

Combining molecular marker technology and participatory techniques: A case study for drought-tolerant rice in eastern India. I: Molecular breeding strategy

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Abstract

Participatory plant breeding (PPB), like conventional breeding, is predicted to give a low response to selection for traits with low heritability. These include many traits that farmers consider desirable, such as drought resistance. The technology of marker-assisted selection (MAS) can be used to make selection for traits with low heritability more effective. We describe how MAS can be used to assist in the breeding of drought tolerance in upland rice.

Introduction

We are carrying out MAS for drought-tolerance-related traits. We have done this on large populations of young rice plants grown in the glasshouse at Bangor, or on rice plants grown in India from which DNA has been sampled. We are following three different strategies for combining PPB with molecular marker techniques. These are:

1. Backcross-MAS to pyramid root QTLs (quantitative trait loci) that should impart tolerance to drought, and a QTL controlling aroma in the grain.
2. Modified single large-scale MAS, to create bulk populations for PPB.
3. Marker-evaluated selection in which changes in allele frequency are assessed in a range of PPB products to enable the design and creation of ideotype varieties.

Strategy 1. MAS for root QTL and aroma

Drought is a serious abiotic constraint for rice farmers of Eastern India (Pandey *et al.*, 2000). Their land is often drought-prone because of sloping fields and shallow soils, and rainfall is unpredictable. These farmers predominantly grow landraces or varieties that are selections from landraces. Modern, improved varieties often have poorer drought tolerance than landraces. This can, in part, be due to poorly developed root systems, so farmers prefer to grow local landraces with deep root systems (Ekanayake *et al.*, 1985; Lafitte *et al.*, 2001). PPB and participatory varietal selection (PVS) in eastern India have been successful (Virk *et al.*, this volume) indicating that farmers appreciate being given a

greater choice of varieties and are willing to adopt them if they are superior.

The popular variety Kalinga III is susceptible to lodging and has a poor root system, making it prone to early-season drought. Several mapping studies have identified QTL for root traits in segregating populations and have identified QTLs in the variety Azucena that increase root length, thickness and penetration ability (Price and Tomos, 1997; Price *et al.*, 2000; Price *et al.*, 2002; Yadav *et al.*, 1997).

We targeted four QTLs controlling root traits (on chromosomes 2, 7, 9 and 11) that had large effects and were stable across experiments. These regions have all been shown to contain QTLs for drought-related traits in several crosses evaluated by other workers (for review see Zhang *et al.*, 1999). We selected for these four target regions in a backcross marker-assisted selection (BC-MAS) programme using Kalinga III as the recurrent parent. A fifth, highly heritable QTL for aroma on Chromosome 8 was also selected. The flanking markers used for selection were restriction fragment length polymorphisms (RFLP) and simple sequence repeats (SSR or microsatellites). Selection was made for the target regions in BC1, BC2 and BC3. At the BC3 generation, lines having at least two of the target regions were also screened with 30 background markers at non-target regions; two lines with higher frequencies of Kalinga III alleles at background regions were selected. These lines were advanced to fix the target Azucena alleles. Crosses between lines containing different target regions were made in order to pyramid all five target QTLs in the Kalinga III genetic background (Figure 1a).

Root screening of partial pyramid lines (containing 1, 2 or 3 target root QTLs) has been carried out in soil-filled pipes under field conditions

in Bangalore, India, (HE Shashidar, pers. comm.) and in root boxes under glasshouse conditions in Aberdeen, UK (A Price, pers. comm.). Some combinations of Azucena alleles at root QTL were shown to significantly increase root length, thickness and volume compared with Kalinga III.

A locus in the target root QTL on Chromosome 7 was found to considerably delay flowering in the glasshouse at Bangor, and experiments are planned to test if this is due to linkage drag or pleiotropy.

Strategy 2. SLS-MAS

The approach of single large-scale MAS (SLS-MAS) has been described (Ribaut and Betrán, 1999). This interactively combines the use of DNA markers and conventional breeding. It involves screening a large population of F₂ or F₃ plants for favourable loci while still maintaining, as much as possible, the allelic segregation in the rest of the genome. We used a population of more than 500 lines, derived from the previously selected BC₂, and screened them for markers at the four target root QTLs (Figure 1b).

In contrast to the conventional SLS-MAS

method, we only attempted to identify lines with one root QTL. We detected, on average, 25 lines that were fixed (homozygous) for each Azucena target locus. The selected lines with at least one root QTL were then screened with a marker linked to aroma to identify a subset of lines containing aroma. Of these, four were homozygous and seven were heterozygous for the aroma locus. The lines having QTLs identified by the markers were used to make bulks for PPB (Table 1). All the bulks were segregating for Azucena alleles in the non-target genomic regions.

Strategy 3. Marker-evaluated selection

In PPB we have used material from Kalinga III crossed to:

- IR64 and IR36 - both are elite modern varieties for irrigated conditions;
- Vandana, an Indian upland variety;
- Radha 32, a popular Nepalese variety (Gyawali *et al.* this volume).

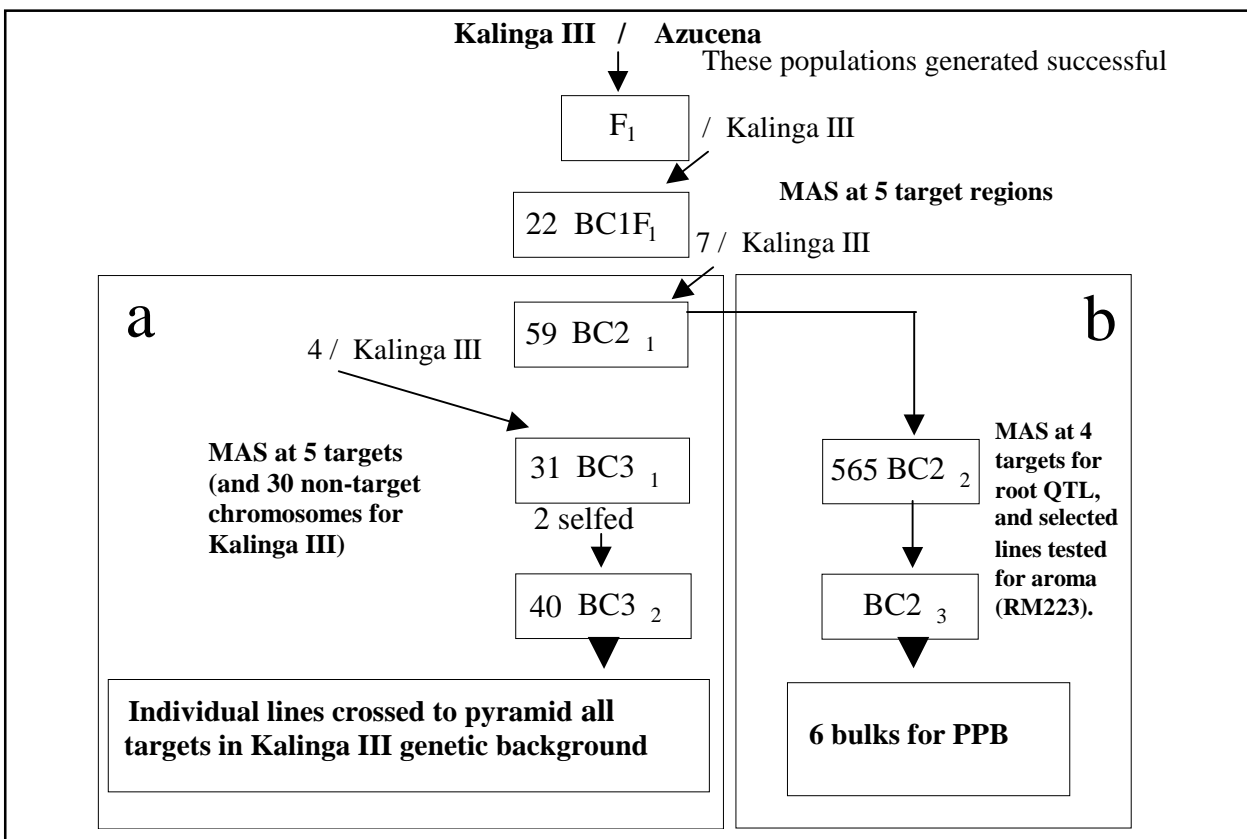


Figure 1. Backcross-marker-assisted selection programme used to transfer five target regions from Azucena to Kalinga III, indicating the number of plants genotyped at each generation. a) Selection and advancement of BC₃ lines, to pyramid QTL in Kalinga III genetic background. b) Modified single large-scale MAS used to derive five bulks, each containing a different target QTL, and one control bulk.

Table 1. Bulks selected via modified SLS-MAS for PPB, indicating target regions from Azucena present.

Bulk	Target regions present
I	None (control)
II	Root QTL (Chromosome 7)
III	Root QTL (Chromosome 9)
IV	Root QTL (Chromosome 11)
V	Root QTL (any one target) and aroma (Chromosome 8)
VI	Root QTL (Chromosome 2)

genotypes in Eastern India (Kumar *et al.*, 2000) and Nepal. Some of the lines and bulks performed well across both countries, and in different seasons and ecosystems. These lines and bulks represent a significant genetic resource for testing the usefulness of marker-evaluated selection. The most promising lines selected by farmers may hold the key to the best choice of future PPB crosses. We are currently using molecular markers to evaluate 62 populations from these crosses (bulks and pure lines), which were selected in PPB.

Farmer-preferred traits are those having adaptive or commercial value. Markers linked to these farmer-preferred traits can theoretically be identified using marker-evaluated selection, and we aim to test this hypothesis and develop the technique.

DNA fingerprint linkage blocks (DFLBs, Zhu *et al.*, 1999) are being used to determine farmer-preferred genomic regions. If marker allele frequency in the PPB-selected population differs significantly from the allele frequency in a non-selected population derived from the same cross, it is assumed to be linked to a locus that contributes to a farmer-preferred trait. This is a one-tailed selective genotyping (OTSG) approach, similar to the trait-based analysis for detection of marker-QTL linkage of Lebowitz *et al.* (1987).

QTLs explaining 30% of the genetic variance may be detected in samples of 15 or more selected lines, but linkages involving QTL of smaller effects are unlikely to be detected (G Atlin, pers. comm.). We expect that loci controlling plant height and flowering time will definitely be detected, and that other farmer-preferred traits might also be identified. The unselected population used as a control will prevent detection of false DFLBs, which differ in

allele frequency due to segregation distortion. Segregation distortion in rice mapping populations is often found in regions containing gametophytic gene loci and/or sterility loci (He *et al.*, 2001).

By exploring the graphical genotypes of farmer-selected lines, 'ideotype' plants can be designed for specific environments and crosses will be made to select for those ideotypes in the progeny using MAS. The optimum combinations of genomic regions for farmer-preferred traits and root QTL could be readily transferred, through MAS, to any farmer-preferred variety.

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