DFID P*lant* S*ciences* R*esearch* P*rogramme*

FINAL TECHNICAL REPORT

R7434

Innovative Methods for Rice Breeding – Combining Participatory Plant Breeding (PPB) with Molecular Marker Technology

October 1999-July 2002

Report submission date: **31st October 2002**

Author of this report:	Katherine Steele, CAZS
Contributors:	Daljit Virk & John R. Witcombe, CAZS
Collaborators:	J. S. Gangwar & S.C. Prasad, Gramin Vikas Trust (East), Ranchi, India. S. Gyawali, M. Subedi & S. Sunwar, Li-BIRD, Pokhara, Nepal. Ravi Kumar& D.N. Singh, Birsa Agricultural University, Ranchi, India. H. E. Shashidhar, University of Agricultural Sciences, Bangalore, India. Jiahui Zhu, John Innes Centre, Norwich, UK.
Technical Support:	Julian Bridges, Gwen Edwards (from August 2000) & Beverly Moore (until January 2000), CAZS.

1. Executive Summary

The project combined PPB with marker-assisted selection (MAS) by continuing the marker-assisted backcross programme begun in project R6673 to introduce QTLs for root growth and drought resistance into Kalinga III. Bulks generated through marker-assisted backcrossing were used for participatory plant breeding. Farmers made selections in the bulks in 3 seasons and pure bulks were obtained which out-performed Kalinga III. A control bulk with no QTLs was just as acceptable to farmers as bulks with root QTLs. This indicates that other Azucena alleles contribute favourably in the backcross material.

Advanced backcross lines containing root QTLs were screened for root traits in soil-filled pipes under field conditions and in growth-room conditions in order to test for the effects of QTLs. Results of these studies were inconclusive although the material studied was not necessarily fixed at all alleles which could have influenced the very large standard errors obtained. Crosses were made to pyramid root QTLs into single genotypes and seeds are now available for farmers which contain all four root QTLs in the Kalinga III genetic background.

PPB in this project, and in other DFID-PSRP projects, was done using progeny from a number of different crosses and also with products from mutation breeding. Farmers in India and Nepal across a range of rice environments identified many successful lines or bulks. The project combined PPB with molecular markers by evaluating the products of PPB for marker variation. Initially AFLPs were used, but these were not useful for comparative mapping. Methods for increasing the through-put of microsatellites (simple sequence repeats, SSRs) were tested and this method was used to analyse successful PPB lines. Towards the end of the project the method of SNPs (single nucleotide polymorphisms) became available and a small number were used to test PPB lines and bulks. These studies have been used to illustrate graphically the parental contribution of certain individuals or sets of selected material. Marker analysis has started to reveal some of the effects of farmer selection at certain chromosome regions or individual markers.

This has led us to develop the hypothesis that markers can be used to identify farmer-preferred chromosome regions. These could be cross-specific or ecosystem-specific. The method used for analysis of PPB products has been called marker-evaluated selection (MES). A strategy has been identified which will use MES to isolate makers which could be linked to traits of specific importance to farmers in certain ecosystems. They can then be used in MAS to improve existing lines which do not contain these markers so that they should perform even better in the target environment.

2. Background

Upland rice is an important crop in the DFID bilaterally funded project areas of the Gramin Vikas Trust (GVT, formerly KRIBHCO Indo-British Rainfed Farming Project, KRIBP) where end-of season (terminal) drought is common. Previous projects identified and popularised a farmer-preferred variety, Kalinga III through participatory varietal selection. Kalinga III has poor roots, weak straw and poor drought resistance, so improvement for these traits is desired. In a review of opportunities for improving drought resistance in rice Fukai and Cooper (1995) suggested that improved root growth would be useful for upland rice.

Surveys of the varieties grown by farmers in both rainfed marginal areas and high potential production systems consistently showed that farmers grow a few, old varieties and hence are forgoing the potential benefits offered by more modern varieties (for a review see Witcombe 1999). Farmers of rainfed uplands in particular have not benefited greatly from the outputs of modern plant breeding. These farmers apply low levels of inputs because they wish to avoid risk and the available cultivars are non-responsive and are susceptible to pests and diseases. Participatory methods of plant breeding can help overcome these constraints by more rapidly producing and placing in farmers' hands new varieties with greater utility to farmers. The participatory process allows farmer preferences to be fully taken into account.

This project sought to apply participatory plant breeding (PPB) to a range of crosses and to evaluate the effectiveness of the participatory approach using molecular markers. Some crosses, for example Kalinga III x IR64, were targeted for a range of situations including upland, lowland and irrigated conditions. The end products from these crosses in both India and Nepal were tested with molecular markers. The project aimed to test the approach of using DNA fingerprint linkage blocks to identify farmer-preferred genomic regions and regions that determine adaptation to the contrasting environments used for selection.

However, for traits of low heritability selection efficiencies are lower on farmers' fields than when selection is carried out under the more controlled conditions of the research station. Molecular markers offer the opportunity to select for improved genotypes in a way that is independent of the environment. Molecular marker selection has the potential to greatly improve the efficiency of PPB.

DFID PSRP projects R4631 and R6673 carried out in CAZS, Bangor in collaboration with IRRI identified molecular markers for genes contributing to root growth and drought resistance traits in rice (Price *et al*. 1997; and Price and Tomos, 1997). Project R6673 began a marker-assisted backcross programme on Kalinga III to introduce the most useful QTLs from Azucena.

3. Project Purpose

DFID programme purpose

Novel methods of aiding conventional plant breeding to overcome biotic or abiotic constraints developed, tested, piloted and promoted.

Project Purpose

Participatory methods for varietal selection and breeding developed and tested.

- To demonstrate the value of marker-assisted selection and DFLB fingerprint linkage blocks when used in conjunction with participatory plant breeding.

- To use these methods to introduce QTLs for desirable root traits and desirable DFLBs into farmer-preferred varieties.

4. Research Activities

Advanced lines produced and tested by PPB

Segregating generations from several crosses (Table 1) were advanced and used for consultative PPB. Many farmers in villages throughout the GVT project areas in eastern India (Jharkhand, Orissa and West Bengal) and LI-BIRD districts in Nepal were invited to participate after completing a participatory rural appraisal questionnaire. The number of farmers involved in eastern India and Nepal increased each year of the project. In March 2000 the number of villages included in the GVT area was 205 in 60 clusters. Farmers were given seeds and asked to grow them using their standard management practices. They were given no training or advice on selection by scientists. Selection was made by farmers in eastern India and Nepal on segregating bulks in the main growing seasons in 1999, 2000, 2001 and 2002. Farmers in Nepal also carried out PPB for early (*Chiate*) season rice.

Simultaneous on-station collaborative trials under farmer-management conditions were conducted at the GVT/BAU upland farm in Ranchi with material from all crosses and with lines and bulks produced through marker-assisted back crossing. Some of the lines and bulks were advanced in the off-season (*rabi*) in India at CRRI, Cuttack. Successful farmer-selected varieties were tested in official trials for official state release.

Table 1. Summary of the crosses used for PPB.

Cross (parents)	Planned Activities	Actual activities	Number selected (and names).
IR64/Kalinga III	PPB eastern India and Nepal	PPB in eastern India and Nepal.	8 India (Ashoka) 18 Nepal (Barkhe or Judi)
IR36/Kalinga III	PPB in eastern India and Nepal.	PPB in eastern India and Nepal.	3 India (Sudha)
Sathi/Kalinga III F_3 RM	PPB in eastern India and Nepal	Consultative PPB in 1999 in western India showed disease susceptibility, therefore the cross was dropped from PPB. Some lines were used for crossing in eastern India.	Not selected
Sathi/Kalinga III/Kalinga III	PPB in eastern India and Nepal	Consultative PPB in 1999 in western India showed disease susceptibility, therefore the cross was dropped. Some lines were used for crossing in eastern India.	Not selected
WAB 56-104 (*Oryza sativa*)/ CG14 (*Oryza glaberrima*) DH	Consultative PPB in eastern India.	Consultative PPB in 1999 only. Dropped from PPB in 2000 and best lines used for crosses in 2000.	18 Ganesha
Kalinga III/Azucena/Kalinga III BC_2	PPB in eastern India and Nepal	Six bulks were made available for collaborative and consultative PPB in K2000 in eastern India.	6 India (MAS or Richa)
Kalinga III/Azucena/Kalinga III BC_3	PPB in eastern India and Nepal	Aromatic lines and pyramid lines included in consultative PPB in eastern India. Some lines already tested by PPB in Nepal.	Still under evaluation.

Table 1. continued.

Kalinga III/ Vandana	Not initially planned in this project.	PPB in Eastern India.	5 India (Komal)
Kalinga III/ Radha 32	Not initially planned in this project.	PPB in Nepal.	16 Nepal (Barkhe or Judi)

Molecular marker genotyping of parental and selected varieties

Three different types of PCR-based molecular markers have been used in this study with 10 parent varieties (Table 2) and progeny lines that have performed well in PPB. Data has been obtained and analysed with AFLPs and SSR and is summarised in Table 3 During the project a new capillary electrophoresis capacity for analysis of AFLPs and SSRs was set up at CAZS. This enables multiplex PCR analysis and medium-to high throughput of samples compared to conventional gel electrophoresis.

SNPs are a relatively new marker system in rice can be more high-throughput than AFLPs and SSRs. The SNP PCR fragments can be analysed using mass-spectroscopy to detect the relative frequency of each allele in a mixed sample of DNA from many different individuals. The analysis of parents and progeny with SNPs is currently underway and remains to be completed.

Table 2. Rice parent varieties tested for polymorphism.

	Variety
1	Kalinga III
2	IR64
3	IR36
4	Sathi 34-36
5	WAB56-104
6	CG14 (*O. glaberrima*)
7	Radha 32
8	Vandana
9	Azucena
10	Pusa Basmati 1

Table 3. Summary of molecular screens of PPB lines and bulks.

Number of lines or bulks	**Origin of samples**	**Markers tested**
18 Ganesha	PPB (India)	104 AFLPs & 13 SSRs
3 Ashoka	PPB (India)	104 AFLPs & 13 SSRs
3 Sudha	PPB (India)	104 AFLPs & 13 SSRs
10 Irradiated Pusa Basmati	PPB (Nepal)	18 SSR
2 round grain lines and 5 bulks from IR64/KIII	PPB (Nepal)	12 SSR
44 from various crosses with Kalinga III	PPB (India and Nepal)	18 SSR & 12 SNPs

Marker assisted backcross (MABC) programme

In project R6673 a MABC programme was started. 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 contain QLTs for drought-related traits in several different mapping populations (for review see Zhang *et al*., 1999). A fifth target was a QTL for aroma on Chromosome 8. We used a marker-assisted backcrossing with using Kalinga III as the recurrent parent. The flanking markers used for selection were restriction fragment length polymorphisms (RFLP) and simple sequence repeats (SSR or microsatellites) and their map positions are shown in Figure 1. Figure 2 shows the selection made at each generation. $BC3F_2$ and their progeny were used to develop partial pyramids, full pyramids and near-isogenic lines (NILs) containing different introgressed target regions.

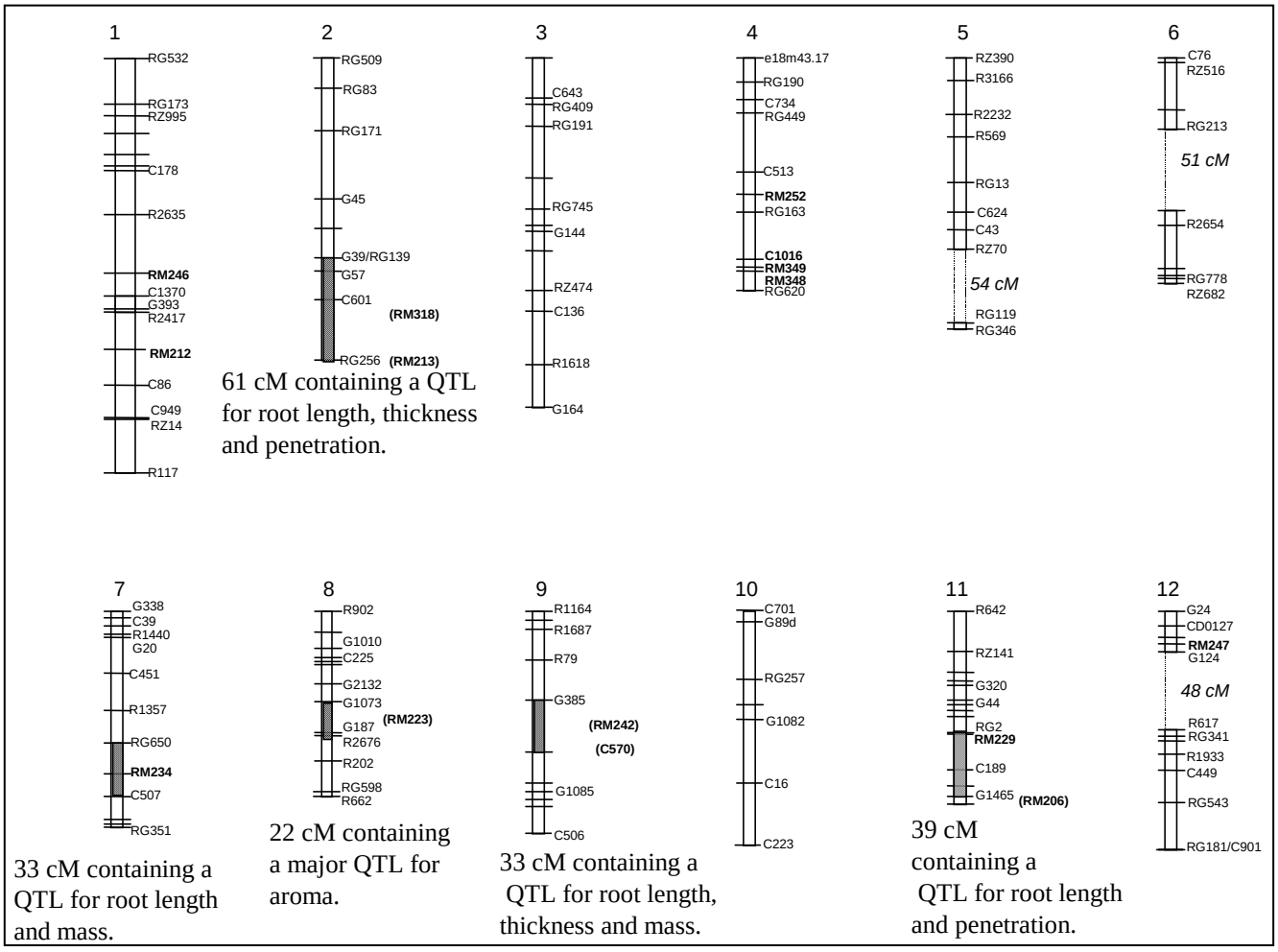

Figure 1. Targets for marker-assisted selection. The map shows the position of RFLP and SSR markers mapped in the Azucena/Bala RILs. Markers that have been used for selection, but are not yet added to this map are indicated in brackets.

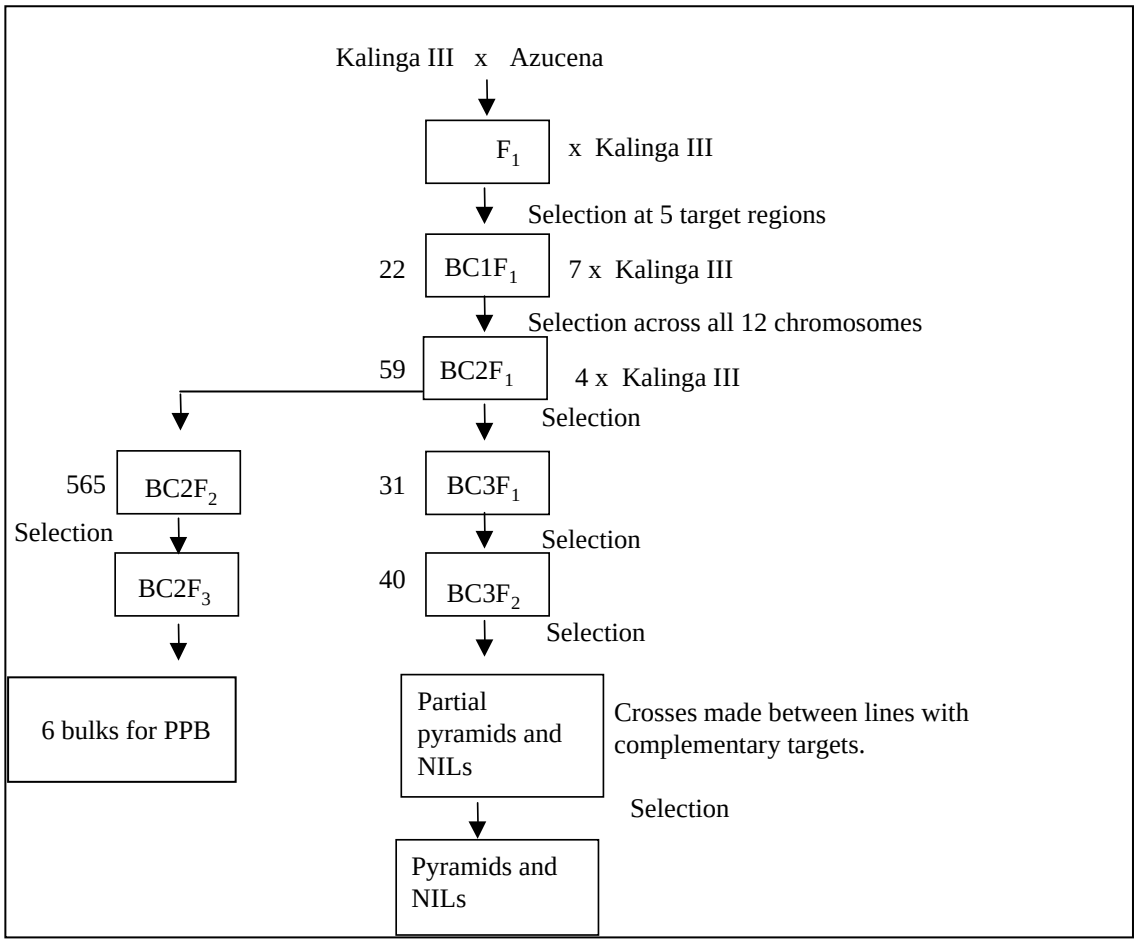

Figure 2. Scheme used for marker-assisted backcrossing (MABC) to generate pure pyramids and partial pyramids containing root QTLs and aroma from Azucena in a Kalinga III genetic background.

Pyramid lines

At the BC_3 generation, lines having at least two of the target regions were also screened with thirty background markers at non-target regions; two lines with mainly Kalinga III alleles at background regions were selected. These lines were selfed 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. The generations used to select the complete pyramid are shown in Table 4. RFLP markers were replaced with SSR markers as the project progressed. The number of markers tested indicates the basic number used for selection. Once a plant was selected it was tested with extra markers at specific regions. During this programme many partial pyramids and near-isogenic lines were identified. In addition to 50 $PY2F_3$ lines genotyped in Summer 2001, more than 100 progeny from the BC3 at the F_3 and F_4 generations were advanced and genotyped. All these plants were phenotyped for flowering time in the glasshouse in Summer 2001.

Table 4. Total number of plants of each generation screened for production of pyramid lines with 5 target QTLs in a Kalinga III genetic background.

Generation	Date	No plants genotyped	Number of markers tested	No plants selected	Selected plants used to:
$BC3F_1$	Summer 1999	31	36 RFLP & 20 SSR	2 1	Self Self (control)
$BC3F_2$	Autumn 1999	30	9 RFLP & 8 SSR	2 11 4	Cross - for pyramids. Self (aroma fixed) Self (one QTL fixed)
$BC3F_3$	Spring 2000	47	5 SSR	3	Cross with $PY1F_1$
$PY1F_1$	Spring 2000	5	8 SSR	1	Cross with $BC3F_3$
$BC3F_3$	Spring 2001	90	Various SSR	28	Self - for NILs
$PY2F_2$	Summer 2001	9	5 SSR	4	Self
$PY2F_3$	Summer 2001	50	12 SSR	1	Self
$PY2F_4$	Spring 2002	33	1	all	Self – for PPB

Modified SLS-MAS to select bulks with fixed targets for PPB

The $BC2F_1$ generation (see figure 2) was selfed and 565 $BC2F_2$ lines were screened with SSR markers at the 5 targets and used to select 6 bulks. This was similar to the SLS-MAS method of Ribaut and Betrán (1999). In contrast to the SLS-MAS method, we only attempted to identify lines with one root QTL which were used to make bulks for PPB (Table 5). These bulks are genetically close to Kalinga III (87.5%), they are variable (segregating for Azucena alleles in the non-target genomic regions) and have one fixed QTL from Azucena. The aromatic bulk V was made from a mixture of lines with different root QTL fixed, but only four of them were fixed (homozygous) for RM223 and seven were heterozygous for RM223 (i.e. only 68% of alleles were from Azucena at the aroma target in this bulk).

Table 5. Bulks selected via modified SLS-MAS for PPB, indicating target regions from Azucena present.

	Target Chromosome	Markers selected with Azucena alleles	Number of $BC2F_3$ lines in bulk	Bulk criteria
Bulk I	-	none	30	control (no QTL)
Bulk II	7	RM234	22	Root QTL
Bulk III	9	RM242	21	Root QTL
Bulk IV	11	RM229	21	Root QTL
Bulk V	8 + another target	RM223 + other	11	Aroma plus root QTL
Bulk VI	2	RM213	28	Root QTL

Screening of MAS lines for root traits

PPB products (Ashoka 228 and 200F), parents and control lines along with lines developed through MABC, have been screened for root traits and flowering time in the following screens:

- Four screens for roots in soil-filled pipes under field conditions in Bangalore, India, in 2000, 2001 and 2002 (by H.E. Shashidhar).
- One screen for roots in soil-boxes under glasshouse conditions in Aberdeen, UK (by A.H. Price).
- One screen for flowering time in a glasshouse in Bangor, in Summer 2001 (by K.A. Steele).

Development of future strategy for DFLB-directed varietal improvement

Preliminary studies used AFLPs and SSRs to study pure lines and bulks derived through PPB. These were 18 Ganesha, 3 Ashoka, 3 Sudha, 10 Irradiated Pusa Basmati, 2 round grain lines and 5 bulks from IR64/KIII. Following on from these small studies a larger study was started using SSRs and SNPs to screen 44 products of PPB from different crosses, all with Kalinga III as one parent. The aim was to identify farmer-preferred genomic regions. These are likely to be QTL influencing any trait of agronomic benefit to farmers.

5. Outputs (Results)

Advanced lines produced and tested by PPB

Participatory plant breeding has been successful with four out of six crosses. The crosses which were not successful were Sathi 34-36/Kalinga III and the IR36/Kalinga III. The Sathi 34-36-derived material was susceptible to disease in eastern India and therefore not suitable for dissemination. Both of these crosses have produced lines with desirable traits which are being used as parents in new crosses. The IR36 cross did produce some fair material for eastern India, however, perhaps the main reason why this material was dropped was poor management of the material on the research station.

Four crosses have produced successful material and the IR64/Kalinga III cross has produced varieties which are suitable for upland conditions in India, uplands, medium-lands and upland *Ghaiya* conditions in Nepal.

Two varieties (Table 6) have been released for farmers in eastern India as a direct result of selection by PPB. Ashoka 228 was developed through consultative PPB and Ashoka 200F was developed by one farmer in collaborative PPB.

Table 6. Released varieties from PPB in eastern India.

Name of line or bulk	Generation tested in AICRIP	Method	Year Released	Variety name
Ashoka 228	F_5	Modified pedigree bulk selection	2001	Birsa Vikas Dhan 110
Ashoka 200F	F_5	Modified pedigree bulk selection	2001	Birsa Vikas Dhan 109

Effects of selection identified by DNA fingerprinting.

Effects of PPB selection in O. sativa / O. glaberrima *wide cross*

Initially 105 double haploid (DH) lines were tested in consultative PPB. Selections were made in Nepal and India in 1999 and 2000. The 18 most preferred lines (Ganesha) were tested with AFLP markers and data was obtained for 59 polymorphic markers (46 AFLP and 13 SSR). The results showed that the 18 Ganesha lines each contained between 50.9% and 87.5% WAB 56-104 alleles. The overall contribution of each parent to the 18 preferred lines is summarised as a graphical genotype in figure 3. It is possible to draw a similar diagram for each individual line.

After 3 years of consultative PPB this material was dropped from the study because none of the DH lines were suitable for farmers in Eastern India or Nepal. However some of the traits were of interest to farmers such as grain characteristics, in particular women farmers liked the golden grain colour. The most preferred line (#45), with 77.6% WAB 56-104, was used in a cross with Ashoka 157. This material was advanced and the F_4 generation was made available for collaborative PPB in three states in the *kharif* season 2002.
A control set of 18 unselected DH lines was originally planned for screening with the same markers but this work was not completed because the Ganesha lines were dropped from the PPB programme.

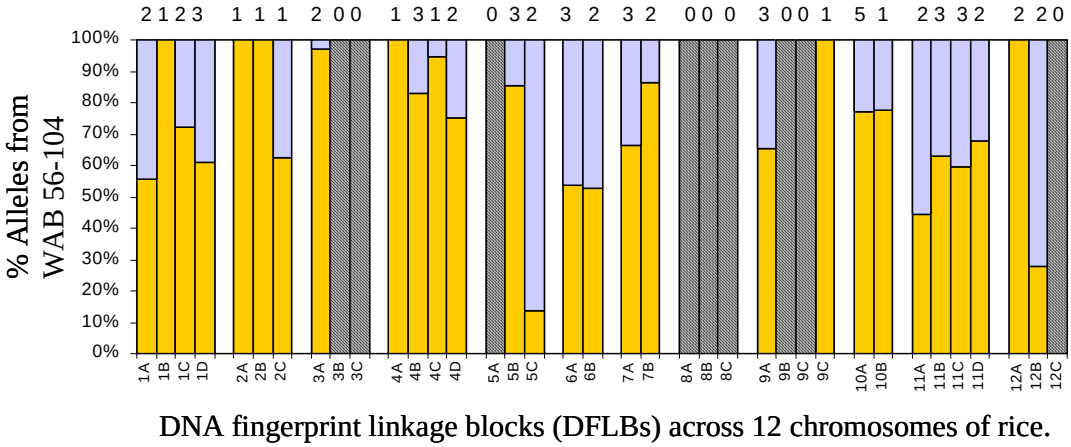

Figure 3. Graphical genotypes at DFLBs, showing percentage contribution of WAB 56-104 alleles in the 18 best DH lines (Ganesha) for eastern India (yellow bars). The numbers above each block show the total number of markers tested in that region. At only two DFLBs, 5C and 12B, there was a greater proportion of CG14 (the *O. glaberrima* parent, lilac bars) alleles than *O. Sativa* alleles.

Effects of PPB selection in lines derived from mutation breeding

Pusa Basmati-1 was used for mutation breeding in another DFID PSRP project and lines derived from it were selected for plant height and aroma in collaborative PPB in Nepal. The ten best performing lines were tested with 18 SSR markers to determine their similarity to Pusa Basmati-1 and two checks (Kalinga III and IR64). A dendrogram was constructed using hierarchical cluster analysis on Jaccard's coefficients for each pair using average linkage between groups (Figure 4.). The results indicated that there was a high level of molecular genetic variation between the selected lines. This was higher than expected in material derived from the same M_1 generation.

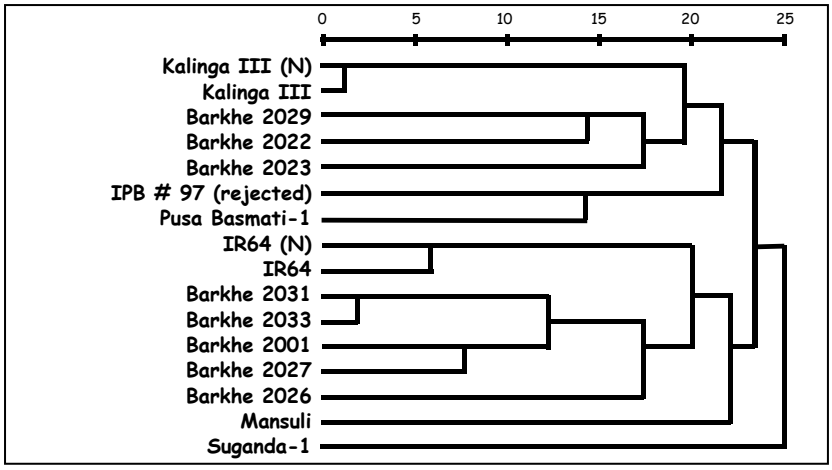

Figure 4. The ten best performing lines derived from mutation breeding of Pusa Basmati-1 selected PPB in Nepal were tested with 18 SSR and compared with the same markers tested in Pusa Basmati-1 and two checks (Kalinga III and IR64). Kalinga III and IR64 DNA was tested from seeds grown in both eastern India and in Nepal (N). Fragments of different sizes were scored for presence or absence and used to find Jaccard's coefficients. The dendrogram was constructed using hierarchical cluster analysis on Jaccard's coefficients for each pair using average linkage between groups.

Effects of PPB selection in crosses with Kalinga III as one parent

Twenty-nine successful lines and bulks from the IR64/Kalinga III cross were compared with molecular markers. Preliminary results with 18 SSR markers have shown that some markers might be unconsciously selected more frequently by farmers in certain ecosystems. The SSR markers RM237 and RM5 that are more likely to be inherited from Kalinga III than IR64 in material selected for upland ecosystems in the progeny derived from IR64/Kalinga III (Figure 5).

It is interesting to note that of the 18 markers tested, from across 9 different chromosomes, the two that have shown significant differences in allele frequency in response to selection by farmers are both located on chromosome 1. The semi-dwarfing locus *sd-1* is located on chromosome 1 at approximately 190 cM. IR64 is a semi-dwarf variety and Kalinga III is tall (120cm). The farmers in uplands state that they prefer tall varieties so these results could be due to linkage drag caused by greater selection pressure for taller plants (Kalinga III alleles at *sd-1*) in the uplands.

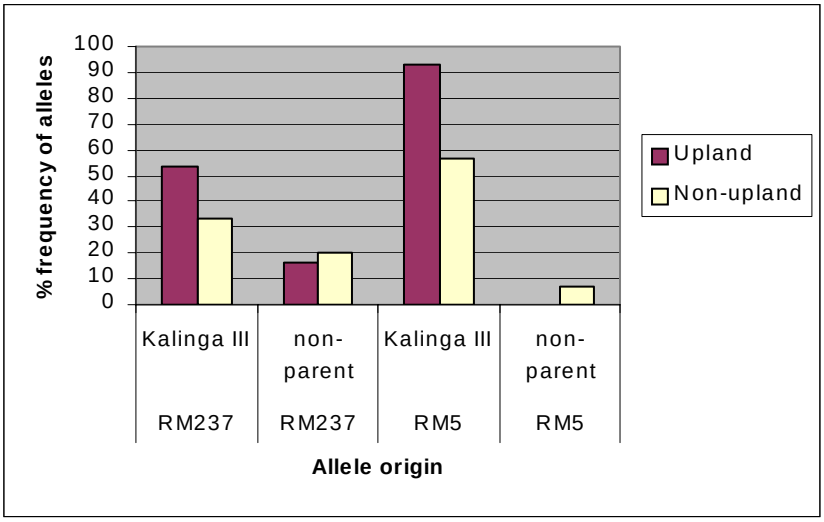

Figure 5. Allele frequencies detected in 14 upland and 15 non-upland selected bulks or lines at two loci on chromosome 1; RM5 (at approx. 89 cM) and RM237 (at approx. 110 cM). Alleles were either from Kalinga III, IR64 or non-parent (from a small proportion of out-crossing in the field).

Molecular markers in Ashoka 228 and Ashoka 200F

In total, 57 polymorphic markers (28 AFLPs and 29 SSRs) were used to screen bulks or individual plants of Ashoka 200F and Ashoka 228. These results are shown in Appendix Table 1. Ashoka 228 contains approximately 38% IR64 alleles and Ashoka 200F contains approximately 30% IR64 alleles. IR64 alleles were detected on 10 chromosomes. At 10 AFLP markers both Ashoka 228 and Ashoka 200F have both inherited the same IR64 allele. SSR or SNP markers these regions must now be compared in other PPB upland material from this cross to analyse the frequency in selected material. At 7 markers an IR64 allele is present in A200F that is not found in A228. At 9 markers an IR64 allele is present in A228 that is not found in A200F. There was slightly more heterogeneity for IR64 alleles in Ashoka 200F, although Ashoka 228 contains heterogeneity for some unknown alleles (possibly from out-crossing, or from heterogeneity in the original parents used for this cross).

It is interesting to note that these two selected bulk populations have inherited Kalinga III alleles at most of the markers we are using for MAS in the Azucena/KIII backcross material.

Future strategy for marker directed varietal improvement identified

A new strategy, marker evaluated selection (MAS) has been developed during this project. Initially the aim was to identify DNA fingerprint linkage blocks for use in selection of ideotype varieties. This strategy has been refined so that the allele frequency at individual markers can be found and used for graphical genotyping.

Marker-evaluated selection is being applied to study of PPB lines from India and Nepal. PPB products were derived from at least 4 different ecosystems, two different countries (India and Nepal) and two growing seasons. Results will be used in the next breeding strategy with the aim of combining all the best characteristics (via linked markers) for a particular ecosystem into one ‘ideotype’ variety using MAS. This work will be continued in the follow-up project R8200 to develop ideotype varieties for specific ecosystems.

Advanced lines produced by MAS developed and tested

Pyramid lines from MABC

Of 50 pyramid (PYF_3) lines screened for flowering time, one was selected with markers as a full pyramid. It was fixed for Azucena at the target regions: RM6 and RM318 on chromosome 2, RM351 and RM234 on chromosome 7, RM201 and RM242 on chromosome 9 and RM229 and RM206 on chromosome 11. It was segregating for RM223 (aroma) on chromosome 8 and RM213 on chromosome 2. It was also fixed for Azucena at one non-target region on chromosome 1 (RM237 and RM5). At 10 other markers tested on this plant in non-target regions it was fixed for Kaligna III.

This individual was selfed and 33 pyramid lines at the F_4 generation were tested for with the marker RM213 which is located below the target region, approximately 5 cM below RG256.). chromosome 2. Lines with and without this extended introgressed section can now be compared. The progeny lines (F_5), some with all 4 root targets and some with all 5 targets (including aroma), will be advanced at in the *rabi* season 2002-03 and used for PPB.

Near-isogenic lines from MAS

At the $BC3F_2$ generation potentially useful material was selected with molecular markers. Eleven lines which were fixed for aroma (some also contained root QTLs) were made available for PPB in both India and Nepal. Four partial pyramid lines, containing combinations of two or three target root QTLs, plus one control line in the same generation with no target markers were made available for PPB in India.

Eighty-five lines derived from 31 MABC lines with different combinations of root QTLs and aroma (including 1 control line with no QTL) were tested on-station at Ranchi in *kharif* 2001. Forty of these lines were selected by researchers and farmers and were advanced in the *rabi* season 2001-2002.

At the $BC3F_3$ generation lines which were fixed for Kalinga III at non-target regions were used for selection for near-isogenic lines and 28 were selected which contained a range of different combinations of alleles at the five target regions. Their progeny were used in root screens, flowering time screens and for the production of sub-NILs.

Production of Sub-NILs

A fourth round of backcrossing to Kalinga III was carried out using progeny of two of the selected plants ($BC3F_4$ generation). These plants were NILs containing different target QTLs in a Kalinga III genetic background and they are listed in Table 7. Kalinga III was used as the male parent to generate the BC4 generation, which was advanced to $BC4F_2$. This seed is a valuable genetic resource for future fine mapping of these regions for root traits in a method similar to that described by Monforte and Tanksley (2000).

A cross with progeny of a third plant was attempted, it was a NIL for the QTL on chromosome 7, but there were difficulties in making the backcross because the introgressed region contained the Azucena allele at RM248 which delayed flowering and made crossing very difficult.

Table 7. Near isogenic lines selected to backcross with Kaligna III to produce BC4 which can be used for fine mapping each of the target QTLs.

Line i.d. number	Generation	Target QTLs
21-01-03-11-25	$BC3F_4$	2 and11
42-01-05-10-07	$BC3F_4$	8 and 9
42-01-05-02-08	$BC3F_4$	7

Six bulks from modified SLS-MAS

Six bulks produced by modified-SLS-MAS were tested in collaborative and consultative PPB (Figure 6.). The results of collaborative selection in the six bulks on ten farms in the *kharif* season 2001 are summarised in Tables 8 and 9. All MAS bulks were preferred over Kalinga III, hence the Azucena genome (with or without root QTL) contributes positively to Kalinga III in a heterogeneous $BC2F_4$. We have to find out if selected bulks with 1 root QTL are superior to selected control (0-QTL) under droughted conditions to prove validity of MAS for root traits. Three of the bulks were recommended for AICRIP trials in Kharif 2002, but only one (Bulk II re-named Richa II) was actually entered in the multi-location trials in 2002. We expect the other two bulks to be tested in 2003.

Aroma is a trait that is highly desirable to the farmers. Bulk V was noted as aromatic in the field during flowering by the farmer OR1 (in Udali, Orissa) during *kharif* 2001. The frequency of aroma alleles (RM223) was assessed in 29 panicles harvested from individual plants in this plot. The frequency decreased from 68% in the original bulk V, to 12% after two seasons of selection by farmers, indicating unconscious selection against aroma, probably because aroma is associated with higher insect attack.

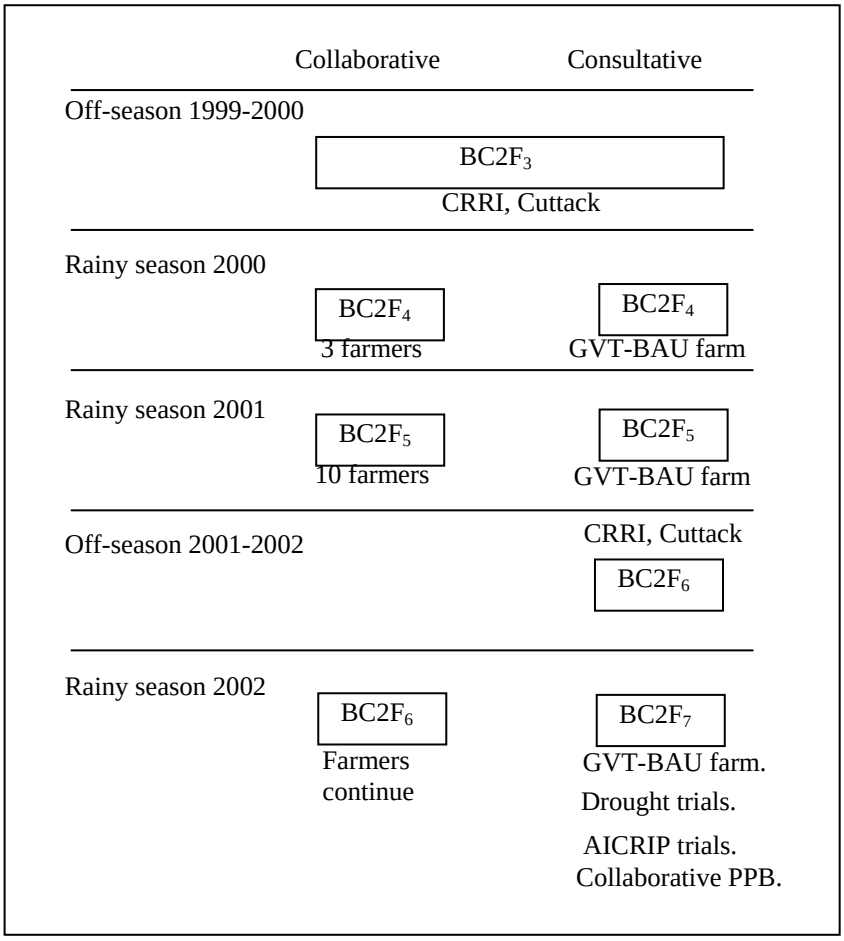

Figure 6. Collaborative and consultative PPB with six SLS-MAS bulks. Each generation consisted of only selected material from the previous generation, and all six bulks were advanced simultaneously.

Table 8. Summary of MAS-PPB trials in farmers' fields, in *kharif* 2001. Farmers were asked to grow all 6 bulks alongside Kalinga III using their usual practices.

	Farmer code (and village)	**Seed harvested**	**Yield data obtained**	**Farmers intend to continue with PPB**
Jharkhand	JH1 (Mehru)	no	no	no
	JH2 (Mehru)	yes	yes	?
	JH3 (Mehru)	yes	yes	?
	JH4 (Urguttu)	yes	no	?
West Bengal	WB1 (Pasro)	yes	yes	no
	WB2 (Baldangri)	yes	yes	yes
Orissa	OR1 (Udali)	yes	yes	yes
	OR2 (Udali)	yes	yes	no
	OR3 (Barbilla)	yes	yes	no
	OR4 (Barbilla)	yes	no	no
Total	10	9	7	2

Table 9. Data recorded for six bulks from 7 farms in the *kharif* season 2001, including farmers' subjective matrix ranking scores.

				Matirx ranking								
Variety	**Days to 50% fiowering**	**Plant ht.(cm.)**	**Harvest index**	**Maturity**	**Height**	**Lodging resistance**	**Disease resistance**	**Insect resistance**	**Straw**	**Grain colour**	**Grain type**	**Overall ranking**
Bulk I	63.0	99.2	0.45	12	14	21	24	16	12	11	12	19
Bulk II	64.3	97.1	0.46	9	15	19	24	13	13	9	6	22
Bulk III	60.0	101.1	0.45	17	16	20	24	19	16	17	9	15
Bulk IV	62.0	98.2	0.44	21	14	19	24	23	15	19	17	14
Bulk V	67.3	103.2	0.43	15	18	23	24	13	17	21	19	12
Bulk VI	64.7	95.0	0.45	12	12	16	24	10	16	13	10	18
Kalinga III	58.7	98.0	0.42	23	18	22	24	20	20	15	14	7

Effectiveness of MAS for improving root traits assessed

Screening products of MABC for root traits and flowering time was carried out as shown in Table 10, which shows the lines used and the number and types of treatments in each screen.

It must be noted that the pyramid lines and NILs used for these screens were often still segregating at a few markers (either target or non-target). The reason for this was the lack of time available for further generation advances. The data from Bangalore were often highly variable across replicates for each sample giving large standard errors.

Results of Bangalore root screens are inconclusive. In 2000 there was no significant difference between the stress and non-stress treatments. The only MAS line which showed significant difference from Kalinga III was that containing root QTLs 7 and 9; it had longer roots than Kalinga III, but root volume was not different. In the other Bangalore screens Kalinga III was not included so could not be used for comparison. In 2001 there was a significant difference for root volume between lines containing QTLs 2 and 11 and the control line with no QTLs, however in this study the line with QTLs 7 and 9 had shorter roots than the control with no QTLs. In 2002 there were no clear differences between lines with root QTLs and those without for any of the traits under either treatment. Two near-isogenic lines which contained QTL 9 but differed for aroma (chromosoem 8) had long roots (>70 cm at maturity), but one had long roots only under stress and the other had long roots only under watered conditions. The PPB lines Ashoka 228 and Ashoka 200F, with no Azucena alleles also had good roots in this experiment.

In the Aberdeen root box screen two of the near-isogenic lines out performed Kalinga III for root-shoot ratio. None of the other results were significantly different from Kalinga III, however in this screen Kalinga III scored well for most root traits. Kalinga III had roots of 62.59±10.99 cm and Azucena had roots 69.14±15.02 cm long. We can conclude that Kalinga III does not have significantly worse roots in well-watered soil-box conditions.

In the flowering time screen at Bangor a locus from Azucena at RM248 in the target root QTL on Chromosome 7 was found to considerably delay flowering.

Table 10. All of the screens carried out for roots and flowering time on MAS and PPB lines selected during this project.

Location and screen	Date sown	MAS entries and QTLs present		Variety entires	No. reps	Number of treatments
Bangalore (Root screen in soil-filled pipes)	July 1999			Kalinga III Azucena Bala Sathi Sudha 280 Sudha 228 Sudha 265 Sudha 165 Ashoka 228 Ashoka 200F Ashoka 238 Moroberekan Vanamaradi-Nellu Karidoddi DBN IR64	4	1. Watered daily.
Bangalore (Root screen in soil-filled pipes)	July 2000	21-01-03-01 21-01-03-06 42-01-05-12 21-01-03-08	9 2, 11 7, 9 9, 11	WAB 56-104 CG14 Kalinga III K.D. Doddi Basavraja	4	1. Sampling at 60 days with low moisture stress from days 45-60 2. Sampling at maturity with low moisture stress from days 45-60. 3. Sampling at 60 days watered daily. 4. Sampling at maturity, watered daily.
Bangalore (Root screen in soil-filled pipes)	July 2001	21-01-03-03 42-01-05-12 21-01-03-06-44 21-01-03-11-17 21-01-03-06-02 21-01-03-11-07 42-01-05-11-10 42-01-05-10-07 21-01-03-11-25 42-01-05-11-05 21-01-03-11-09 42-01-05-10-08 PY2F2 (1) PY2F2 (3) PY2F2 (8) 21-01-03-03	11 7,9 2,8,9,11 2,11 2,8,9,11 2,9,11 9 8,9 2,11 9 2 8 7,9,11 2,7,11 2 0	Ashoka 228 Ashoka 200F Moroberekan	3	1.Sampling at flowering (watered daily). 2.Sampling at time for grain harvest (watered daily).
Bangalore (Root screen in soil-filled pipes)	Jan. 2002	Same entries as in July 2001		Same entries as in July 2001	3	1.Sampled at 70 days, watered daily. 2.Sampled at 70 days, low moisture stress from day 45-60 3.Sampled at maturity, watered daily. 4.Sampled at maturity, low moisture stress from day 45-60.
Aberdeen (Root screen in soil-boxes)	Jan 2001	21-01-03-06 21-01-03-06-02 21-01-03-06-46 21-01-03-06-44 21-01-03-08 42-01-05-12 21-01-03-01	2,8, 11 2,8, 9,11 2, 8, 9, 11 2,8, 9,11 9,11 7,9 9	Kalinga III Azucena Bala IR64	12	1. Watered daily.
Bangor (Flowering time screen in pots)	June 2001	18 BC3-derived 10 PY2-derived	Various combinat-ions	Kalinga III IR64 Azucena Ashoka 228 Ashoka 200F	5	1. Watered daily.

6. Contribution of Outputs

- Advanced lines from 4 out of 6 crosses were selected by farmers in Eastern India through PPB. Selected material out-performs local varieties and is already available for farmers to grow.

- The varieties Ashoka 200F and Ashoka 228, developed from PPB from the cross IR64/Kalinga III were released for upland farmers in eastern India. These contain less than 40% of their alleles from IR64, but at nine AFLP markers and one SSR they have both inherited the allele from IR64. Root systems of these two varieties are similar to those of the bulks selected for root QTLs.

- Advanced bulks derived through single large-scale MAS and containing QTLs for roots and aroma have been selected and purified for upland ecosystems by farmers in eastern India. One bulk has been tested in multi-location state trials.

- A strategy for the use of markers for evaluation of PPB lines by marker-evaluated selection has been identified and parents have been identified for crossing to produce ideotype varieties for upland ecosystems.

- Advanced lines and bulks containing aroma have been produced using combined MAS and PPB. This is the first time an aromatic variety suitable for the rainfed uplands has been available in the three states of eastern India.

- Molecular analysis of 10 lines selected by farmers in Nepal from a population obtained from mutation breeding shows that out-crossing occurred in this population because there was more molecular variation than expected from single point mutations of the parent genotype.

- Molecular results for two round-grain lines selected from the IR64/Kalinga III population revealed that they were not extreme transgressive segregants but selected volunteers or off-types.

- The marker RM248 in the target QTL on chromosome 7 is linked to flowering time. In bulks selected by upland farmers from the crosses IR64/Kalinga III and IR36/ Kalinga III this marker was more likely to have been inherited from Kalinga III. Two other markers on chromosome 1 (near to the semi-dwarfing gene found in IR64) were also preferentially selected for Kalinga III by farmers in the upland ecosystems.

- Root screening has shown that there is little effect of individual root QTLs on root traits and results from lines with several QTLs in different combinations and genetic backgrounds have been inconclusive.

- Near-isogenic lines are available at the BC4 generation which will be crucial for future fine mapping studies of the five target regions.

- MES and selection of ideotypes is planned in the follow-up project R8200.

7. **Dissemination**

Project web page: **http://www.cazs.bangor.ac.uk/ricemas/index.htm**

Witcombe, J.R. and Steele, K.A. (2000). A case study in Participatory Plant Breeding in Rice I: Theory of Participatory Plant Breeding and the use of Marker Assisted Selection. Paper presented in the symposium, *'Scientific basis of participatory plant breeding and conservation of genetic resources, Oaxtepec, Morelos, Mexico, October 8-14, 2000*. University of California Divisions of Agriculture and Natural Resources Conservation Program, Davis, USA. Abstracts Report No 24, p 25.

Steele, K.A., Moore, B.J., Price, A.H. and Witcombe, J.R. (2000). Molecular markers for improvement of drought resistance in upland rice. Poster presented at the Annual Main Meeting of the Society for Experimental Biology, 27th-31st March 2000, Exeter, UK.

Steele, K. A., Virk, D. S., Kumar, R., Singh, D.N., Prasad, S.C., Gangwar, J.S., Shashidhar, H. E. and Witcombe J.R. (2001). Combining participatory plant breeding (PPB) with molecular marker assisted selection to improve rice grown by resource poor farmers in eastern India PSP Annual Report 2000 Research Highlights, pp55-62.

Steele, K.A., Edwards, G. and Witcombe, J. R. (2002) Combining molecular marker technology and participatory techniques: A case study for drought tolerant rice in Eastern India. Part 1: Molecular Breeding Strategy. Paper presented at a Workshop on breeding rice for drought-prone environments: integrating conventional and participatory plant breeding in south and south-east Asia, March 2002, IRRI, Los Baños, DFID PSP & IRRI

Steele, K.A, Singh, D.N., Kumar, R., Prasad, S.C., Virk, D.S., Gangwar, J.S. and Witcombe, J.R. (2002) Combining molecular marker technology and participatory techniques: A case study for drought tolerant rice in Eastern India. Part 2: PPB and Marker-assisted Selection Paper presented at a Workshop on breeding rice for drought-prone environments: integrating conventional and participatory plant breeding in south and south-east Asia, March 2002, IRRI, Los Baños, DFID PSP & IRRI

Steele, K.A., Edwards, G., Shashidhar, H.E., Macmillan, K. and Price, A.H. (2002). Marker-assisted selection for four root growth QTL and aroma to improve upland rice variety Kalinga III. Paper presented in the General Environmental Physiology session of the Annual Main Meeting of the Society for Experimental Biology, 8th-12th April 2002, Swansea, UK.

8. Acknowledgements

The authors wish to thank:

- Dr S. Sivaramakrishnan of ICRISAT, Patancheru, India for the use of his laboratory for DNA extractions with the technical help of Mr R. Lucas.
- Dr Adam Price of Aberdeen University for root screening of MAS-derived lines.
- Ms Jwala Bajracharya (PhD student funded by IPGRI, at NARC, Nepal) for assistance with SSR analysis of some PPB lines.

9. Glossary

AFLP	Amplified fragment length polymorphism
BAU	Birsa Agricultural University
BC	Backcross Population
CAZS	Centre for Arid Zone Studies
Chaite	February sown rice (Nepal)
DFID	Department for International Development
DFLB	DNA fingerprint linkage blocks
DH	Double haploid population
GVT	Gramin Vikas Trust (India)
IRRI	International Rice Research Institute
JIC	John Innes Centre (Norwich, UK)
Kharif	Main rice growing season (India)
LI-BIRD	Local Initiatives for Biodiversity, Research and Development (Nepal)
Main	Main rice growing season (Nepal)
MABC	Marker-assisted backcross
MAS	Marker-assisted selection
MES	Marker-evaluated selection
PCR	DNA amplification via polymerase chain reaction
PPB	Participatory Plant Breeding
PSRP	Plant Science Research Programme
PY	Pyramid (multiple targets introgressed in the same line)
QTL	Quantitative Trait Loci
Rabi	Off-season for second rice crop (India)
RFLP	Restriction Fragment Length Polymorphism
RM	Random Mating Population
SSR	Simple sequence repeats or microsatellites
WARDA	West Africa Rice Development Association

10. References

Fukai S. and Cooper M. (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Research* 40: 67-86

Monforte A.J. and Tanksley S.D. (2000). Fine mapping of a quantitative trait locus (QTL) from Lycopersicon hirsutum chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. *Theoretical and Applied Genetics* 100: 471-479.

Price A.H. and Tomos A.D. (1997). Genetic dissection of root growth in rice (*Oryza sativa* L.) II: Mapping quantitative trait loci using molecular markers. *Theoretical and Applied Genetics* 95, 143-152

Price A.H. and Virk, D.S. and Tomos, A.D. (1997). Genetic dissection of root growth in rice (*Oryza sativa* L.) I: A hydroponic screen. *Theoretical and Applied Genetics* 95:132-142

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

Witcombe J.R. (1999). Do farmer-participatory methods apply more to high potential areas than to marginal ones? *Outlook on Agriculture* 28: 65-71.

Zhang J., Chandra Babu R., Pantuwan G., Kamoshita A., Blum A., Wade L., Sarkarung S., O'Toole J.C. and Nguyen H.T. (1999). Molecular dissection of drought tolerance in rice: from physio-morphological traits to field performance IN Genetic Improvement of rice for water-limited environments. Proceedings of the Workshop on Genetic Improvement of Rice for Water-Limited Environments, 1-3 December 1988, Los Banos, Philippines. Manila (Philippines): International Rice Research Institute. pp 331--343.

Zhu J.H., Stephenson P., Laurie D.A., Li W., Tang D. and Gale M.D. (1999). Towards rice genome scanning by map-based AFLP fingerprinting. *Molecular and General Genetics* 261, 184-195

Appendix Table 1. Results of molecular studies on the two PPB upland varieties released in eastern India. 'I' indicates IR64 allele detected and 'K' indicates Kalinga III allele detected. Where alleles from neither parent were detected this has been indicated with a '?'. The approximate map positions of markers are from maps in Gramene (http://www.gramene.org/index.html) and the AFLP maps in Zhu *et. al* (1999).

Chromo-some	Marker i.d.	Marker type	Approximate position (cM)	Ashoka 228	Ashoka 200F
1	RM5	SSR	89.8	K	K
1	RM246	SSR	110.7	no data	K
1	RM237	SSR	110.7	K	K
1	RM212	SSR	147.8	K	no data
2	E12-M35-430	AFLP	33	I	I
2	RM262	SSR	73.8	K	K
2	RM6	SSR	151.7	K	K
2	RM213	SSR	183.1	K	K
3	E12M48-173	AFLP	7.6	K	K
3	RM251	SSR	76.8	K	K+I
4	E12M48-184	AFLP	15	K	K
4	E12M35-295	AFLP	17.5	K	K
4	E23M84-665	AFLP	21.8	I	K
4	E23M63-181	AFLP	23.2	K	K
4	E23M63-193	AFLP	24.3	I	K
4	E23M63-228	AFLP	68.5	K	I
4	E23M84-260	AFLP	73.3	I	I
4	RM252	SSR	78	K	K
4	RM226	SSR	91	K	K+I
4	E12M35-272	AFLP	115.5	I	I
4	RM349	SSR	200.3	K +?	no data
5	E23M63-122	AFLP	0	I	I
5	RM13	SSR	28.8	I	I
5	E12M35-540	AFLP	71.1	I	I
5	RM164	SSR	75.4	K	K
6	WAXY	SSR	4	K	no data
6	RM225	SSR	32.7	K	I
6	E12M35-365	AFLP	36	no data	I
6	E23M63-108	AFLP	39.1	I	K
6	E23M63-330	AFLP	46.4	I	K
6	E12M35-248	AFLP	93.6	I	I
7	E12M48-226	AFLP	0	K	K
7	RM2	SSR	42.1	I	K
7	RM11	SSR	50.7	K	K+I
7	RM234	SSR	98.2	I	K
7	RM248	SSR	129.9	K	K
8	RM337	SSR	0	K +?	K
8	RM350	SSR	86	K	I
8	RM223	SSR	90.3	K	K
9	RM219	SSR	9.6	K	K
9	E12M35-191	AFLP	24.5	I	I
9	RM242	SSR	70.1	I	K
10	E12M35-303	AFLP	5	I	I
10	E22M48-06?	AFLP	18.6	I	K
10	E12M35-536	AFLP	44.4	I	I

Appendix Table 1. continued.

Chromo-some	Marker i.d.	Marker type	Approximate position (cM)	Ashoka 228	Ashoka 200F
10	RM258	SSR	62.3	K	K
11	E22M48-14	AFLP	20.1	K	K
11	E23M63-254	AFLP	23.1	I	I
11	E12M48-178	AFLP	29.1	I	I
11	RM202	SSR	54.6	K +?	K
11	E23M63-174	AFLP	59.6	I	K
11	E22M48-07	AFLP	60.8	K	K
11	RM229	SSR	77.8	K	K
11	E12M48-133	AFLP	81.7	K	K
11	RM224	SSR	120	K	K+I
12	E12M74-450	AFLP	28.3	K	K
12	RM17	SSR	103.4	no data	K