Combining PPB and marker-assisted selection: strategies and experiences with rice

Article · January 2002

7 authors, including:

Katherine Steele
Bangor University
32 PUBLICATIONS **1,301** CITATIONS
SEE PROFILE

Daljit Virk
Bangor University
74 PUBLICATIONS **912** CITATIONS
SEE PROFILE

J. R. Witcombe
Bangor University
123 PUBLICATIONS **2,691** CITATIONS
SEE PROFILE

Extended Abstract for: The Quality of Science in Participatory Plant Breeding Workshop at IPGRI, Rome, Italy, September 30-October 4, 2002.

Theme VI. Future Horizons - Linking DNA marker technology and PPB

Combining PPB and marker-assisted selection: strategies and experiences with rice

K.A. Steele[1], D.S. Virk[1], S.C. Prasad[2], R. Kumar[3], D.N. Singh[3], J.S. Gangwar[2] and J.R.Witcombe[1]

1Centre for Arid Zone Studies, University of Wales, Bangor, LL57 2UW UK,
2Gramin Vikas Trust, 280 Kanke Road, Ranchi, Jharkhand, India
3Department of Plant Breeding and Genetics, Birsa Asgricultural University (BAU), Kanke, Ranchi, Jharkhand, India,

Summary

Participatory plant breeding (PPB) should not preclude the use of modern biotechnological techniques. We have tested whether the two approaches can be effectively combined to enhance one of the advantages of PPB - selection in the target environment that can give improved adaptation to stress conditions. Marker-assisted selection (MAS) can further improve the efficiency of selection for stress tolerance traits that typically have a low heritability. We combined the two approaches by using MAS to increase the initial frequency of favourable alleles in bulk populations which farmers then selected in their own fields. We are using three different strategies to combine MAS with PPB for rice. In one we use bulk populations, in the second pure-line breeding, and in the third we are using markers to evaluate the results of selection to optimise the next cycle of selection.

Background of PVS and PPB

Participatory varietal selection (PVS) has been conducted in eastern India and Nepal and has identified many new varieties for farmers in different ecosystems. One successful variety, Kalinga III, was identified for Western India through PVS (Joshi and Witcombe, 1996). It is preferred by many upland farmers in western and eastern India for its yield, earliness and slender grains. However it is susceptible to early-season drought and has poor roots and weak stems. No substitute variety was identified through PVS and many farmers continue to grow their traditional, low-yielding but drought-tolerant varieties.

PPB was initiated in both India and Nepal to breed superior alternatives to varieties identified by PVS. The PPB is co-ordinated through a local NGO (Gramin Vikas Trust) in India and carried out by a local NGO (LI-BIRD) in Nepal. In collaborative PPB farmers grow the segregating material in their own fields with no scientist intervention and they make their own selections. In consultative PPB the populations are grown on station using cultivation practices typical of those practiced by farmers, and farmers visit the plot at several dates during the growing season to make selections.

Marker-Assisted Selection (MAS) for Root Traits and Aroma

MAS is useful for selection of any trait that can be linked to molecular markers and that is difficult to select for in traditional field screening. Selection for root traits by MAS could help drought resistance breeding in upland rice (Price and Courtois, 1999). Our MAS programme has targeted quantitative trait loci (QTLs) associated with improved roots that should result in better drought resistance. Selection was applied for RFLP (restriction fragement length polymorphism) and SSR (simple sequence repeats or microsatellites) markers at four target chromosomal regions (QTLs) that determine root traits. MAS backcross breeding methods were used to transfer these QTLs from the variety Azucena to the upland variety Kalinga III. The aim was to improve the performance of Kaligna III in the field during early season drought by the introduction of a more efficient root system, while maintaining the high yield and good grain quality of

Kalinga III. A secondary aim was to introduce a major QTL for aroma from Azucena into non-aromatic Kalinga III.

To identify root QTLs a mapping population of recombinant inbred lines (RILs) was genotyped with markers. The population was phenotyped for root traits including root length and thickness, root penetration ability and root mass at depth. The association between markers and traits was carried out using the mapping software Mapmaker QTL and QTL cartographer (Price et al., 2000, Price et al., 2002). The four target QTLs for root traits were chosen for the improvement of Kalinga III because these QTLs were highly heritable and of large effect. Also these four target regions were in the same regions as similar QTLs detected in other mapping populations. A molecular marker linked to aroma was identified from comparative mapping.

Strategy 1: Single Large- Scale-MAS

The first strategy used modified single large-scale marker-assisted selection (SLS-MAS, Ribaut and Betrán, 1999) to generate six bulks. Each bulk was either selected for a root QTL, or for aroma and a root QTL, and a control bulk from the same generation was selected to contain no QTLs from Azucena. These bulks were genetically close to Kalinga III (85%) although all except the control bulk were fixed for at least one QTL from Azucena, and all bulks contained other (non-selected) Azucena genes which were still segregating.

The bulks were given to three upland farmers in eastern India, in the main season of 2000, at the BC_2F_4 generation. Farmers selected within the bulks over two seasons. While some of these original participating farmers may continue with farm-saved seed, we have added new farmers to the PPB programme using seed we multiplied in the off-season. In the main season in 2002 more than 50 farmers across 3 different states grew at least one bulk alongside Kalinga III. (Figure 1: Bulk VI alongside Kalinga III growing in farmer Gansi Devi's field). To compare consultative and collaborative methods of PPB a parallel consultative approach is also being followed in the same set of bulks.

Early results from the main growing season of 2002 showed that all six MAS bulks were preferred by farmers over Kalinga III. Most of the rice in the upland farms in the trials was droughted, but in all plots visited by one or more of the authors the bulks were performing as well as, or better than Kalinga III and a reasonable harvest was expected from the bulks. The Azucena genome (with or without the root QTL) appears to contribute positively to Kalinga III in a heterogeneous BC_2F_4. The next stage is to determine if the selected bulks with one root QTL are superior to the selected control with no QTLs under drought conditions. This tests the validity of MAS for root traits.

Strategy 2: MAS Pyramid Breeding

The second strategy used MAS to generate pure lines with combinations of root QTLs or root QTLs and aroma. These have been derived from the same cross between Azucena and Kalinga III, but a third backcross to Kalinga III was made. The BC_3 generation was used for additional crosses to produce lines (pyramids) containing all four root QTLs, with and without aroma, in the Kalinga III genetic background. These are being tested at the upland breeding station in consultative PPB. These lines can be used to evaluate the value of the root QTLs both individually and combined in pure lines. Comparison of bulk and pure-line breeding will identify which is most efficient for speeding the selection for drought tolerance.

Graphical Genotyping of PPB Products

Strategy 3: Marker Evaluated Selection

In a third strategy, marker evaluated selection (MES), PPB is conducted first then markers are used to analyse products of farmer-selection so that these traits can be selected by MAS in future crosses. We are evaluating the best performing varieties (i.e., most preferred by farmers) from PPB from four different crosses with molecular markers to identify ‘farmer-preferred’ genomic regions. Markers such as SSRs, AFLPs (amplified fragment length polymorphisms) and SNPs (single nucleotide polymorphisms) were used

to sample genomic regions. DNA was used from individual plants or samples of bulk DNA from up to 10 plants. Bands of the same size are considered to have a common ancestor and represent the same allele at that marker locus. PPB lines selected by several different farmers in a common agroecological situation are being tested. If an allele from one of the parent lines is found to be more frequent than that of the other parent then the marker is likely to be linked to a QTL influencing a trait of agronomic benefit to those farmers.

The first stage was to carry out a PPB programme in specific target environments using different crosses with at least one parent in common. PPB products were derived from 4 different rice ecosystems, two countries - India and Nepal - and two growing seasons – early and main (Table 1). The marker frequency in 52 PPB products has been found for 18 SSR markers. Additionally a control population of non-selected F_2 lines from one of the crosses has been tested to determine the allele frequency without selection. Each marker is assessed for shifts in frequency between the control and the farmer-selected PPB lines. Shifts in frequency according to ecosystem, country and season in response to selection by farmers are assessed. Finally the graphical genotypes of each PPB line can be drawn. From this information it is possible to draw a graphical genotype of an ideotype variety for a particular situation. PPB lines which differ from the ideotyope at certain markers can then be improved by MAS for this marker. The results will be used to design a breeding strategy that combines all the best characteristics for a particular ecosystem into ideotype varieties using MAS.

Results with 18 SSR markers used to test 27 lines from one cross (IR64/Kalinga III) have shown that some markers might be shifting in frequency because they are linked to traits that farmers have successfully selected for. The SSR marker, RM5, is more likely to be inherited from Kalinga III than from IR64 in material selected for upland ecosystems (Figure 2). Allele frequency between all three ecosystems was tested for RM5 using the G-test (Sokal and Rohlf, 1995) and there was significant difference between ecosystems ($G = 22.0$, $P = 0.0002$). The allele frequency for RM5 was not significantly different between the F_2 control population and the PPB lines or bulks from the same cross, nor

was it different between the lines selected in India and Nepal, and lines selected for Main season or *Chiate* season. RM5 is located on chromosome 1 and is approximately 100 cM from the semi-dwarfing locus *sd-1* at approximately 190 cM. IR64 is a semi-dwarf variety and Kalinga III is tall (120cm). The farmers in uplands prefer tall varieties, so the higher frequency of Kalinga III alleles at RM5 could be due to linkage drag on chromosome 1 caused by greater selection pressure for taller plants (Kalinga III alleles at *sd-1*) in the uplands.

Conclusions

We have combined molecular marker-assisted selection with PPB in several different strategies. Farmers have shown that they prefer bulks derived from SLS-MAS compared to local and non-PPB varieties. This modified backcross breeding strategy has been successful, however the influence of target root QTLs and aroma still remains to be tested.

Additional markers from throughout the genome are being tested on 52 PPB lines and bulks for MES. These will be analysed and used to identify ideotype graphical genotypes for the different situations. We speculate that some genes conferring a positive effect for agronomic traits will have a large enough effect to be detected by shifts in marker allele frequency.

References

Joshi A and Witcombe JR (1996). Farmer participatory crop improvement. II. Participatory varietal selection, a case study in India. *Experimental Agriculture* 32: 461-477.

Price AH and Courtois B (1999). Mapping QTLs associated with drought resistance in rice: Problems, progress and prospects. *Plant Growth Regulation* 29: 123-133.

Price AH, Steele KA, Moore BJ, Barraclough PB and Clarke LJ (2000). A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root penetration ability. *Theoretical and Applied Genetics* 100:49-56.

Price A.H., Steele K.A., Moore B.J. and Wyn-Jones G. (2002) Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: II. Mapping QTL for root morphology and distribution. *Field Crops Research* 76, 25-43.

Ribaut J-M and Betrán J (1999). Single large-scale marker-assisted selection SLS-MAS. *Molecular Breeding* 5: 531-541.

Sokal R.S. and Rohlf F.J. (1995) Biometry : the principles and practice of statistics in biological research, 3rd edition, Freeman, New York.

Figures and Tables

Figure 1. Bulk VI (left side) generation BC_2 F_7 selected for a root QTL from Azucena on chromosome 2, and Kalinga III (right side) growing in a field belonging to Gansi Devi, in Borogora, Jharkhand.

Figure 2. Allele frequency at the SSR marker RM5 in 13 upland, 6 medium upland and 8 lowland PPB bulks or lines from the cross Kalinga III/IR64 and a control non-selected F_2 population of 48 individuals. Alleles detected were either from Kalinga III, IR64 or neither parent (from a small proportion of out-crossing in the field).

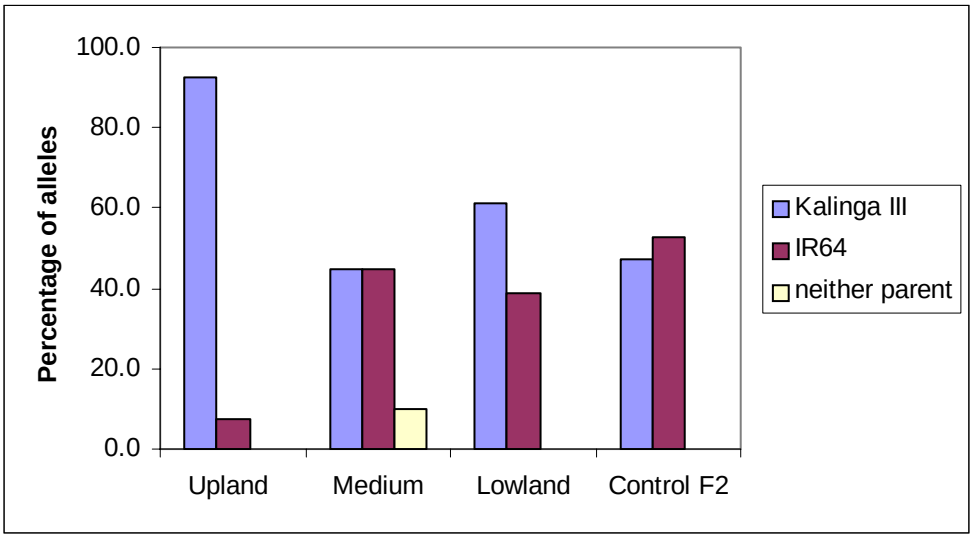

Table 1. Summary of 52 different lines and bulks selected by farmers in PPB from four different crosses with Kalinga III for three ecosystems in India and Nepal (*Chaite* is the spring or early season in Nepal). These 52 lines or bulks were evaluated with molecular markers.

Pedigree (Kalinga III crossed with)	**Country**	**Season**	**Number of lines or bulks sampled from ecosystem**		
			Upland	**Medium upland**	**Lowland**
IR64	Nepal	*Chaite*	4	0	2
IR64	Nepal	Main	1	5	6
IR64	India	Main	8	1	0
Radha 32	Nepal	*Chaite*	2	5	8
Radha 32	Nepal	Main	1	0	0
IR36	India	Main	3	1	0
Vandana	India	Main	5	0	0
Total			**24**	**12**	**16**