Theor Appl Genet (2006) 112: 208–221
DOI 10.1007/s00122-005-0110-4

ORIGINAL PAPER

K. A. Steele · A. H. Price · H. E. Shashidhar
J. R. Witcombe

Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety

Received: 28 June 2005 / Accepted: 2 September 2005 / Published online: 6 October 2005

Abstract A marker-assisted back-crossing (MABC) breeding programme was conducted to improve the root morphological traits, and thereby drought tolerance, of the Indian upland rice variety, Kalinga III. This variety, the recurrent parent in the MABC, had not previously been used for quantitative trait locus (QTL) mapping. The donor parent was Azucena, an upland *japonica* variety from Philippines. Five segments on different chromosomes were targeted for introgression; four segments carried QTLs for improved root morphological traits (root length and thickness) and the fifth carried a recessive QTL for aroma. Some selection was made at non-target regions for recurrent parent alleles. We describe the selection made in three backcross (BC) generations and two further crosses between BC_3 lines to pyramid (stack) all five target segments. Pyramids with four root QTLs were obtained in eight generations, completed in 6 years using 3,000 marker assays in a total of 323 lines. Twenty-two near-isogenic lines (NILs) were evaluated for root traits in five field experiments in Bangalore, India. The target segment on chromosome 9 (RM242-RM201) significantly increased root length under both irrigated and drought stress treatments, confirming that this root length QTL from Azucena functions in a novel genetic background. No significant effects on root length were found at the other four targets. Azucena alleles at the locus RM248 (below the target root QTL on chromosome 7) delayed flowering. Selection for the recurrent parent allele at this locus produced early-flowering NILs that were suited for upland environments in eastern India.

Communicated by Qifa Zhang

K. A. Steele (✉) · J. R. Witcombe
CAZS Natural Resources, University of Wales, Bangor,
LL57 2UW, Gwynedd UK
E-mail: k.a.steele@bangor.ac.uk
Tel.: +44-1248-382346
Fax: +44-1248-364717

A. H. Price
Department of Plant and Soil Science, University of Aberdeen,
Cruickshank Building, St Machar Drive,
Aberdeen, AB24 3UU UK

H. E. Shashidhar
Marker-Assisted Selection Laboratory,
Department of Genetics & Plant Breeding,
College of Agriculture, University of Agricultural Sciences,
Bangalore, 560 065 India

Introduction

Selection for a well-developed root system with long, thick roots should improve the drought tolerance of upland rice because the plant would avoid water stress by absorbing water stored in the deep soil layers (Yoshida and Hasegawa 1982). Phenotypic selection for root morphological traits in conventional breeding programmes is unfeasible, so in the mid-1990s several mapping populations were developed to detect quantitative trait locus (QTLs) influencing root morphology and other drought-related traits that could then be used in marker-assisted selection (MAS) to improve upland varieties (Champoux et al. 1995, Yadav et al. 1997; Price and Tomos 1997). Courtois et al. (1996) confirmed that tropical *japonica* rice cultivars consistently have thicker and deeper roots than *indica* cultivars, so tropical *japonicas*, such as Azucena, should contribute to improved rooting if crossed to *indicas*. Selection for root depth QTLs was conducted by Shen et al. (2001) with doubled haploid (DH) lines from the IR64/Azucena (lowland/upland) mapping population as donor parents and IR64 as the recurrent parent. Here we report the results of a marker-assisted back-crossing (MABC) breeding programme where the recurrent parent was an Indian upland variety that had not been previously used in mapping studies.

In Eastern India, upland rice is directly sown without transplanting and is completely rainfed as no irrigation is available. Few modern varieties exist for these conditions. The upland variety Kalinga III was chosen for

root improvement because, although it escapes end-of-season drought through early maturity, it is susceptible to early and mid-season drought. Azucena was chosen as a donor parent because its roots were superior to those of Kalinga III. In addition it had been shown to carry root QTLs when used as a parent in a mapping population with Bala, another Indian upland variety that is no longer cultivated. An additional objective of this breeding programme was to introgress a single QTL for aroma from Azucena into non-aromatic Kalinga III.

We describe the results of a MABC and pyramid crossing scheme used to introgress four QTLs for root morphology and one QTL for aroma from Azucena into Kalinga III. We discuss the results in relation to other MABC programmes for quantitative trait introgression.

Materials and methods

Development of NILs: choice of target QTLs

Five target chromosome segments (Table 1) from Azucena, an upland *japonica* were chosen for introgression into Kalinga III, an upland *indica*. Four QTLs (QTL2, QTL7, QTL9, QTL11) were chosen for improved rooting ability on the evidence from three mapping populations: IR64/Azucena (Yadav et al. 1997, Zhang et al. 1999); Bala/Azucena (Price and Tomos 1997; Price et al. 2000); and CO39/Moroberekan (Champoux et al. 1995). They conferred various quantitative root morphology traits with low heritability and explained between 5 and 30% of the variation of each trait. In the Bala/Azucena cross, subsequent mapping studies in different environments confirmed that these four chromosome segments carried QTLs for root morphology (Price et al. 2002a, b). In the lowland cross, CT9993-5-10-1-M /IR62266-42-6-2, identical segments on chromosomes 2, 7 and 11 were shown to carry root QTLs (Kamoshita et al. 2002). In this same population Zhang et al. (1999) detected a QTL on chromosome 11, near to RM21, which explained 23% of phenotypic variance for grain yield under drought stress.

The fifth target was a QTL for aroma (QTL8) on chromosome 8 identified by Lorieux et al. (1996), in a DH mapping population derived from IR64/Azucena (Azucena was the aromatic parent). This QTL is approximately 6.2 cM above the RFLP locus RG1 (R^2 (%) = 69) and it carries a major recessive gene for concentration of 2-acetyl-1-pyrroline (AcPy), the main compound associated with rice aroma. Although this region was not associated with improved roots it was chosen because it could improve grain quality (fragrance) and thereby the potential market value of upland rice.

Development of NILs: Nomenclature

During the breeding programme the lines were numbered according to their ancestry. In the first back-cross there were 22 BC_1 lines numbered $BC_1$1 to $BC_1$22. In the next back-cross generation, for example, back-cross progeny of $BC_1$21 were numbered $BC_2$21-01 to $BC_2$21-n. After the third back-cross all the lines were selfed so that, for example the pedigree of line $BC_3F_3$21-01-03-06-02 can be traced as: $BC_1$21: $BC_2$21-01: $BC_3$21-01-03: $BC_3F_2$21-01-03-06: $BC_3F_3$21-01-03-06-02.

In order to pyramid the target chromosome segments an initial pyramid cross was made between two BC_3F_3 lines to produce progeny coded as PY_1F_1. Only one line, called $PY_1F_1$was used to cross to a BC_3F_3 and the nine progenies (PY_2F_1) were numbered $PY_2$1 to $PY_2$9. Four were selected (PY_2 1, 3, 5 and 8) to contribute to the following PY_2F_2 generation. In this generation suffixes were added to the plant numbers according to plots in a greenhouse experiment (from 24 to 33) and then according to plant number (1–5). For example, PY_2 $F_2$8-33-5. In this PY_2F_2 generation one plant, $PY_2F_2$3-26-5, was selfed to produce 33 PY_2F_3 progeny plants that were given the suffixes 1-33.

Genotypes at specific loci were designated K (Kalinga III homozygotes), H (heterozygotes) and A (Azucena homozygotes).

Back-crossing and selection

Kalinga III was used as the recurrent parent for three generations of back-crossing (Fig. 1). The initial cross and first backcross were made at the International Rice Research Institute (IRRI). In 1997, BC_1F_1 seeds were supplied to CAZS (UK) where all subsequent crosses were made under greenhouse conditions. Selection was carried out using both (RFLP) and simple sequence repeat (SSR) microsatellite markers to introgress Azucena alleles at five target segments and to maintain recurrent parent alleles in a few non-target regions.

In the first three back-cross generations selection was made with RFLPs that had been mapped in the Bala/Azucena population and flanked, or were within, the regions containing the target QTLs. In addition, the RFLP marker C570 was used. This marker was polymorphic between Azucena and Kalinga III (but not between Azucena and Bala) and mapped near to QTL9. Genomic DNA from every line in the same generation was assayed for RFLPs together on the same Southern blot hybridised with one probe at a time (for methods see Price et al. 2000). Selection in BC_1–BC_3 was for lines with the greatest number of heterozygotes at the target segments.

For the later generations (BC_3F_2 and beyond) more flexible and cheaper PCR-based SSR markers were used. The map positions of SSRs were estimated by comparative mapping (http://www.gramene.org) and some were confirmed by mapping in Bala/Azucena RILs.

Two BC_3F_1 lines ($BC_3$21-01-03 and $BC_3$42-01-05) were selected because they were heterozygotes at complementary targets (Fig. 2). They were advanced by selfing to the BC_3F_2 and 15 lines derived from each one were genotyped. Three of these lines were selected for making pyramid crosses (Fig. 1 and next section).

Table 1 Chromosome segments containing QTLs which were selected in MABC and pyramids

Chromosome	Nearest or flanking mapped marker(s)	QTL	R^2%	Reference	Selectable RFLPs	Selectable SSRs
(a) *Targets for introgressions from Azucena*						
2	C601	Root penetration	18.0	Price et al., (2000)	G39	RM221
		(ratio of penetrated roots to total roots).	10.8	Price et al., (2002a)	G57	RM6
		Deep root weight (well-watered treatment).	15.8		C601	RM318
		Root thickness (well-watered treatment)			RG256	RM213
7	RG650-C507	Total root weight	4.8	Yadav et al., (1997)	RG351	RM234
		Deep root weight	14.7		RG650	RM351
		Deep root per shoot ratio	22.3		C507	(RM248)
		Deep root weight per tiller	18.7		(RG351)	
		Maximum root length	17.7			
8	RG28-RG1	Aroma	69.0	Lorieux et al., (1996)	G1073	RM223
					G187	
					R2676	
9	G385	Deep root thickness (well-watered treatment)	18.1	Price et al., (2002a)	G385	RM242
	G1085	Deep root thickness (drought-stressed)	13.0		C570	RM201
11	RG2	Root length (hydroponics)	29.8	Price & Tomos (1997)	C189	RM229
	C189	Root penetration	7.2	Price et al., 2000	G1465	RM206
		(ratio of penetrated roots to total roots)				
(b) *Selection for recurrent parent*						
2	RG171-G45	Root thickness		Price et al., (2000)	RG171	
		(Azucena alleles reduce thickness)			G45	
5	RG13-RZ70	Root length, thickness and penetration		Price et al., (2000)	RG13	
		(Azucena alleles reduce thickness and penetration)			C43	
					RZ70	
7	RM248	Flowering time				RM248
		(Azucena alleles delay flowering)				
8	R202	Osmotic adjustment		Lilley et al. (1996)	G56	
		(Azucena alleles reduce osmotic adjustment)				

(a) Four target root morphology QTLs and one aroma QTL (positive effect alleles donated by Azucena) mapped in IR64/Azucena and/or Bala/Azucena populations. Selectable RFLPs flanking the target QTLs were used for selection in one or all of three generations of back-crossing and SSR markers located within the target segments were used after pyramid crosses had been made for selection of NILs. The loci in brackets were selected for Azucena alleles in the first three generations only. (b) Four regions where recurrent parent selection was carried out to eliminate unwanted QTLs from Azucena

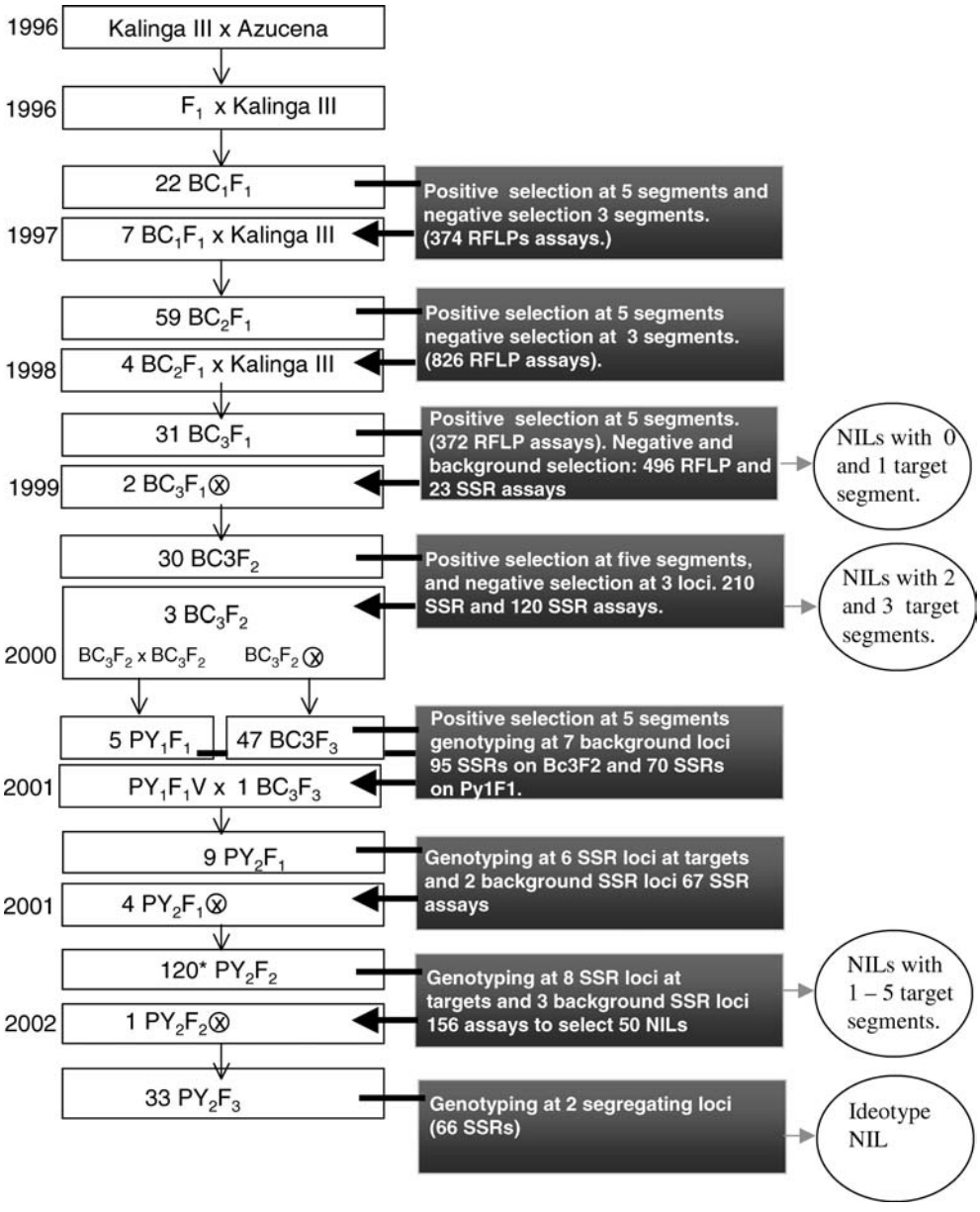

Fig. 1 Marker-assisted backcross and pyramid crossing scheme to introgress five target chromosome segments from Azucena into Kalinga III. The year each cross pollination or self pollination (indicated by an *circled x*) was made is shown on the *left side*. The *shaded boxes* show the genotyping and selection direction; positive selection was for Azucena alleles and negative selection was for Kalinga III. In the later generations it was not necessary to genotype every line at every locus; selfed progeny from lines that were homozygote at a locus were not tested again at that locus

A third BC_3F_1 line ($BC_3$23-01-06) was selected as a control because it did not carry any Azucena alleles at the five targets for introgression; it was genotyped at 15 further SSR loci and was heterozygous at the top of chromosome 7 (at RM2) and on chromosome 6 (at RM225 and RM3). It was advanced to the $BC_3F_2$and used as a control NIL to evaluate root traits.

Selection for recurrent parent alleles near QTLs

Kalinga III alleles were selected at four chromosome segments in the MABC programme (Table 1). Three (listed below) were targeted for selection in the first three backcross generations.

i. Below the aroma QTL, near RG1 on chromosome 8. A QTL for osmotic adjustment conferred by *indica* genotypes was mapped to this location by Lilley et al. (1996). Bala is known to be able to osmotically adjust much more than Azucena (Lilley and Ludlow, 1996), so Bala may have the favourable allele at the locus on chromosome 8; indirect evidence of performance under drought in the field supports this possibility (Price et al. 2002b). Even though osmotic adjustment has not been tested in Kalinga III, it was prudent to assume that Kalinga III also had this favourable osmotic adjustment allele. Hence, Kalinga III alleles were selected at RFLP G56 to prevent linkage drag in this region.
ii. In the Bala/Azucena population Azucena alleles at RG171 contributed to thinner roots (Price and Tomas, 1997). Further experiments showed that two root QTLs with opposing effects were detected on chromosome 2 (Price et al. 2000) and, in the segment

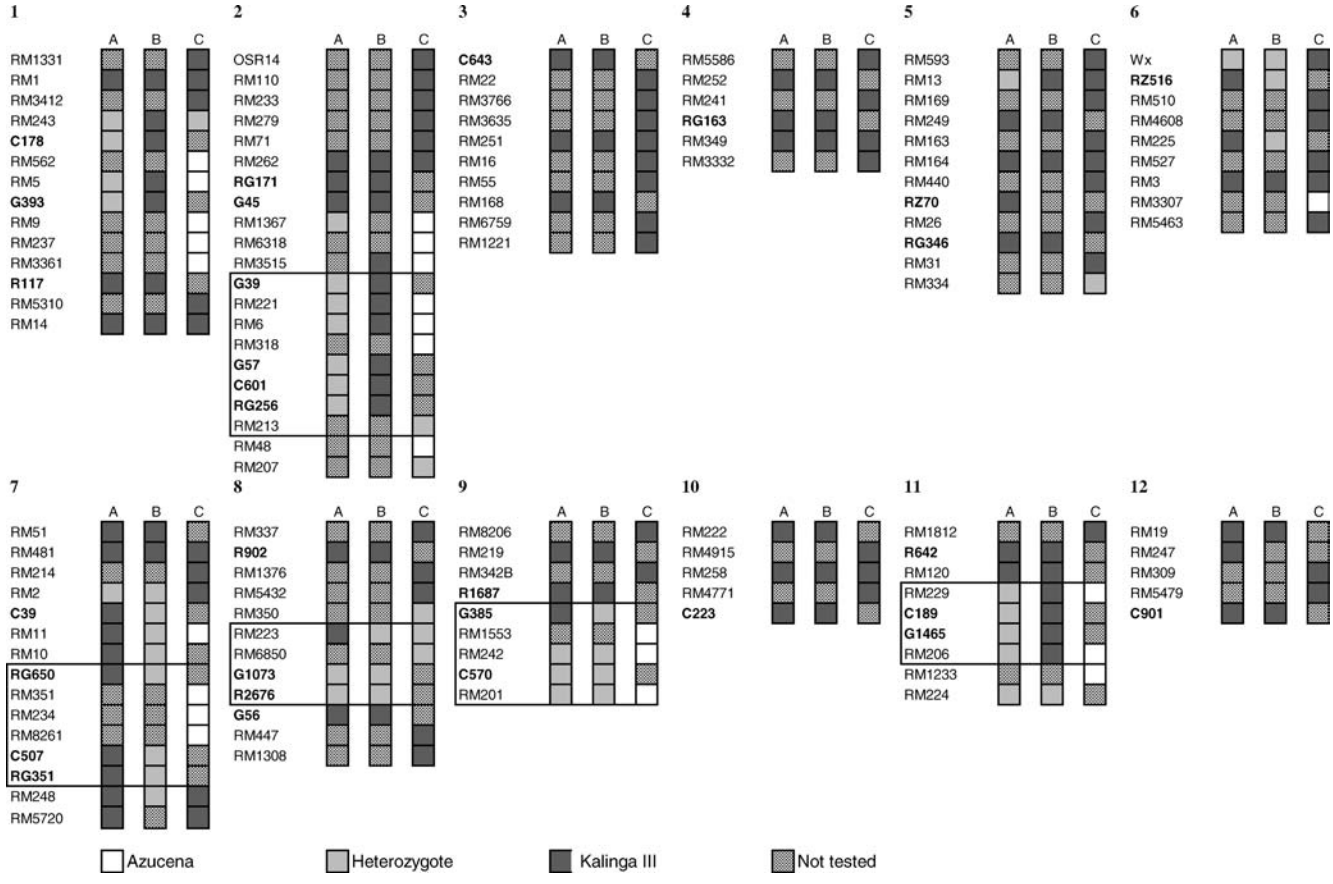

Fig. 2 Graphical genotypes of (**a**) BC_3F_121-01-03, (**b**) BC_3F_142-01-05 and (**c**) PY_2F_33-26-05-18. A and B are the two BC_3lines selected to contain all target regions between them and they were used as the parents from which all subsequent NILs (except the control NIL) and pyramids were derived. C was chosen for genotyping because it was the pyramid line most suited to upland conditions in eastern India. SSR and RFLP (bold) markers are shown in their relative order along chromosomes according to comparative mapping using http://www.gramene.org. The five target chromosome segments are boxed

spanning RG171-G45, *indica* alleles were most likely to improve roots, approximately 20 cM above the target QTL (G39-C601). Kalinga III alleles were selected for at RG171 and G45 to prevent linkage drag.

iii. At a QTL on chromosome 5 (RG13-RZ70) where Bala alleles increased root penetration (Price et al. 2000) Kalinga III alelles were selected using RFLPs.

Kalinga III alleles were selected at a fourth segment after the pyramid crosses at the end of the programme to prevent linkage drag for lateness from Azucena. The greenhouse evaluation (see later section) revealed that the SSR locus RM248 (at the base of the introgressed segment of chromosome 7) was associated with delayed flowering. A similar effect at this locus on maturity was observed in progeny of a cross between Kalinga III/IR64 where IR64 alleles at RM248 also delayed flowering ($r_2\% = 83$; Steele et al. 2004).

Selection for recurrent parent alleles at other regions

All thirty-one BC_3F_1 lines that were produced were genotyped at background loci. On non-target chromosomes these were RFLPs: C178, G393 and R117 (the latter is linked to the semi-dwarfing locus (*sd1*) in IR64 and Bala); C643 and R1618 on chr3; RG163 and C734 on chr4; RG346 on chr5; RZ516 on chr6; C223 on chr10; C901 on chr12 and at SSR locus RM247 on chr12). On target-carrier chromosomes in non-target regions the RFLPs tested were: RG256 on chr 2; C39 on chr 7; R902 on chr 8; R1687 on chr 9 and R642 on chr 11. The presence of Kalinga III alleles at these regions was taken into consideration during selection, but with the limited number of lines it meant that it was not possible to eliminate all donor parent alleles.

Forty BC_3F_2 lines were produced in total and they were genotyped with one SSR at each of the target regions. Twenty-five of these lines were then selected for genotyping with background RFLPs. Further selection identified BC_321-01-03, BC_342-01-05 for genotyping with additional background SSRs.

Pyramid crossing and selection

In order to pyramid the five target segments from Azucena crosses were made between the BC_3F_242-01-05-12 with either BC_3F_221-01-03-01 or BC_3F_221-01-03-06

(see Table 2 for genotypes). The progeny (first generation pyramid, PY_1) lines were genotyped with SSRs at all possible segregating loci in the five target segments: one was heterozygous for all targets. The probability (at $P \leq 0.95$) of getting a line with at least four target segments in a homozygous state through selfing was low at only one in 765. Therefore, instead of selfing, more efficient crossing between complementary lines was used. These lines were derived by selfing $BC_3F_2$21-01-03-06 (A at targets QTL2 and QTL11 and H at targets QTL8 and QTL9); 47 of its progeny (BC_3F_3) were genotyped at RM223 (QTL8), RM242 and RM201 (QTL9). Three lines ($BC_3F_3$21-01-03-06-02, $BC_3F_3$21-01-03-06-44 and $BC_3F_3$21-01-03-06-46) were A at these loci. The selected PY_1 (H at all five targets) was crossed to these three complementary BC_3F_3 lines to produce the second pyramid (PY_2) generation.

Nine PY_2 lines were obtained and they were genotyped at the four root QTLs and at SSR markers on chr1 and chr6. All but one of the PY_2F_1 lines carried Azucena alleles (homozygous or heterozygous) at RM5. In the PY_2F_2, 120 seedlings were grown and genotyped with SSR markers at segregating loci to select ten lines each represented by the most uniform five plants. Genomic DNA was extracted from seedlings using Extract 'N'Amp kits (Sigma-Aldrich, Poole, Dorset, UK). Genotyping was carried out at up to two of the following SSR loci: RM221 (QTL2), RM234 (QTL7), RM201 (QTL9), RM229 and RM206 (QTL11). These plants were grown to maturity in the greenhouse (see below).

Thirty-three selfed progeny lines from $PY_2F_2$3-26-5 were genotyped at the two remaining segregating target loci (RM213 and RM223) and lines 3 (K at RM213) and 7 (A at RM213) were selected as NILs to be included in root evaluation experiments from December 2002. Line 18 (H at RM213) was genotyped at further SSR loci throughout the genome (Fig. 2).

Phenotypic evaluation

Lines selected at the BC_3F_2 generation and beyond were phenotypically evaluated and regarded as NILs (Table 2) although some were still segregating at both target or non-target regions.

Greenhouse experiment

A replicated experiment was conducted in a greenhouse at Pen-y-Ffridd Research Station, University of Wales, Bangor, to evaluate above-ground traits in NILs. The two purposes were: (1) to identify chromosome regions from Azucena that delayed anthesis so that they could be selected against and (2) to identify linkage drag or pleiotropic effects of target loci on other non-root traits.

Seed of 28 NILs (including ten PY_2F_2 lines) and both parent varieties were soaked overnight in aerated sterile distilled water and sown in John Innes No 2 compost on 14 June 2001. After 25 days the seedlings were transferred to 4l pots filled with John Innes No 2 compost mixed with Multicote 6 fertiliser (N 18: P 6: K 12) at 5 g l^{-1} . The pots were randomised in five replicate blocks; each NIL represented once per block and each block containing 33 plants on one of five adjacent benches in the greenhouse. They were watered daily by filling each pot to the rim with tap water. The following were recorded: plant height (on days 28, 49, 91 and at grain maturity); number of tillers (on day 28); the number of days to anthesis; the total number of days to reach maximum height; number of days to grain maturity; number of panicles; panicle length; and presences or absence of awns. In replication 5 only, grain length and grain width were measured with callipers (to 0.01 mm) for ten seeds at random from each NIL. All individuals were genotyped at 11 SSR loci.

Field experiments to evaluate roots

The seeds used for root experiments passed UK phytosanitary tests for official export to India. Five independent experiments were sown on five dates between July 2000 and March 2003, under field conditions at the research station of the University of Agricultural Sciences, Bangalore, India (Table 3). Different NILs were evaluated in each of the experiments. In 2001, 16 NILs were chosen because these were predicted to be the most suitable for rainfed upland environments, and two pyramid lines were tested when they became available later. Parent lines were not included in all experiments. The treatments and sampling strategies were slightly different in each experiment. All five experiments included well-watered (WW) treatments. Drought treatments (DT) were tested in three experiments (July 2000, January 2002 and March 2003). Due to limited space the number of entries was reduced to the most 'interesting' nine lines for the final two experiments.

PVC cylinders (1 m long, 180 mm diameter) were filled with soil to 15 mm from the top with sandy loam and well-composted farmyard manure in a 4:1 ratio. The soil was uniformly compacted in each cylinder and they were placed in subsurface pits so that the tops of the cylinders were at ground level. Three seeds were sown in each cylinder and were thinned to one per cylinder after 10 days. The experiments were replicated with all genotypes randomised within a single block. WW treatments were irrigated on every alternate day, while DT treatments were well-watered up to day 40, 50 or 55 after which irrigation was withheld and rainfall prevented with a rain-out shelter for 10 days, or until sampling (S). Sampling was either conducted at a specific time (day 40, 55, 75, 68, or 70), or when each individual plant reached maturity (M).

Phenotypic trait sampling was carried out according to the methods of Venuprasad et al. (2002). The traits recorded were: plant height, tiller number, maximum

Table 2 Genotypes of 24 NILs at SSR loci in five target chromosome segments (approximate positions from http://www.gramene.org) for introgression and one non-target segment

		1	2				7			8	9		11	
Generation	Line	95 cM RM5	144 cM RM221	153 cM RM318	155 cM RM6	186 cM RM213	68 cM RM351	74 cM RM234	97 cM RM248	81 cM RM223	73 cM RM242	81 cM RM201	78 cM RM229	103 cM RM206
BC_3F_2	21-01-03-01*	3	1	-	2	3	3	3	3	2	1	1	2	2
	21-01-03-03	2	2	-	3	2	3	3	3	1 or 2	1	1	2	1
	21-01-03-06*	2	2	-	1	2	3	3	3	2	1	1	1	1
	21-01-03-08	1	2	(2)C601	-	-	3	3	3	2	1	(1)C570	-	(1)G1465
	21-01-03-14	1	2	-	1	1	3	3	3	1	1	1	3	3
	23-01-06-06	3	3	-	3	3	3	3	3	3	3	3	3	3
	42-01-05-09	3	3	-	3	3	1	1	3	2	2	1	3	3
	42-01-05-12*	3	3	-	3	3	1	1	1	2	1	1	3	3
BC_3F_3	21-01-03-06-02	1	1	1	1	1	3	3	3	1	1	1	1	1
	21-01-03-06-44	1	1	1	1	2	3	3	3	1	1	1	1	1
	21-01-03-06-46	3	-	1	1	1	3	3	3	1	1	1	1	1
	21-01-03-11-07	3	2	1	1	2	3	3	3	3	1	1	1	1
	21-01-03-11-09	3	-	1	1	3	3	3	3	3	3	2	3	3
	21-01-03-11-17	3	-	1	1	2	3	3	3	3	3	3	1	1
	21-01-03-11-25	3	-	1	1	3	3	3	3	3	3	3	1	1
	42-01-05-10-07	3	3	3	3	3	3	3	3	1	1	1	3	3
	42-01-05-10-08	3	3	3	3	3	2	2	2	1	3	3	3	3
	42-01-05-11-05	3	3	3	3	3	3	3	3	3	1	1	3	3
	42-01-05-11-10	3	3	3	-	-	3	3	2	3	1	1	3	-
PY_2F_2	1-24	2	-	-	1	1	3	3	3	-	2	2	2	2
	3-26	1	2	1	1	2	2	2	3	2	2	2	1	2
	5-29	3	3	-	3	1	3	3	2	-	3	2	1	1
	5-31	3	2	-	2	1	1	1	2	-	2	1	1	1
	8-32	2	2	1	1	3	3	3	3	-	3	3	3	3

Azucena 1, heterozygote 2 and Kalinga III 3. Lines indicated with an asterisk were used as parents for pyramid (PY) generations. Dash indicates not genotyped, brackets show the genotype at the linked RFLP locus indicated

Table 3 Means and standard error of means for root length (mm) of parents and 19 NILs measured in soil-filled cylinders at UAS, Bangalore in five root experiments

Experiment sowing date Treatments and sampling	July-00 1 DT55-75 S75 2. WW S75	August-01 1. WW S55 2. WW SM	January-02 1. DT 40-60 S70 2. DT 40-60 SM 3. WW S70 4. WW SM	December-02 1 WW S40	March-03 1. WW SM 2. DT 50-68 S68	All experiments	
Number of replications	4	3	3	10	3		Rank
Kalinga III	600 ± 100	-	-	340 ± 110	550 ± 160	493 ± 30	12
Azucena	-	-	670 ± 200	300 ± 170	800 ± 230	597 ± 60	2
21-01-03-03	-	380 ± 120	500 ± 170	-	-	461 ± 40	18
23-01-06-06	-	430 ± 90	490 ± 190	-	-	469 ± 40	17
42-01-05-09	-	-	-	370 ± 210	580 ± 150	486 ± 40	14
42-01-05-12	920 ± 190	420 ± 110	640 ± 240	400 ± 110	570 ± 320	595 ± 40	3
21-01-03-06-02	-	480 ± 100	560 ± 150	-	-	535 ± 30	9
21-01-03-06-44	-	400 ± 110	570 ± 220	-	-	519 ± 50	10
21-01-03-11-07	-	510 ± 190	560 ± 260	-	-	541 ± 55	7
21-01-03-11-09	-	440 ± 170	410 ± 180	-	-	424 ± 40	20
21-01-03-11-17	-	360 ± 60	480 ± 190	260 ± 130	450 ± 110	395 ± 30	21
21-01-03-11-25	-	390 ± 40	470 ± 180	-	-	444 ± 40	19
42-01-05-10-07	-	410 ± 100	740 ± 250	340 ± 160	580 ± 200	539 ± 40	8
42-01-05-10-08	-	350 ± 110	550 ± 180	-	-	481 ± 40	15
42-01-05-11-05	-	400 ± 90	640 ± 170	-	-	559 ± 45	5
42-01-05-11-10	-	400 ± 60	680 ± 260	430 ± 180	780 ± 260	605 ± 40	1
PY_2F_11	-	410 ± 160	660 ± 180	-	-	582 ± 50	4
PY_2F_13	-	420 ± 210	620 ± 320	-	-	550 ± 50	6
PY_2F_18	-	350 ± 110	610 ± 200	-	-	518 ± 50	11
PY_2F_33-26-5-3	-	-	-	310 ± 110	660 ± 270	481 ± 0	16
PY_2F_33-26-5-7	-	-	-	410 ± 90	550 ± 130	487 ± 8	13

A dash indicates that the line was not included in a experiment. WW well-watered, DT55-75 no water from day 55 to day 75, S40 sampling 40 days after sowing, SM Sampling at maturity. For 'all experiments' standard errors were calculated from the pooled error after model fitting, and the overall order of ranking for root length (longest = 1) shown in the final column

root length, total root number (not in January 2002), root dry weight (not in July 2000 and August 2001), shoot dry weight (not in July 2000 and August 2001) and total root volume (not in January or December 2002).

Statistical analysis

Statistical analysis was carried out using Minitab (v. 14) and a significance level at $P < 0.001$ used unless otherwise indicated. Initially the trait data from each of the greenhouse and root experiments were analysed in separate two-way ANOVAs to establish overall genotype effects and treatment effects (except for the root evaluation sown in Dec 2002 and the greenhouse experiment which only had one treatment). A combined analysis was done to investigate genetic effects where no treatment effects were observed.

For the greenhouse experiment, lines were coded according to loci at seven markers: RM5 (C1), RM6 (C2) RM213 (C2), RM248 (C7), RM234 (C7), RM242 (C9) and RM229 (C11) according to three classes: A (Azucena homozygous), K (Kalinga III homozygous) or H (heterozygote). All traits were tested separately using multifactor ANOVA (GLM) to detect any significant effects of Azucena alleles at these loci (single marker analysis). The effect of the chromosome 8 target was not tested.

Data from the five root experiments at Bangalore were pooled with replicate, treatment and sampling differences included. Single factor ANOVAs (GLM) were used for each trait and standard errors were calculated from the pooled error. The effect of Azucena alleles at these regions was tested using multi-factorial anova (GLM) by using the most common genotypes at loci in each of the six regions (RM5 for chr1; RM221, RM6, RM318 and RM213 for QTL2; RM351, RM234 for QTL7; RM223 for QTL8; RM242 and RM201 for QTL9; RM229 and RM206 for QTL11) as separate fixed factors in the analysis. Two analyses were done because loci that were heterozygous in the previous generation were either (1) analysed by assuming to be 'A' in all replications for that line, or (2) analysed by assuming to be a third class, 'H', in all replications for that line. The genotype with the greatest number of target alleles at loci within each segment was used. Parent lines Azucena and Kalinga III were not included in this analysis. The model included all six segments. Stages at which lines were sampled (day (if vegetative) or maturity), treatment (DT or WW) and root experiment and interactions were tested. Least squared means were used to indicate the direction and effect of the factor on the variable where significant differences were detected.

Results

The programme successfully produced an ideotype line (PY_2F_33-26-5-18) that had all five target regions from

Azucena introgressed in a predominantly Kalinga III genetic background (Fig. 2). It had Kalinga III alleles at RM248, within the QTL7 target region (but probably just below the root QTL), selected to prevent linkage drag for late maturity. It was, however, homozygous for Azucena alleles at two non-target regions (chr1 between RM562 and RM3361; and chr6 at RM334). Approximately 3,000 plant/locus assays were made in the breeding programme to produce this plant.

Greenhouse experiment: non-root traits

All measured traits showed significant line effects indicating that they were influenced genetically in this cross (Table 4). The locus RM5, within a segment of chromosome 1 that was unintentionally introgressed in some NILs, had no significant effect on any traits except grain width and Azucena alleles decreased width ($P < 0.05$). Significant effects on the following traits were detected at target root QTLs:

QTL2

Tiller number on day 28: NILs with K and H at RM6 had five more tillers than those with A. Final plant height: Kalinga III alleles at RM213 increased height by 0.09 m. Growth rate (increase in height between days 28 and 49): Kalinga III alleles at RM213 increased growth rate ($P < 0.05$) and H at RM6 increased growth rate ($P < 0.05$). Number of days to anthesis: Azucena homozygotes at RM6 ($P < 0.05$) and RM213 ($P < 0.05$) were 4 days later. Number of days to grain maturity: Azucena alleles at RM6 ($P < 0.05$) delayed harvest by 3 days in H and by 8 days in A. Grain length and width: Azucena alleles at RM213 increased length by 0.3 mm ($P < 0.05$) and width by 0.1 mm; Azucena alleles at RM6 decreased length by 0.3 mm.

QTL7

Tiller number on day 28: Kalinga III homozygotes (K) had five more tillers than A or H at RM248. Plant height on day 91: Azucena homozygotes (A) at RM234 were 0.04 m taller than K or H, while heterozygotes (H) at RM248 were 0.09 m shorter than A or K. Final plant height: A and H were taller than K at both RM248 and RM234; Azucena alleles at RM248 increased height by > 0.4 m. Growth rate (increase in height between days 28 and 49): Azucena alleles at RM234 increased growth rate ($P < 0.05$). Number of days to anthesis: Azucena alleles at RM248 delayed anthesis. This was by 63 days in H and 71 days in A. (Note that Azucena flowers approximately 73 days later than Kalinga III, so this locus accounts for a very large proportion of the variation of this trait in this cross). Number of days to grain maturity: Azucena alleles at RM248 delayed maturity by 57 days. Length of panicles: Azucena alleles at RM248 increased panicle length by 40 mm. Grain length and width: Azucena alleles at RM248 decreased length by 0.5 mm.

QTL9 at RM242

Tiller number on day 28: Kalinga III alleles increased tiller number, with K and H having five more tillers than A. Plant height on day 91: A were 0.04 m taller than K or H. Number of panicles: Kalinga III alleles (K and H) increased panicle number by ten. Grain length and width: Azucena alleles decreased length by 0.3 mm and width by 0.1 mm.

QTL11 at RM229

Plant height on day 91: Kalinga III homozygotes (K) were 0.04 m taller than A or H. Final plant height: Kalinga III alleles (K or H) increased final plant height by 0.09 m. Length of panicles: Azucena alleles decreased panicle length. Grain width: Azucena alleles increased grain width by 0.05 mm ($P < 0.05$).

The control NIL, $BC_3$23-01-06-06, that had Kalinga III alleles (K) at all five targets produced significantly more tillers than Kalinga III, but it did not differ from Kalinga III in any other trait. The three tallest NILs ($PY_2F_2$5-31, $PY_2F_2$5-30 and $BC_3F_3$42-01-05-12) had significantly longer panicles than Kalinga III and were the only ones segregating for presence or absence of

Table 4 Mean and standard error of tiller number, maximum plant height and days to anthesis measured in the greenhouse experiment for different generations and five plants of each NIL

Generation	NIL derived from line	Number of NILs tested	Number of tillers on day 28	Maximum height (m)	Number of days to anthesis
Azucena		1	9 ± 0.7	1.53 ± 0.02	146 ± .6
Kalinga III		1	27 ± 1.7	1.12 ± 0.01	73 ± 1.2
BC_3F_3	BC23	1	36 ± 3.3	1.09 ± 0.03	72 ± 0.9
BC_3F_3	BC21	5	21 ± 1.8	1.08 ± 0.02	73 ± 1.0
BC_3F_3	BC42	2	21 ± 2.0	1.53 ± 0.02	104 ± 5.5
BC_3F_4	BC21	7	15 ± 1.2	1.07 ± 0.02	78 ± 1.5
BC_3F_4	BC42	3	16 ± 1.4	1.30 ± 0.05	93 ± 5.6
PY_2F_2	PY1	2	16 ± 1.3	1.08 ± 0.04	81 ± 1.7
PY_2F_2	PY3	4	21 ± 2.0	1.21 ± 0.03	95 ± 1.7
PY_2F_2	PY5	2	22 ± 2.5	1.43 ± 0.06	121 ± 9.1
PY_2F_2	PY8	2	19 ± 1.7	1.06 ± 0.02	73 ± 1.2

glume awns. However, they were late maturing (141–183 days to reach anthesis) and were therefore, unsuitable for Indian upland conditions; hence they were not included in the root experiments.

Field experiments: root length

Root length was the only trait that differed significantly between Azucena and Kalinga III ($T_{(48)} = 2.077$, $P < 0.05$) in the Bangalore field experiments. There was variation for root length between experiments and harvest dates (Table 3) so the overall ranks have limited value. Although before day 55 Kalinga III did not have significantly shorter roots than Azucena, it did from day 68 to maturity. Analysis of the NILs sampled only at maturity showed significant genotype and treatment effects but no genotype by treatment interaction. The NIL which showed the biggest increase in root length under DT compared to WW was $BC_3F_3$42-01-05-11-05. Four NILs showed no significant difference between the two treatments, including $BC_3F_2$23-01-06-06 that had none of the five target regions.

The genotypes at six chromosome segments (five targets plus chr1) inferred from genotyping in the previous generation were used as fixed factors in a combined ANOVA with data from all five experiments. When three classes ('A', 'H' or 'K') were used, Azucena alleles at QTL9 increased root length by 96 mm (in A) and by 40 mm in H; Azucena alleles at QTL11 ($P < 0.05$) increased root length 20 mm only in H. When the analysis was carried out using two classes ('A' or 'K', with heterozygotes assumed to have donated only A to the generation used for these experiments) the significance of the effect of QTL9 increased, but the effect of QTL11 was not significant. Hence we can be confident that the root length QTL on chromosome 9 was expressed in the novel Kalinga III genetic background and it had a detectable effect (Table 5).

Drought had a significant effect on root length; the overall least squares mean for DT was 576 ± 24 mm and for WW 509 ± 18 mm. There was no interaction of drought treatment with the introgressed segment on chromosome 9.

Field experiments: other root and non-root traits

Although none of the other traits measured were significantly different between Azucena and Kalinga III it was possible to relate genetic effects of some traits to target segments. Azucena alleles at QTL9 significantly increased the root length in relation to plant height ($P < 0.05$) and increased final plant height by 0.07 m when the analysis used 'A' or 'K' classes. The unintentionally introgressed segments from Azucena on chromosome 1 ($P < 0.05$) reduced plant height by 0.07 m, as did the target QTL2. When the analysis used classes 'A', 'H' or 'K', the effect of chromosome 1 on plant height was not significant, while the effects of QTL2 and QTL9 were more pronounced with H having intermediate heights. QTL7 also had a significant effect with Azucena alleles increasing height by 0.06 m in H and 0.08 m in A. No effect of any introgressed segment was found on root number, tiller number or root dry weight.

No significant difference was found between the parents for root number. The control NIL $BC_3$23-01-06-06 had the greatest number of roots (181 ± 20.7), significantly more than seven NILs with Azucena alleles at root QTLs, and at least 40 more roots than any other line.

The line $PY_2$1 had the most tillers (12 ± 1.0) and was the only line with more tillers than the control NIL $BC_3$23-01-06-06 (10 ± 1.0), but neither were significantly different to other lines for tiller number. Both tiller number and root dry weight were significantly greater in DT than WW treatments. All of these traits differed across experiments and sampling time.

Discussion

Efficiency of MABC for pyramiding quantitative traits

Our programme used two 'pyramid' crosses between BC_3 lines to stack five targets. Five target regions is probably the limit of efficient MABC breeding (Hospital and Chacosset 1997; Servin et al. 2004), but we successfully selected an ideotype with all five regions from Azucena introgressed into the predominantly Kalinga III genetic background, with almost complete line conversion. We used both flanking markers and markers within the target regions, so it is unlikely that recombination occurred within the target regions to revert them to the recurrent parent genotype. However marker coverage on non-target chromosomes was incomplete. The pyramid crosses might have been avoided if the programme had started with pre-existing RILs, or if it had tested more lines at each back-cross generation (Ribaut et al. 2002).

We employed over 3,000 marker assays (2548 RFLP and approximately 700 SSR) to test 323 plants, although more than 1,000 were for selection of background regions throughout the genome. Hence, we used approximately 600 assays per QTL target. This compares favourably with other MABC programmes using RFLPs. Schmierer et al. (2004) tested 450 plants over three BC generations with four RFLP loci flanking two regions carrying QTLs for yield in barley and successfully introgressed them to non-mapped cultivars. The exclusive use of PCR-based markers by Lecomte et al. (2004) enabled sequential selection of QTLs in tomato: only 800 marker assays were employed and 300 plants at each generation were evaluated to detect plants carrying donor alleles at five chromosome regions. We started out MABC with RFLP markers. If we could have started with the SSR markers that became available to us later then the total number of marker assays would have been

significantly reduced. This is because SSRs permit the sequential selection or rejection of lines on a locus-by-locus basis that is not practical with RFLPs.

We evaluated the resultant NILs for expression of root length QTLs and other related QTLs, including root number. The root experiments used were different from those used for the initial detection of QTLs in the Azucena/Bala population because they were designed to be more akin to upland rice growing conditions in India. Reyna and Sneller (2001) pointed out the potential pitfalls involved with attempting to re-capture the value of QTL alleles when they are introgressed into different genetic backgrounds or tested in different environments. However, the four targets carrying rice root QTLs were chosen because they were stable across several experiments and environments and most had been observed in more than one mapping population. Our results show that one root length QTL was stable in this novel genetic background when tested in a novel testing environment. Even when genetic background and testing environment are constant many QTLs do not behave as expected, as was shown by Bouchez et al. (2002) in maize. They introgressed three segments containing QTLs for earliness and yield using RILs selected from a mapping population as a starting point for MABC. QTL mapping was carried out in the BC_3 population under the same environmental conditions as the initial mapping in RILs and, although some regions showed a constant effect, one QTL for silking date was not significant in the $BC_3$and a QTL for yield had an inverse effect.

Phenotypic effects of introgressed segments

Each one of the NILs used in the greenhouse experiment described here were genotyped, but the plants used in the root experiments were the progeny of lines that had been genotyped at an earlier stage in the programme. Some of them were segregating at some of the target loci, hence, the root experiments were less sensitive than the greenhouse experiment for detecting effects conferred by introgressed regions. The initial mapping populations used to detect Azucena root QTLs had either Bala or IR64 as the shorter rooted parents. However, Kalinga III, our shorter rooted parent, generally has longer and thicker roots than both Bala and IR64 under droughted and watered conditions (unpublished). Although Kalinga III shared the same allele as Bala at nine target RFLP loci, it might not be allelic for all root QTLs which would explain why some introgressed segments did not significantly increase root length. It might be the case that Azucena and Kalinga III actually share the same root-morphology alleles at these QTLs. We discuss the effects of each segment below.

Table 5 Multi-factor analysis of variance for root length for five experiments conducted at UAS, Bangalore on 19 NILs with the effect of Azucena alleles at six chromosome segments in a Kalinga III genetic background

Factor	DF	F	P
Chromosome 1 RM5 (A/H or K)	1	0.00	0.952
Chromosome 2 RM221-RM213 (A/H or K)	1	0.21	0.649
Chromosome 7 RM351-RM234 (A/H or K)	1	0.04	0.847
Chromosome 8 RM223 (A/H or K)	1	1.36	0.244
Chromosome 9 RM242-RM201 (A/H or K)	1	21.72	0.000***
Chromosome 11 RM229-RM206 (A/H or K)	1	2.23	0.137
Stage at analysis (< 56 days, < 76 days or maturity)	2	34.58	0.000***
Treatment (DT or WW)	1	6.22	0.013*
Experiment	4	3.61	0.007**
Error	434		
Total	447		

Chromosome 2 (RM221-RM213)

In the Bala/Azucena mapping population this region showed the maximum effect in experiments for root penetration using a layer of wax (Price et al. 2000). QTLs for maximum root length were also detected when the Bala/Azucena population was evaluated in thin chambers of soil (Price et al. 2002a) and have more recently been identified as a relatively small, but stable QTL for maximum root length in a study evaluating the environmental interaction of root-growth QTLs (unpublished data). Importantly, in these experiments the QTL has a bigger impact on root thickness and root distribution at depth than maximum root length. Thus the lack of impact of this target region on maximum root length in Kalinga III may reflect the homology of Azucena and Kalinga III alleles or the relatively small size of the QTL for root length. Nevertheless, it may have produced a greater (but unmeasured) mass of roots at depth.

For agronomic traits, other than roots, the introgression of Azucena alleles in this region was detrimental: Azucena alleles reduced plant height and tiller number and delayed flowering. Our results suggest that this target region is undesirable in ideotypes for the rainfed uplands of eastern India where tall, early plants are required. Shen et al. (2001) targeted the region immediately above our target where a QTL for maximum root length had been mapped (by Yadav et al. 1997 in Azucena/IR64); a region where we carried out background selection to retain Kalinga III (see Recurrent parent allele selection, ii, above). They did not find any effect in NILs from this QTL and explained it through repulsion between two linked QTLs.

Chromosome 7 (RG650-RM234-RG351)

This target region spans around 40 cM because the confidence interval for this root QTL is large. This region was also targeted by Shen et al. (2001), but the

region near to RM234 was lost in most of their NILs. We have maintained RM234 whilst deliberately selecting against the lower end of the chromosome at RM248 where linkage drag for Azucena alleles delays flowering time. We detected no effect on root length but some effects on plant height (both increase and decreases were detected, suggesting phenological interactions) conferred by Azucena alleles. This is considered the weakest of the four root-growth QTLs used here, and subsequent testing confirms that it is less regularly detected in the Bala/Azucena population than those on chromosomes 2 and 9. The QTL for root length and mass on chromosome 7 did not have a pleiotropic effect on later maturity in the Bala/Azucena population, but it might have a pleiotropic effect in the Kalinga III background. If the QTL is actually located near RG351 and RM248 it would have been lost in most of the NILs.

Chromosome 9 (G385-RM242-RM201)

In IR64/Azucena DH lines, a root length QTL on chromosome 9 has been detected repeatedly in the same type of root cylinder experiments. Under low-moisture stress the segment between RZ206-RZ422 (close to G385) conferred 15% of the variance for root length (Hemamalini et al. 2000), whilst the locus RM201 was linked to maximum root length in single marker analysis (unpublished). When this region (RM242-RM201) was transferred into NILs with an IR64 genetic background it increased root length (Shen et al. 2001) although Venuprasad et al. (2002) found no evidence that it also influenced grain yield.

In our NILs with a Kalinga III genetic background, Azucena alleles at this region increased root length, plant height and root length in relation to plant height. These effects, however, were not expressed before day 55. This target QTL is beneficial for the improvement of Kalinga III for upland environments. This region increased deep root thickness in the Bala/Azucena population, however, we have not investigated the expression of this trait in the NILs.

Chromosome 11 (RM229-RM206)

This region was shown to have the greatest effect (30%) on root length in an experiment where the Bala/Azucena F_2 population was evaluated in hydroponics (Price et al. 1997). It has been linked to improved yield under drought (Zhang et al. 1999). We did not demonstrate any significant benefit due to introgression of this region from Azucena into Kalinga III in NILs, and there was some evidence that it decreased panicle length.

Chromosome 8 (RM223)

The effect of this region was only tested for the traits evaluated in the root experiments and it did not confer any measurable effects. We have not carried out any formal analysis for aroma in the NILs, but it must be noted that many of them that were Azucena homozygotes for RM223 did have noticeably fragrant flowers and grain.

Non-target regions and traits

Some of the NILs had Azucena alleles at RM5 (chromosome 1), but this did not influence any of the root traits measured, although it slightly reduced plant height. It did not influence tiller number, suggesting that the non-target introgressed region on chromosome 1 does not span the region containing QTLs for tiller number identified in Azucena/IR64 (Yan et al. 1998) or Bala/Azucena (Price et al., 2000).

Yan et al. (1998) observed 15–20 genomic regions across eight chromosomes which influenced tiller number at various growth stages. Two of them were in our target regions; QTL tn 7 where Azucena decreases tillering 50 days after transplanting (we observed decreased tiller number at RM248 on day 28) and QTL tn 11-1 where Azucena increases tillering between 20 and 30 days after transplanting. The control NIL (with Kalinga III alleles at all five target regions) had the most tillers and significantly more than Kalinga III. This NIL was predicted to contain approximately 15% Azucena alleles at non-target regions, so Azucena alleles at other regions (possibly the QTL tn 6 of Yan et al. 1998) may contribute positively to tillering in this line. Shen et al. (2001) reported that most IR64/Azucena NILs introgressed with Azucena alleles at root QTLs had no increase in tiller number.

Implications for breeding

This programme led to the production of NILs with specific introgressed segments containing root QTLs and it is unlikely that they could have been obtained without the use of MAS. It involved a huge effort: the initial mapping, the back-crossing to Kalinga III, over 3000 marker assays and the final testing to validate the effects. Selectable PCR-based markers became available during the programme, but if they had been available from the start many fewer assays would have been necessary. A relatively small number of lines were assayed compared with other MABC programmes, and the disadvantage from this was that two non-target regions were introgressed in the final ideotype line. Some of the introgressed regions were large, giving an increased chance of linkage drag for unwanted traits. The glasshouse experiment was designed to check for linkage drag or pleiotropy for several non-root traits, and it successfully identified an Azucena allele that delayed flowering.

Differences between the genetic backgrounds of Kalinga III and Bala could explain why the improvement in root length gained from pyramiding these root

QTLs into Kalinga III was so little. A study to compare the rooting ability of lines containing the same pyramided introgressions from Azucena in both the Kalinga III background and the Bala background would be useful to clarify the effectiveness of pyramiding these QTLs. However, from an applied breeding perspective there is little to be gained from the improvement of Bala (released in 1970) which is no longer cultivated in India, whereas Kalinga III (released in 1983) is still being widely cultivated across India (Witcombe et al, 1999).

The root QTL targets had relatively low heritability and only one of them significantly improved root length in one testing environment in the novel genetic background. This and other target regions affected traits such as plant height, maturity and panicle length and some had a negative effect on other traits that are beneficial in upland rice. These effects could be due to either pleiotropy or linkage drag. The effect on root traits of multiple introgression of QTLs was disappointing, although the programme did achieve its aim of producing lines suited to the uplands of eastern India that out-performed Kalinga III under drought. Lines carrying approximately 15% Azucena alleles (at QTLs or otherwise) have better phenotypes than Kalinga III for upland environments and we are currently testing many of these NILs in field trials in eastern India. Our results demonstrate that backcross breeding using a wide cross as a tool for varietal improvement can be successful, even without marker-assisted selection. This agrees with our results from another wide cross where strong selection pressure in one environment biased the genome of adapted progeny towards that of the parent adapted to the same environment (Steele et al. 2004).

Acknowledgements The authors thank Brigitte Courtois and Modesto Amante for making the first cross and backcross at IRRI, Philippines. We thank Beverley Moore, Gwen Edwards, Elly Rodriguez and Julian Bridges for technical support on molecular and greenhouse experiments in Bangor, and James M. Gibbons for statistical advice. This document is an output from a project (Plant Sciences Research Programme R7434 & R8200) funded by the UK Department for International Development (DFID) and administered by CAZS Natural Resources for the benefit of developing countries. The views expressed are not necessarily those of DFID.

References

Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favourable alleles at quantitative trait loci between maize elite line. Genetics 162:1945–1959

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

Courtois B, Chaitep W, Moolsri S, Sinha PK, Trebuil G, Yadav R (1996) Drought resistance and germplasm improvement. Ongoing research in the Upland rice research consortium. In: Piggin C, Courtois B, Schmit V (eds) Upland rice research in Partnership IRRI, Los Baños, IRRI Discussion paper Series 16:154–175

Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimens at peak vegetative stage in rice (*Oryza sativa* L). Euphytica 112:69–78

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469–1485

Kamoshita A, Zhang J, Siopongco J, Sarkarung S, Nguyen HT, Wade L (2002) Effects of phenotyping environment on identification of Quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci 42:255–265

Lilley JM, Ludlow MM (1996) Expression of osmotic adjustment and dehydration tolerance in 61 diverse rice lines. Field Crops Res 48:185–197

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. J Exp Bot 47:1427–1436

Lorieux M, Petrov M, Huang N,Guiderdoni E, Ghesquiere A (1996) Aroma in rice: Genetic analysis of a quantitative trait. Theor Appl Genet 93:1145–1151

Price AH, Tomos AD (1997) Genetic dissection of root growth in rice. II Mapping quantitative trait loci using molecular markers. Theor Appl Genet 95:143–152

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

Price AH, Steele KA, Moore BJ, Jones RGW (2002a) Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: II Mapping QTL for root morphology and distribution. Field Crops Res 76:25–43

Price AH, Cairns JE, Horton P, Jones HG, Griffiths H (2002b) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53:989–1004

Reyna N, Sneller CH (2001) Evaluation of marker-assisted introgression of yield QTL alleles into adapted soybean. Crop Sci 41:1317–1321

Ribaut J-M, Jiang C, Hoisington D (2002) Simulation experiments on efficiencies of gene introgression by backcrossing. Crop Sci 42:557–565

Schmierer DA, Kandemir N, Kudrna DA, Jones BL, Ullrich SE, Kleinhofs A (2004) Molecular marker-assisted selection for enhanced yield in malting barley. Mol Breed 14:463–473

Servin B, Martin OC, Mezard M, Hospital F (2004) Toward a theory of marker-assisted gene pyramiding. Genetics 168:513–523

Shen L, Courtois B, McNally KL, Robin S, Li Z (2001) Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theor Appl Genet 103:427–437

Steele KA, Edwards G, Zhu J, Witcombe JR (2004) Marker-evaluated selection in rice: shifts in allele frequency among bulks selected in contrasting agricultural environments identify genomic regions of importance to rice adaptation and breeding. Theor Appl Genet 109:1247–1260

Venuprasad R, Shashidhar HE, Hittalmani S, Hemamalini GS (2002) Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (*Oryza sativa* L) under contrasting moisture regimes. Euphytica 128:293–300

Witcombe JR, Petre R, Jones S, Joshi A (1999) Farmer Participatory crop improvement IV The spread and impact of a rice variety identified by participatory varietal selection. Expl Agric 35:471–487

Yadav R, Courtois B, Huang N, McLaren G (1997) Mapping genes controlling root morphology and root distribution on a double-haploid population of rice. Theor Appl Genet 94:619–632

Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998) Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L). Theor Appl Genet 97:267–274

Yoshida S, Hasegawa S (1982) The rice root system: its development and function. In: Drought resistance in crops with emphasis on rice, International Rice Research Institute, Los Banos, Laguna, pp 97–114

Zhang J, Chandra Babu R, Pantuwan G, Kamoshita A, Blum A, Wade L, Sarkarung S, O'Toole JC, Nguyen HT (1999) Molecular dissection of drought tolerance in rice: from physiomorphological traits to field performance. In: Ito O, O'Toole J, Hardy B (eds) Genetic improvement of rice for water-limited environments. Proceedings of the workshop on genetic improvement of rice for water-limited environments, 1–3 December 1998, Los Banos,. Los Banos Philippines:International Rice Research Institute, pp 307–317