FINAL TECHNICAL REPORT

R7435

Analysis of environmental effects on expression of root penetration QTLs in upland rice, and development of PCR markers for QTL selection in drought resistance breeding

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1. Executive Summary

This project drew the previous work on mapping rice root traits and field-drought-reaction QTLs to a conclusion. The project addressed the specific issue of the effect of the environment on QTL expression in the field. Prior to this project most root studies in this population had been carried out in artificial, non-field conditions. In particular this project tackled the influence of the soil in field situations. The field site at WARDA where previous QTL mapping for drought reaction traits had been carried out was one of the sites compared for soil physical properties. Other fields at WARDA and IRRI were analysed and root trait analysis was done in fields with contrasting soil penetration resistance. Roots studies were for the parents (Azucena and Bala), the mapping population (recombinant inbred lines, RILs) and for near-isogenic lines (NILs) derived from marker-assisted selection to introgress Azucena root QTLs into both Kalinga III and Bala.

The results were complex, underlying the complex nature of QTLs for roots and drought reaction. There was some agreement for QTLs detected in the field and previously detected QTLs. A QTL on chromosome 2 for root density in the field matched root penetration QTLs detected using a wax layer in pots. Detection of QTLs in the field was found to be more difficult than in artificial conditions because of the very large environmental effect. This was exemplified in the lack of significance in detection of improved root distribution in NILs containing Azucena root growth QTLs. The QTL x environment interaction was investigated and is discussed in the resulting publications, which also include the QTL maps for the traits studied. The molecular map and trait data is freely available for researchers on the internet and this information can now be used by rice and other cereals breeders wishing to study or select root and drought related traits.

The molecular linkage map was improved by the addition of 19 microsatellite (SSR) markers. These linked split linkage groups completing 9 of the 12 chromosomes to the target of mapped markers at every 20 cM or more. Only one chromosome (chromosome 12) is now left as two separate linkage groups, and chromosome 8 still lacks markers at the top end. The additional markers enabled more precise QTL analysis of existing and new trait data to be completed with this map. Some of these SSR markers are now being used in marker-assisted selection in rice breeding projects.

2. Background

Although considerable progress has been made by a number of different groups in identifying regions of the rice genome of potential value in marker-assisted improvement of varieties for drought resistance, much more work is still required on the environmental stability of these QTLs (QTL x environment interaction) if their true potential is to be identified and exploited. This project aimed to address this requirement by analysing how QTLs for root growth traits and reaction to drought are influenced by the soil environment.

Rice breeders in Africa and Asia require reliable methods of introducing drought resistance into high yielding varieties for rainfed environments. Upland rice breeders need to confirm the reliability of QTLs across environments so that marker-assisted selection of can be targeted at the most effective QTLs.

In previous DFID-PSRP projects, R4631 and R6673, a rice mapping population based on recombinant inbred lines derived from a cross of varieties Bala and Azucena was developed and used to identify quantitative trait loci (QTLs) for traits related to drought resistance. This project aimed to explore the effects of the environment on the expression of root growth QTLs in this population and in lines derived from marker assisted selection for Azucena-derived root QTLs. In project R6673 and R7434 four root QTLs were targeted in a breeding programme to improve the rooting ability of the upland variety Kalinga III.

Root penetration QTLs were identified previously in laboratory screens and they appeared to be associated with root thickness. Confirmation that these QTLs do indeed contribute to penetration through hard soils in the field was needed. Soil properties vary between different fields and even within the same field, so attempts were made to characterise these differences and test the population in fields with contrasting soil properties. Other traits had been measured previously and compilation of the full data-set was necessary, particularly with a more complete linkage map.

The molecular linkage map constructed in previous projects was not saturated to the required level of one marker every 20 cM, so addition of markers to the map was needed. The PCR-based marker system of microsatellites/simple sequence repeats (SSRs) was chosen because these markers are easier and cheaper to use than RFLPs and they can be readily selected in MAS.

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

Techniques for improving drought resistance in upland rice developed and improved.

The aims were:

- To identify relatively environmentally stable target QTLs
- To identify associated PCR-based markers available for use in MAS
- To disseminate data and QTL maps for use by other researchers.

4. Research Activities

Genetic map of Bala x Azucena improved with PCR-based markers.

Activity 1.1

Information on microsatellites (SSRs) mapped in many different rice populations is publically available. Primer sequences are available on the Gramene web page (formerly Ricegenes: http://www.gramene.org/microsat/) and on the Monsanto web page (http://www.rice-research.org/rice_ssr.html). SSR primers located in regions of low-marker-density on the Azucena/Bala map or at MAS-target regions were tested for polymorphism between Bala and Azucena.

Activity 1.2

Polymorphic microsatellites (SSRs) were screened on at least 150 RILs from the Azucena/Bala mapping population.

Markers were analysed using either high resolution agarose with ethidium bromide detection or capillary electrophoresis with dye-labelled primers and Beckman Coulter fragment analysis CEQ 2000 software.

PCR-based markers transferred to other varieties targeted for marker-assisted selection.

Activity 2.

SSR primers located at five MAS-target regions were tested for polymorphism between Kalinga III and Azucena.

Impact of hard soils in the field on root growth and drought resistance QTLs quantified and compared to previous lab screens for root penetration.

Activity 3.1

Ten upland field sites at WARDA (Côte d'Ivoire) and eight upland field sites IRRI (Philippines) were surveyed for soil physical and chemical properties.

Activity 3.2

Detailed soil surveys of the three selected contrasting sites at both WARDA and IRRI were conducted.

Activity 3.3

The effect of soil impedance on root growth of Bala and Azucena in the field was evaluated at WARDA in dry season 2000.

Activity 3.4

114 RILs were screened for drought performance in at least two sites, contrasting in their soil physical properties in dry season 2001.

Activity 3.5

The rooting profiles of 114 RILs were analysed in the field by digging trenches and counting the number of roots at a depth of 30 cm.

Activity 3.6

QTLs were mapped for drought performance and root distribution traits. These were compared with previously identified QTLs.

Activity 3.7

Root growth and drought performance was evaluated for the products of marker assisted selection. These were seven lines derived from Azucena/ Kalinga III marker-assisted backcross breeding and five pairs of NILs with complementary introgressed QTLs from each of the parents Bala and Azucena. These were screened at IRRI in the dry season of 2002.

QTL maps for root growth and drought reaction produced including quantification of QTL x environment interaction

Activity 4.1

All existing and new trait data for the RIL mapping population was checked. Where necessary it was corrected for spatial variation and normalised. It was compiled into spreadsheets for analysis with mapping software.

Activity 4.2

QTLcartographer was used for composite interval mapping.

Activity 4.3

Software for QTL x Environment interaction was tested to investigate previously identified and newly mapped target QTLs across different environments.

5. Outputs (Results)

SSR marker analysis (activities 1 and 2)

More than two hundred potentially useful microsatellite (SSR) markers were screened for polymorphism between Azucena and Bala; 168 were from Ricegenes (Gramene) and 33 from Monsanto. Of the Ricegenes (designated RM) markers, 125 were tested for polymorphism between Kalinga III and Azucena. Selectable SSR markers were identified at all 5 target QTLs for MAS.

For mapping 24 SSR primers sets were used to screen RILs and 20 new loci were added to the map. RM20 amplified two independent bands and both were mapped. It was not possible to link all of these markers to the map, in some cases (e.g. RM337) they were not linked to the existing linkage group and other markers in the gap are required to join them, however the mapping data for these markers can be added to the spreadsheet. The markers are listed in Appendix Table 1 with comments about their map location, or reasons why they were not mapped.

The map now has 10 out of 12 linkage groups completed, although the gap on chromosome 6 is still 41 cM wide. Two chromosomes (8 and 12) are still split with significant gaps between linkage groups or unlinked markers.

Variation in soil properties of WARDA field station (activity 3.1 and 3.2)

Ten sites throughout the WARDA field station at M'be, Côte d'Ivoire, were surveyed with a penetrometer (20 readings) and auger (3 soil samplings) which identified 2 types of soil, a sandy hydromophic soil and a gravely, clay upland soil. Two fields of the former type and 4 of the latter were surveyed using the penetrometer in more detail. Ultimately, 3 were chosen for future root-growth experiments (see figure 1). There are differences in the increase in PR (penetration resistance) with depth, with the hydromorphic site showing lower PR at depth. Importantly, the two upland sites both have high PR that will probably be very inhibitory to root growth, and these two sites differ slightly, with highest PR on the site previously used for field evaluation of the Bala x Azucena mapping population in project R6673 (Price et al. 2002b).

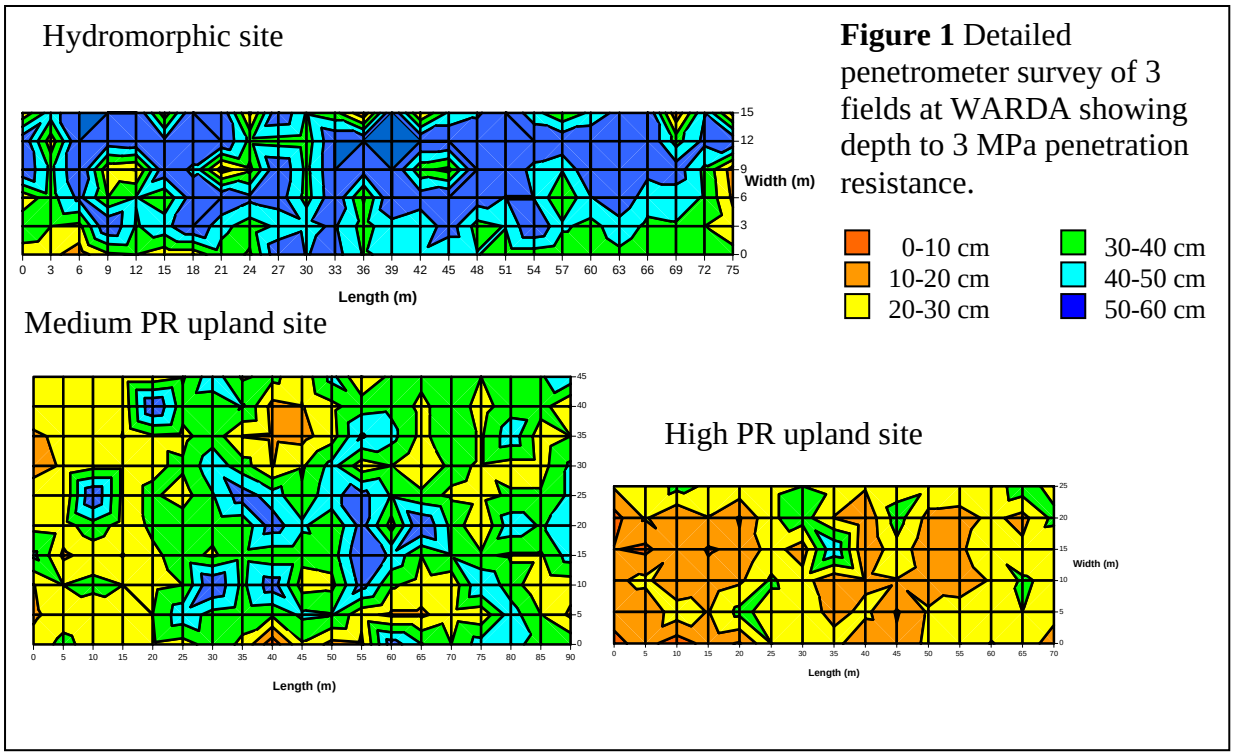

Figure 1 Detailed penetrometer survey of 3 fields at WARDA showing depth to 3 MPa penetration resistance.

Root growth of Bala and Azucena in fields of contrasting soil physical properties (activity 3.3)

The medium and a high penetration resistance site described above were used to grow Azucena and Bala plants under irrigated and drought conditions (4 weeks of vegetative stage drought starting at 35 days after sowing (DAS)) in the dry season. The number of nodal root axes per unit area passing through horizontal transects (root density) was counted at 35, 56, 77 and 98 DAS at 10 cm depth intervals. Results are presented in Appendix Table 2.

Azucena maintained a higher root density at depth. The irrigation tended to increase rooting at depth, but both soils behaved similarly. There was no indication of interaction between rice variety and either site or treatment, indicating that Azucena was consistently better than Bala. The very high PR of the both soils at depth, and in the higher soil layers as the soil dries suggests that the ability to overcome soil PR

will be an important factor determining drought resistance in these soils. The full results have been submitted to the journal Plant and Soil for publication.

QTL mapping root distribution and drought avoidance in fields of contrasting soil physical properties (activity 3.4, 3.5 and 3.6)

Either 2 or 3 replicate plots of 114 lines of the Bala x Azucena mapping population was grown under droughted conditions in two of the sites of contrasting soil physical properties in WARDA in the dry season of 2001 (the hydromorphic and medium penetration resistance upland soil described above). Leaf rolling and leaf drying were recorded at regular intervals during a 4-week drought period (33-65 DAS) as indicators of drought avoidance. After 70 days, root the number of nodal roots passing a horizontal plane at 30 cm depth was measured.

Azucena had more roots at 30 cm depth than Bala under both irrigated and droughted conditions in the medium PR upland site but not in the hydromorphic site (figure 2).

Preliminary QTL mapping has revealed 2 QTLs for root density on chromosome 2 and a putative QTL (LOD score >2<3.2) on chromosome 7 in the upland field, and one putative QTL on chromosome 3 in the hydromorphic field (figure 3). The QTLs on chromosome 2 match root penetration QTLs previously detected using a wax petrolatum layer (Price et al. 2000). Leaf rolling and leaf drying QTLs were also detected on chromosomes 1, 2, 3, 5, 7 and 8 (figure 3). Mostly, these agree with QTLs for leaf rolling and drying detected using this population at IRRI and WARDA (Price et al. 2002). The QTL on chromosome 2 associated with leaf rolling in the hydromorphic field might represent the same gene as the QTL associated with root density in the other field. It is concluded, however, that it is not easy to detect root growth QTLs in the field (because of large environmental variation) and there is little evidence generated here that the root growth QTLs that are detected do act to improve drought avoidance. It must be stressed that the data require more thorough analysis yet, which will be conducted outside the funding of the project in preparation for publication. It must also be emphasised that lack of proof that root QTLs contribute to drought avoidance does not mean they do not- it probably reflects the great difficulty in detecting relatively small effect QTLs in heterogeneous environments and the influence that shoot-related mechanisms of drought avoidance contribute to leaf rolling and drying QTLs in this population (see Price et al. 2002a for a fuller discussion).

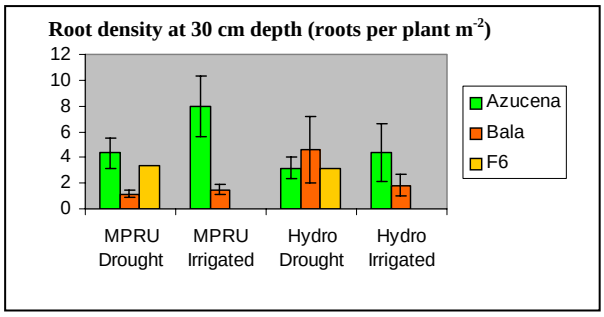

Figure 2. Mean root density of parents and mapping population after 70 days growth under irrigated and droughted treatments in two fields of contrasting soil type, medium penetration resistance upland field (MPRU) and a hydromorphic field (bar = standard error).

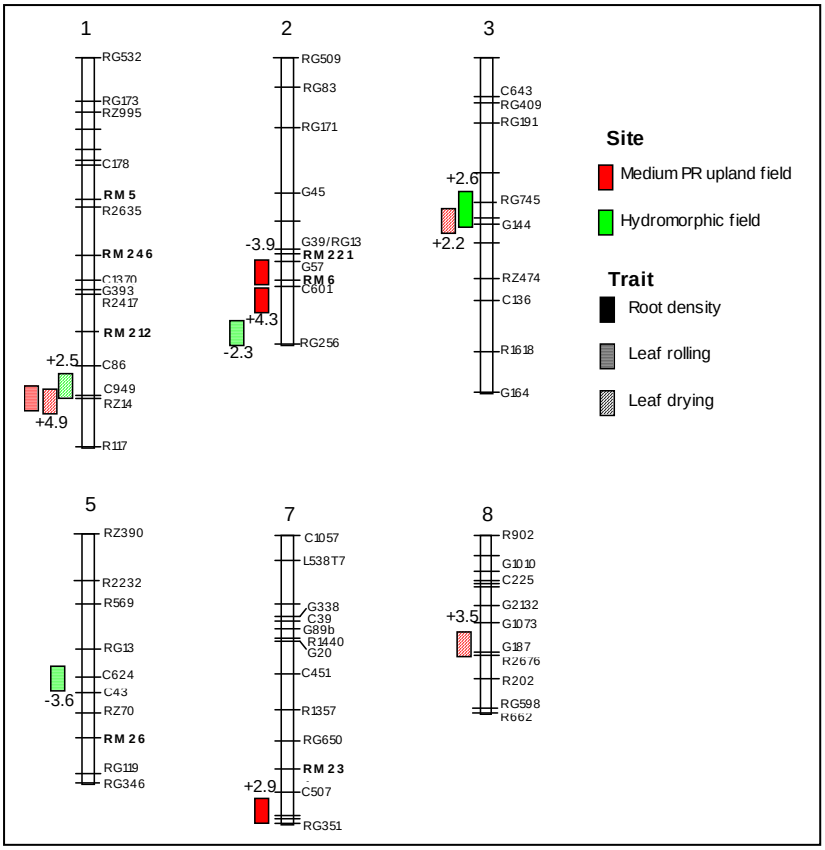

Figure 3. Linkage map with RFLP and microsatellite markers indicated showing quantitative trait loci (QTLs) for root density, leaf rolling and leaf drying in each of two fields. The boxes to the left of the chromosomes represent the left and right -1 LOD interval and the number above or below the box indicates the LOD score for the QTL. A positive LOD value indicates that Azucena alleles increase the trait value, a negative LOD indicates that Azucena alleles reduced the trait value.

Variation in soil properties of IRRI: A detailed survey

Due to deterioration in the security situation in the Cote d'Ivoire, it was decided to switch the final year's experimentation to IRRI, with the formal agreement and collaboration of Drs Gary Atlin and Renee Laffite. This required field surveys for soil physical properties. A total of 8 fields were surveyed for PR and soil properties (using a soil pit). Three fields were selected for future work (see Figure 4). These all differed from those at WARDA, having lower mean PRs and greater depth to PRs considered likely to halt root growth.

Evaluation of root distribution of Near Isogenic Lines in fields of contrasting soil physical properties (activity 3.7)

Based on previous work, 4 QTLs from Azucena were identified as having a positive effect on root morphology (root thickness, maximum root length, penetration ability), on chromosomes 2, 7, 9 and 11. A total of 17 near isogenic lines were tested for root growth in IRRI in the 2002 dry season in the three fields described above. In the each field, 8 replicate subplots were used for each genotype and irrigation was applied throughout the growing season, but in the UR2 field only there was an additional drought treatement plot in which water was withheld for 4 weeks from 35 days after sowing. Root density at 30 and 35 cm depth was measured at 70 days after sowing. Seven near isogeninc lines were the intermediate products of the marker-assisted improvement of root traits in Kalinga III being produced in project R7434 achieved by introgressing Azucena root growth QTLs from chromosomes 2, 7, 9 and 11, in

addition to an aroma gene from chromosome 8. These were lines shown in table 2. A further 10 NILs were pairs produced from residual heterozygousity within the Bala x Azucena mapping population in a BBSRC funded project. There was one pair for each of the QTLs on chromosomes 2, 7 and 9 and two pairs for the QTL on chromosome 11.

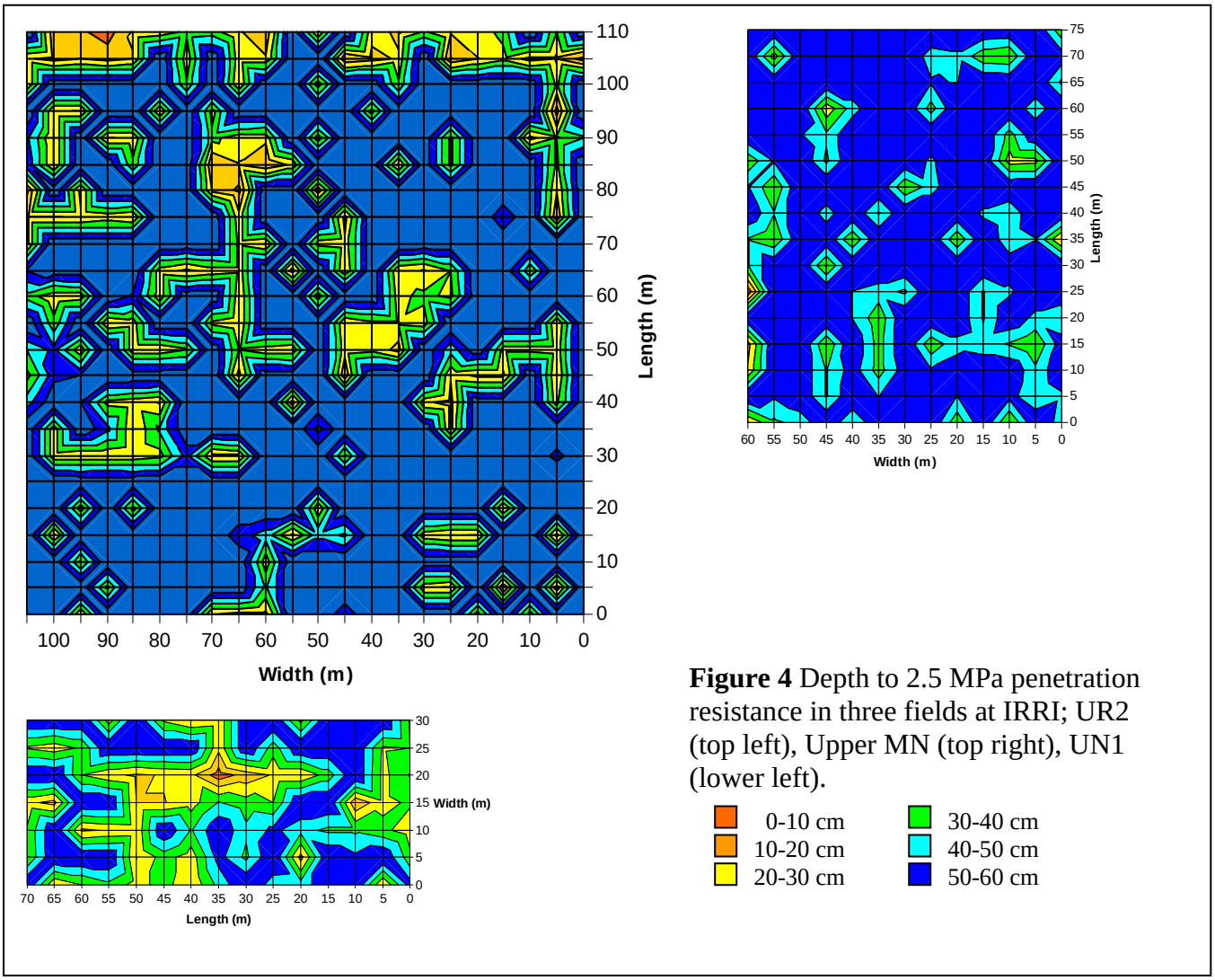

Figure 4 Depth to 2.5 MPa penetration resistance in three fields at IRRI; UR2 (top left), Upper MN (top right), UN1 (lower left).

Table 2. Kalinga III backcross near isogenic lines used for root screens at IRRI.

Line	Code	Homozygous QTLs from Azucena
21-01-03-01	1	Chromosome 9
21-01-03-06	2	Chromosomes 2, 11 and aroma (8)
21-01-03-08	3	Chromosomes 9 and 11
42-01-05-12	4	Chromosomes 7 and 9
21-01-03-06-02	5	Chromosomes 2, 9, 11 and aroma (8)
21-01-03-06-44	6	Chromosomes 2, 9, 11 and aroma (8)
21-01-03-06-46	7	Chromosomes 2, 9, 11 and aroma (8)

No significant differences (based on ANOVA) between Kalinga III and the NILs (Figure 5) were obtained although in some cases there was evidence of improved root distribution at depth based on individual t-tests (e.g. NILs 2 in UR irrigated plots).

With the Bala x Azucena NIL pairs, there was also no significant difference between pairs (results presented in Figure 5) although paired t-test revealed a P value of P = 0.089, indicating significance at the 10% threshold, the Azucena alleles having more roots at 30 cm than the Bala alleles. Only one pair showed strongly significant differences (at the 1% level) in any screen, where Azucena allele of the chromosome 9 QTL pair had more roots than the Bala allele in the UR irrigated plot based on t-test. It is worth noting here that in only one plot did the Azucena and Bala parents differ in root density, a surprising result given the consistency of detectable differences in the WARDA screens. Kalinga III was not noticeably worse than Azucena. The similarity between the parental lines at IRRI indicates a lack of ability to differentiate between genotypes in these sites for which we have no satisfactory explanation at the moment.

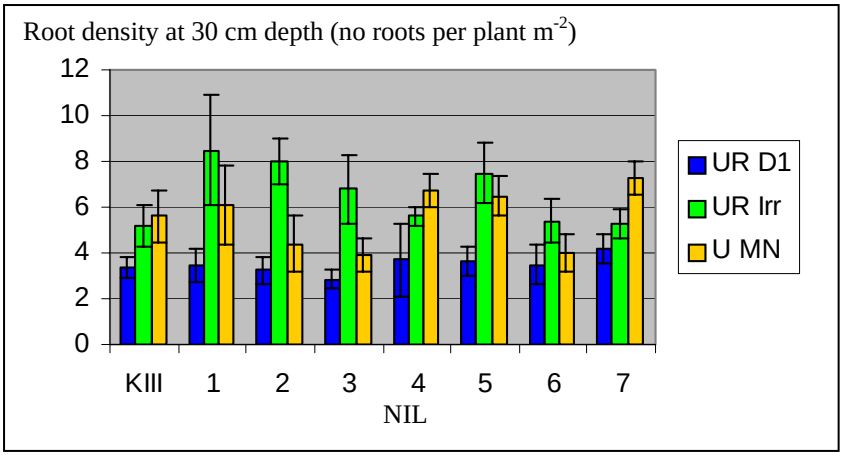

Figure 5 Root density of Kalinga III and 7 NILs derived from it in three sites at IRRI. UR D1 = UR droughted, UR Irr = UR irrigated, U MN = upper MN field. Bar = standard error.

Conclusions from field soil profie analysis and root experiments

1/ Soils in WARDA are hard and could be expected to limit or severely limit root growth. Soils at IRRI are less hard and probably therefore less impeding.

2/ Azucena and Bala differ in root distribution in the field, with Azucena having more roots at depth.

3/ Very large variation in measurements of field root distribution limit the ability to detect relatively small influences on root distribution; hence evidence of site by genotype or treatment by genotype interaction is not detected, and genotype effects themselves are hard to pick up.

4/ QTLs for root distribution can, none-the-less, be detected in the field, and these broadly agree with laboratory/greenhouse screens of root distribution and penetration ability.

5/ General lack of significance in the detection of improved root distribution of near isogenic lines containing one or several Azucena root growth QTLs appears to reflect large variation in measurements (hence large standard errors).

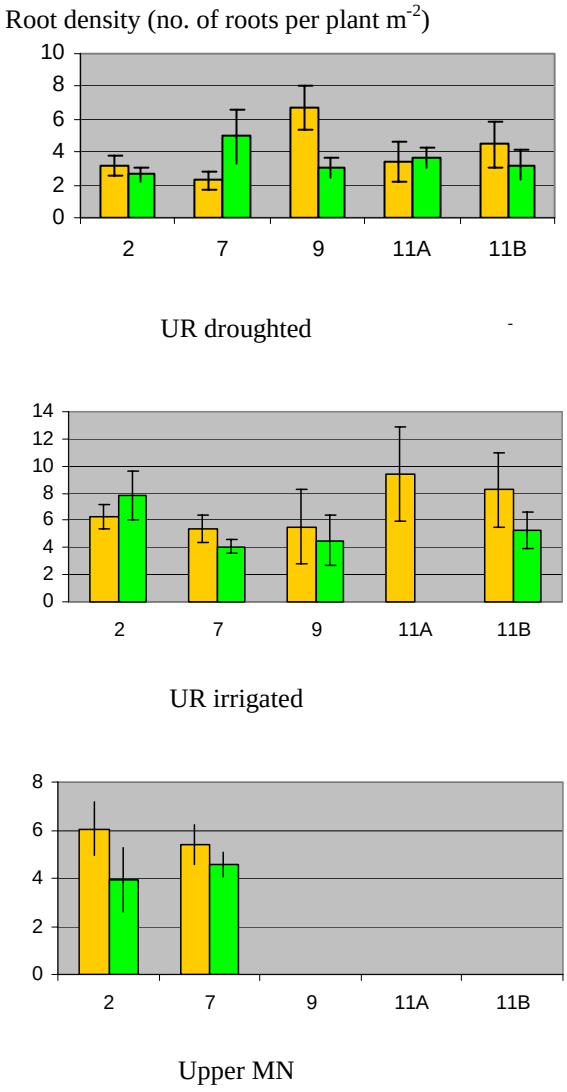

Figure 6 Root density at 30 cm depth for each of five NIL pairs each differing only at one QTL on chromosomes 2, 7, 9 and 11. The NIL with the positive Azucena allele is in yellow, the Bala in green. Three field plots are presented, UR droughted (above), UR irrigated (middle) and Upper MN (below). Bar = standard error

QTL maps for root growth and drought reaction produced (activity 4.1 and 4.2)

The preliminary analysis of QTLs from fields with contrasting soil penetration ratios has been described above.

Existing data obtained from this population has been compiled and the following QTL maps have been published:

- Carbon isotope discrimination (delta ^{13}C) and Specific leaf area at IRRI, 1996 (Price et al. 2002a).
- Root traits in soil chambers in glasshouse experiments at Bangor in 1997 and 1998 (Price et al. 2002d).
- Shoot and drought-related traits studied at WARDA and IRRI (Price et al. 2002b).

See publications for detailed discussion on each trait.

The website: **http://www.abdn.ac.uk/~soi329/** makes data gathered on QTL mapping drought resistance and root growth publicly available as Microsoft Excel and text files.

- MapMaker Raw, Map and Pre files created in July 2002.
- Mapmaker output file created in February 2002.
- Excel graphics of the map with all marker positions indicated with RFLP and microsatellites indicated (created September 2001).
- Root growth screening as described in Price et al.(2002d).
- Drought avoidance screening as described in Price et al. (2002b).

Quantification of QTL x environment interaction (activity 4.3)

Data collected for the Azucena/Bala mapping population from different environments has identified numerous QTLs for roots and drought related traits. The effects of different environments and the interaction of QTLs with the environment has been discussed in the published papers for the drought screens at IRRI and WARDA (Price et al., 2002b) and for roots in soil chambers (Price et al., 2002d).

Attempts were made to evaluate the data for QTL x environment interaction using the software packages PLABQTL and QTLMapper. These were not adequate for the analyses required because they will not detect QTLs that are specific to one environment. Both packages take trait data from multiple environments, look for QTLs based on the average of all environments, and then ask whether the QTLs detected in the average, vary in the individual environments.

Other more valid methods for evaluation of QTL x E are being investigated by Adam Price through informal collaboration beyond this project.

6. Contribution of Outputs

Output 1.

The improved map can now be used for fine-mapping and MAS of drought-related QTLs.

The addition of SSR markers to this map aids comparative mapping.

Output 2.

PCR-based SSR markers at the target regions are more readily applied to MAS than the RAPD markers. They can be used by rice breeders with access to laboratory facilities.

SSR markers screened for polymorphism in this study are currently being used for marker-evaluated selection (MES) in projects R7434 and R8200.

Output 3.

QTLs for root distribution in the field have been identified which show agreement with laboratory root screens.

Results have shown that root growth is probably severely influenced by soil physical properties, and ability to overcome soil penetration resistance is likely to important for drought resistance in fields with soils like those encountered in the upland sites of WARDA.

Detection of root QTLs in the field is confounded by the effects of the environment and the variation in soil physical properties across the field. These problems are likely to be encountered in MAS breeding for root QTLs, where evaluation of introgressed lines is carried out in field conditions.

Output 4.

QTL maps showing genomic regions with the most influence on root growth under different environments have been produced and published. This has supported the choice of 4 target regions for use in the marker-assisted backcrossing programme followed in project R7434.

Data is to be freely available on the internet for access by other researchers.

7. Dissemination

Project web page: **http://www.abdn.ac.uk/~soi329/**

Refereed publications (some are from work done in R6673)

A. H. Price, J.E. Cairns, P. Horton, H. G. Jones and H. Griffiths (2002a) Linking drought resistance mechanisms to drought avoidance in upland rice using a QTL approach; progress and new opportunities to integrate stomatal and mesophyll responses. Journal of Experimental Botany, 53, 989-1004.

A.H. Price, J. Townend, M.P. Jones, A. Audebert and B. Courtois (2002b) Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. Plant Molecular Biology 48, 683-695.

A.H. Price, K.A. Steele, J. Gorham, J. Bridges, B.J. Moore, J.L. Evans, P. Richardson and R.G.W. Jones. (2002c) Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: I. Root distribution, water use and plant water status. Field Crops Research. 76, 11-24.

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

Other dissemination

A Price, J Cairns, P Horton, H Jones and H Griffiths (2001) Genetic basis to drought tolerance in upland rice: Integration of QTL with stomatal and mesophyll responses . Paper presented at the Society for Experimental Botany, Canterbury, 2-6th April 2001.

Adam Price, Jill Cairns, Farhkanda Khowaja, Alison McIntosh, Keith MacMillan, Alain Audebert, Chris Mullins (2002). Towards understanding root growth QTLs in rice: Expression in the field, environmental stability, contribution to drought avoidance and underlying genetic mechanisms. Paper presented at an international workshop on Progress Toward Developing Resilient Crops for Drought-Prone Areas, 27-30 May 2002, IRRI, Los Baños, Laguna, Philippines.

K.A. Steele, G.Edwards, H.E. Shashidhar, K. Macmillan and A.H. Price (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.

J E Cairns, A. Audebert, A. Price and C. E. Mullins. Effect of soil mechanical impedance on root growth of two rice varieties under drought. Manuscript submitted to Plant and Soil.

Acknowledgements

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Technical and laboratory support and was provided by Farhkanda Khowaja, Alison McIntosh, Keith MacMillan (Aberdeen), Gwen Edwards (CAZS, from August 2000) & Beverly Moore (CAZS, until January 2000).

Appendix Table 1. SSR markers used to screen Azucena/Bala mapping population and details of linkage analysis using MAPMAKER /EXP 3.0b. Detection was either by super fine resolution agarose (SFR) or capillary gel electrophoresis using CEQ 2000 software (CEQ).

Chromosome	Marker	Method	Group	Result
1	RM246	SFR	CAZS	Maps 14.9 cM above R2635
1	RM212	SFR	Aberdeen	Maps 23.6 cM above C86
1	RM5	CEQ	CAZS	Maps 2.4 cM above R2635
2	RM213	CEQ	CAZS	Data difficult to score – can't map
2	RM221	CEQ	CAZS	Maps 3.2 cM below RG139/G39
2	RM6	CEQ	CAZS	Maps 5.0 cM above C601
4	RM348	SFR	CAZS	Maps 13.2 cM above RG620
4	RM349	SFR	CAZS	Maps 5.3 cM below C1016
4	RM252 (=RM226)	SFR	CAZS	Maps 10.7 cM above RG163
5	RM26	CEQ	CAZS	Maps 14.8 cM below RZ70
6	MRG1035	SFR	Aberdeen	Maps 2 cM from RG213
6	MRG6488	SFR	Aberdeen	No linkage.
7	L538T7	SFR	Aberdeen	Maps 18 cM below C1057, 28 cM above e12m39.11
7	RM351	CEQ	CAZS	Maps 2.2 cM below RG650
7	RM248	CEQ	CAZS	Maps 0.4 cM above RG351
7	RM234	SFR	CAZS	Maps 14.5 cM above C507
8	RM337	SFR	CAZS	No linkage.
9	RM242	SFR and CEQ	CAZS	Maps 5.4 cm below AFLP e12m39.1
9	RM201	CEQ	CAZS	No Bala allele, so can't map. Linked to RM242 and G1085.
11	RM229	CEQ	CAZS	Maps at RG2
11	RM206	CEQ	CAZS	Maps 2.8 cM above C189
11	RM20B	SFR	CAZS	Maps near R642
12	RM101	SFR	CAZS	Data difficult to score – can't map
12	RM247	SFR	CAZS	Maps 4.9 cM above G124.
12	RM20A	SFR	CAZS	Maps 4.9 cM above G24

Appendix Table 2: Density of axial roots (number of roots m^{-2}) in a horizontal plane at 10 cm depth intervals on the high PR and medium PR sites. Blank spaces in the table represent depths that were not measured. Error bars represent ± 1 s.e ., where there is no error estimated data is from a single subplot. Number of plants per m^2 was 32.

		Irrigated				Droughted			
Variety	Depth (cm)	35 DAS	56 DAS	77 DAS	98 DAS	35 DAS	56 DAS	77 DAS	98 DAS
					High Penetration Resistance Site				
Azucena	10	553 ± 85	960 ± 59	1873 ± 473	2130 ± 570	773 ± 208	903 ± 44	1310 ± 307	1857 ± 237
	20	186 ± 30	770 ± 202	937 ± 241	1346 ± 275	340 ± 79	597 ± 90	847 ± 149	1163 ± 427
	30	0	113 ± 38	123 ± 75	577 ± 128	0*	97 ± 46	417 ± 258	547 ± 218
	40		10	3 ± 3	66 ± 34		0*	50 ± 32	20 ± 11
	50		0*	0*	10 ± 20			0*	0*
	60				0*				
Bala	10	317 ± 20	520 ± 101	1343 ± 478	1230 ± 695	410 ± 26	363 ± 61	773 ± 240	1407 ± 216
	20	117 ± 84	270 ± 55	500 ± 229	940 ± 750	100 ± 35	267 ± 67	193 ± 173	697 ± 150
	30	0	13 ± 3	117 ± 74	247 ± 235	0	0	90 ± 50	270 ± 60
	40		0*	0*	130 ± 160			3 ± 3	100 ± 60
	50				10*				0*
					Medium Penetration Resistance Site				
Azucena	10	283 ± 32	677 ± 168	1940 ± 775	1820 ± 990	290 ± 113	573 ± 240	930 ± 281	2310 ± 171
	20	57 ± 8	297 ± 150	627 ± 98	950 ± 460	93 ± 52	307 ± 162	310 ± 90	1410 ± 385
	30	0	250 ± 185	707 ± 218	706 ± 175	0	160 ± 125	130 ± 25	850 ± 425
	40		57 ± 47	320 ± 181	640 ± 480		30 ± 30	0*	200 ± 112
	50		0*	80 ± 80	105 ± 105		0*		27 ± 9
	60			0*	15*				0*
Bala	10	510 ± 20	400 ± 101	1187 ± 478	1097 ± 695	243 ± 94	360 ± 26	673 ± 35	1557 ± 324
	20	233 ± 274	200 ± 40	410 ± 90	623 ± 292	43 ± 7	93 ± 84	293 ± 124	900 ± 97
	30	0	87 ± 49	163 ± 107	200 ± 66	0	13 ± 13	60 ± 21	290 ± 151
	40		20 ± 20	3 ± 0	147 ± 141		0*	0*	77 ± 70
	50		0*		77 ± 70				0*