from all fertile plants in the RS population after the third round of recombination could be used for a training population after genetic uniformity is

achieved by rapid generation advancement (RGA) facilitation. Based on phenotypic data in the given environment and high throughput SNP data of the training population, QTL affecting the target traits will be identified and genetic prediction model will be set up for each target trait (Figure 3). In the subsequent RS populations, the prediction model can be used to estimate genomic breeding values (GEBV) of individuals for selection based on marker data, thus making more accuracy in selection owing to avoiding phenotyping of the target traits. In practice, both approaches separately involving promising trait-specific ILs in an elite variety background and diverse elite varieties can be used as base parents to cross with DMNS. As compared with the latter, the former has an advantage of synchronous heading date within a RS population which ensures random outcrossing of individuals with the DMNS plants but a relative disadvantage of lesser diversity in its subsequent RS populations.

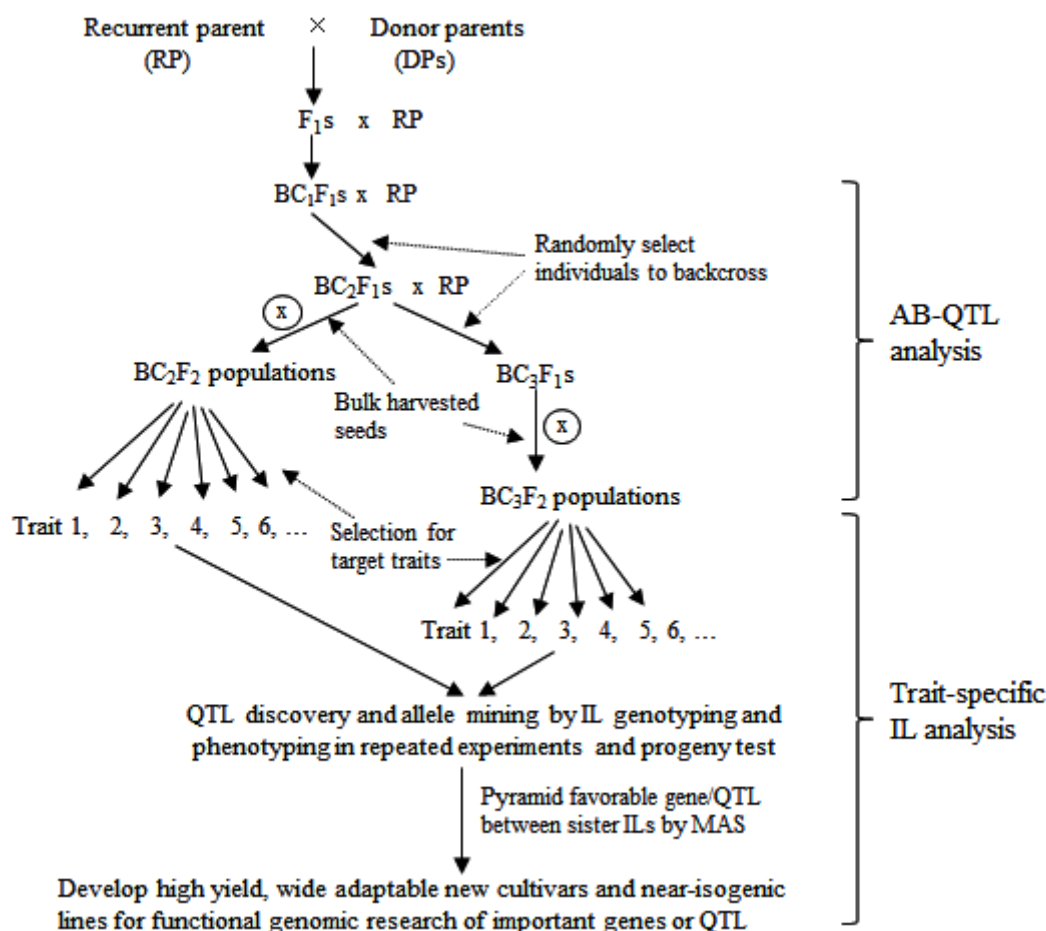


Figure 3: Population improvement by molecular recurrent selection (MRS) based on the dominant male nuclear sterile (DMNS) gene in rice. RS: recurrent selection, GS: genomic selection, SSD: single seed descent

In brief, rapid development of high throughput phenotyping and genotyping technologies has been facilitating identification of QTL affecting important agronomic traits in crops [18]. Backcrossing for allele mining and RS for allele pyramiding by MAS are becoming a high efficient way to integrate QTL mapping with breeding practice. It will greatly promote crop molecular breeding from concept to practice.

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