Final Technical Report

R6373 Screening rice root growth under mechanical impedance

1. Executive Summary

The project purpose was *Screening method for rice varieties with good pan penetration in place* (in IRRI, a target institution). The project addressed the developmental problem of low yields in rainfed rice due to strong soil. In rainfed lowland and upland rice, drought is a major cause of low yields. While deep rooting is known to increase drought resistance, this can be affected by mechanically strong soil. Different techniques for screening the ability of rice root systems to penetrate mechanical barriers were developed, tested and compared. Comparisons were also made with results from fields where hardpans were present. In collaboration with Dr. Adam Price (R6673: Drought resistance in upland rice: genetic analysis and varietal improvement), the root penetration ability of a population of recombinant inbred lines was studied in order to identify quantitative trait loci (QTLs) controlling root penetration. The two main outputs of the project were: (1) an improved wax layer method for measuring root penetration ability in rice in either simulated upland or lowland conditions and (2) the identification of QTLs controlling root penetration ability in upland rice. These outputs contribute to DFID's development goals by giving methods and results that plant breeders can use. New varieties with better root penetration of strong soil would be more drought resistant in rainfed rice-growing environments.

2. Background

Rice is the staple food of over half the world's population, but production is struggling to keep pace with population growth. It is estimated that global rice production will need to increase by 80% in the next 30 years to feed all the new rice consumers born each year. Rice is grown in tropical regions of S.E. Asia, Africa and Latin America under a range of ecosystems defined largely by water supply. Irrigated lowland rice is the most important production system, accounting for 55% of the global rice area and 75% of production. New varieties and intensive management practices have resulted in dramatic yield increases in this system over the last 30 years so that mean yields are now 5 t/ha, but production (in the form of increased area or yield per unit area) is unlikely to expand at the same rate as in the past.

In contrast, IRRI and WARDA have identified that there is great potential for increasing production in less favourable rice ecosystems such as rainfed uplands and lowlands. Yields here are as low as 1 t/ha and it is estimated that some 200 Mha of rainfed lowlands in Africa and Latin America alone are not cropped or are under-utilised. Low or erratic rainfall and poor soil conditions (low water-holding capacity, high strength, acidity, low nutrient status) are key constraints to yield in these ecosystems. The areas are characterised by poverty and over-exploited natural resources as well as poor soils. There is a need to develop better varieties and crop management practices for these ecosystems. Deep rooting is important for drought resistance as it enables plants to utilise subsoil water, but strong or compacted soil can prevent this by decreasing root growth. Studying the behaviour of roots of different rice varieties in response to impedance will allow the identification of varieties with good rooting ability under impeded conditions. This knowledge could then be used by rice breeders to develop varieties which combine a high yield potential with good drought resistance under difficult soil conditions. This would be assisted by the identification of suitable molecular markers for root systems that perform well in strong soil. Application of the knowledge gained in this project would therefore be expected to play a part in increasing rainfed rice production. Previous DFID funded projects investigated other drought resistance mechanisms (R4631) and the physiological basis for root growth in strong soil (R4825).

3. Project purpose

The project purpose was defined as: *Screening method for rice varieties with good pan penetration in place*. It addressed the constraint to development of low yields in rainfed rice due to drought. While it is known that rice varieties with deeper root systems are more drought resistant than varieties with shallow root systems, strong soil can decrease rooting depth in rice. There is therefore a need to breed varieties of rice with good penetration of strong soil, especially in the rainfed lowlands where shallow hardpans are widespread. However, assessing good performing varieties in field experiments is time-consuming and costly, so a rapid and reliable laboratory screen for good root penetration would have considerable potential for speeding up a breeding programme. The rationale behind this project was therefore to develop such a screen for plant breeders to use in the target institutions, IRRI and WARDA. It was also anticipated that the screen could be used to search for quantitative trait loci (QTLs) controlling root penetration.

4. Research activities

Activities 4.1 to 4.4 are the activities in the logical framework set out in the project memorandum form, whereas activity 4.5 and its associated output are additional.

4.1 Select varieties for study and establish optimum conditions for screens (to lead to output "Development and comparison of laboratory screens"

Wax layer screen

This screen uses a wax layer, made from a mixture of paraffin wax and white soft paraffin, to impede root growth. It is based on a published method. Wax layers made of mixtures of white soft paraffin and paraffin wax were installed 50 mm deep in sand cores. The sand cores were watered with nutrient solution and planted with 3-day old rice seedlings. The numbers of root axes that had penetrated the wax layers 24 days after planting and the total number of root axes were counted. Different strengths of wax layers and water regimes were investigated. A mixture of upland and lowland varieties was studied, including those used in field experiments organised by IRRI.

Sand core screen

This uses a method where plants are grown in sand cores loaded with weights to vary the mechanical impedance. This approach allowed mechanical impedance to be varied independently of aeration and water status. Two sizes of sand cores were used, designed to grow plants for either two or four weeks.

4.2 Select varieties for study and assess performance under compaction/drought in controlled environments (to lead to output "screen results related to plant performance in controlled environments")

Moroberekan, Azucena and Bala, three rice varieties with very different root penetration ability in the wax layer screen, were assessed by growing in compacted and uncompacted soil under terminal drought. Water uptake was measured.

4.3 Obtain field data from collaborators (to lead to output "screen results related to field performance")

Screen results from the wax layer and sand core experiments were related to root penetration data obtained from Dr. Len Wade. Dr. Wade and co-workers assessed the root penetration ability of eight rice varieties/lines in rainfed lowland fields in Bangladesh where there was a shallow hardpan. The field data were obtained from several fields with different hydrological conditions. It was not possible to assess varieties under upland conditions as IRRI's focus on strong soil is on the rainfed lowland system where the problem is much greater.

4.4 Measure root growth under combined water/mechanical stress (to lead to output "effect of combined water and mechanical stresses elucidated")

This activity was not carried out. See outputs for explanation.

4.5 Phenotype screen an upland rice mapping population for root penetration ability (to lead to the output "QTLs for root penetration ability in upland rice identified")

A sub-population of 104 F_6 recombinant inbred lines (RILs) from the cross Azucena × Bala were tested for root penetration ability in the non-flooded wax layer screen with an 80% wax layer. The RILs were developed by Dr. Adam Price in the related project 'Drought resistance in upland rice: genetic analysis and varietal improvement', R6673. Dr. Price analysed the phenotype data on root penetration ability to allow the QTLs to be identified.

5. Outputs

5.1 Development and comparison of laboratory screens

Wax layer screen

Different criteria for assessing root penetration ability in simulated upland conditions were investigated in the varieties Azucena, Bala and IR36. Yu *et al.* (1995, Crop Science 35, 684-687) scored the root penetration ability of rice varieties by measuring the ratio of penetrated to total root axes with a 60% wax layer, which they called the 'penetration ratio'. We found clear differences in this parameter between varieties, which indicated that Azucena was superior to both IR36 and Bala in overcoming impedance (Table 1). However, IR36 had more axes penetrating the 60% wax layer than Azucena but its performance was downgraded by using the Yu *et al.* 'penetration ratio' as the ranking criterion because IR36 produced a mean of 82 root axes per plant against Azucena's 28. Low impedance controls with 3% wax layers were used to give an unambiguous measure of the number of root axes that actually reached the wax layer. There were small differences between varieties in the number of axes penetrating the 3% wax layer but large

Table 1. *Effects of 3% and 60% wax layers on root penetration in three rice varieties under simulated upland conditions. Percentage penetration is the number of axes penetrating the 60% wax layer as a percentage of the number of axes penetrating the 3% wax layer. Results shown are means ± S.E. (n=5) of the back-transformed experiment means from five separate experiments. There were five replicates in each experiment.*

Variety	Total number of axes per plant		Number of penetrated axes per plant		Percentage penetration	Ratio of penetrated to total axes	
	3% wax	60% wax	3% wax	60% wax		3% wax	60% wax
IR36	82±7	82±6	20.5±1.4	17.4±1.2	85±3	0.26±0.03	0.22±0.03
Azucena	31±2	28±2	17.6±0.7	12.8±0.8	73±3	0.57±0.03	0.46±0.03
Bala	51±2	50±3	15.0±1.6	8.0±0.8	56±8	0.30±0.03	0.16±0.01

differences between varieties in the ratio of penetrated to total root axes with the 3% wax layer
(Table 1). This shows that there were differences between the varieties in their root depth distribution in unimpeded conditions. The control data also allowed calculation of the penetration of the 60% wax layers as a percentage of the penetration with the low impedance control ('percentage penetration' in Table 1). This parameter showed that IR36 ranked highest, although varietal differences were relatively small.

The Yu *et al.* 'penetration ratio' gave a misleading picture of root penetration ability. The high value measured for Azucena was because its low total root axis number led to a high proportion of its axes reaching the wax layer. The variety with the best root penetration was in fact IR36, which had the greatest number of axes penetrating the 60% wax layer and the highest 'percentage penetration'. The 'percentage penetration' of the three varieties showed that the effect of the 60% wax layer on root penetration was quite modest.

As the 60% wax layer offered little impedance to root penetration, experiments were conducted with 70% and 80% wax layers to develop an improved 'upland' screen. In these experiments, the lengths of all penetrated axes were measured to see how using length measurements as a screening criterion compared with using numbers of penetrated axes. Increasing wax concentration progressively decreased the number of penetrated axes and the total length of penetrated axes (results not shown). The length of the longest penetrated axis was much less sensitive to increased wax concentration. For each impeded treatment, the same ranking of the three varieties was obtained whether number of penetrated axes or total length of penetrated axes was used as the criterion. Using the total length of axes penetrating the 80% wax layer as a screening criterion did not appear to have any advantages over using the number of penetrated axes. An 80% wax layer was chosen for the improved 'upland' screen.

In the improved 'upland' screen, the penetration abilities of Azucena and IR36, whether measured by number of axes penetrating the 80% wax layer or 'percentage penetration', were very similar and about 5 times greater than that of Bala (Table 2). The mean percentage penetration for these three varieties against the 80% wax layer was 19%.

Table 2. *Effects of 3% and 80% wax layers on root penetration in three rice varieties under simulated upland conditions. Percentage penetration is the number of axes penetrating the 80% wax layer as a percentage of the number of axes penetrating the 3% wax layer. Results shown are means ± S.E. (n=3) of the back-transformed experiment means from three separate experiments. There were five replicates in each experiment.*

Variety	Total number of axes per plant		Number of penetrated axes per plant		Percentage penetration
	3% wax	80% wax	3% wax	80% wax	

IR36	69±11	75±10	19.1±1.5	4.6±1.0	25±6
Azucena	29±3	28±2	15.1±1.7	4.2±0.6	28±3
Bala	45±4	43±5	14.4±1.5	0.8±0.3	5±1

As a wax layer screen carried out only under simulated upland conditions might favour upland varieties relative to lowland varieties, experiments were carried out to see how using a flooded treatment to simulate lowland conditions affected behaviour of Azucena, Bala and IR36 in the wax layer system. In all three varieties, flooding increased total root axis number, had little effect on the number of axes penetrating the 3% wax control, but greatly decreased the number of axes penetrating a 60% wax layer (Table 3, overleaf). The 'percentage penetrations' under flooded conditions were correspondingly much lower, with a mean of 26% for the three varieties compared with 76% under non-flooded conditions. Under flooded conditions, the upland variety Azucena showed slightly higher penetration of the 60% wax layer than the lowland variety IR36.

Sand core screen

Mechanical impedance decreased root length of both seminal and nodal root axes in the varieties Azucena, Bala and IR36. There was little difference between varieties in maximum rooting depth under control or impeded conditions, which reflected the behaviour of the seminal root axes (Table 4). However, there were clear differences in the behaviour of nodal root axes between varieties, with the variety Azucena having the longest nodal roots in control and impeded conditions.

Table 4. *Effect of mechanical impedance in sand cores on root characteristics in 13 day old rice plants. Results shown are the means of the back-transformed means from two separate experiments. There were five replicates in each experiment.*

	Seminal axis length (there is one seminal axis per plant)		
	Control	Impeded	% control
Azucena	270	157	58
Bala	227	149	65
IR36	241	126	52

Longest nodal axis length Number of nodal axes

	Control	Impeded	% control	Control	Impeded	%
Azucena	237	111	47	10.8	12.1	112
Bala	160	94	59	17.3	15.8	91
IR36	130	73	56	18.8	16.6	88

Table 3. *Effects of 3% and 60% wax layers on root penetration in three rice varieties under simulated upland (non-flooded) and lowland (flooded) conditions. Percentage penetration is the number of axes penetrating the 60% wax layer as a percentage of the number of axes penetrating the 3% wax layer. Results shown are back-transformed means of two separate experiments. There were five replicates in each experiment.*

Variety	Water regime	Total number of axes per plant		Number of penetrated axes per plant		Percentage penetration
		3% wax	60% wax	3% wax	60% wax	
IR36	non-flooded	81	78	19.1	17.4	91
	flooded	124	121	15.6	4.1	26
Azucena	non-flooded	29	27	16.9	12.5	74
	flooded	40	36	16.8	5.2	31
Bala	non-flooded	50	53	12.3	7.9	64
	flooded	64	71	12.8	2.5	20

In plants that were 27 days old (the age at which the wax layer experiments were harvested), a different picture emerged (Table 5). The longest nodal root axis had overtaken the seminal axis and there was little difference between varieties in maximum rooting depth, whether impeded or unimpeded. This was interesting as Bala and IR36 are often considered to be shallow rooted compared with Azucena. A similar picture emerged when other varietal comparisons were made (results not shown).

Table 5. *Effect of mechanical impedance in sand cores on root characteristics in 27 day old rice plants. Results shown are means from one experiment ± S.E. (n=5).*

	Seminal axis length		
	Control	Impeded	% control
Azucena	525± 6	438±24	83
Bala	348	299±18	86
IR36	464±25	348±55	74

	Longest nodal axis length			Number of nodal axes		
	Control	Impeded	%	Control	Impeded	%
Azucena	522±10	258±25	49	19.8±2.5	21.2±1.8	107
Bala	475±20	298±21	63	44.8±2.7	39.8±3.8	88
IR36	503± 9	238±23	47	58.3±7.1	32.6±6.4	56

Comparison of screens

Comparing the ranking of Azucena, Bala and IR36 in the wax layer and sand core screens leads to the conclusion that the ability to penetrate the wax layer was not related to the ability to grow through strong sand. The wax layer screen seems to be measuring the ability of roots to cope with a discontinuity in the strength of the growth medium, rather than simply the ability to elongate through a mechanically strong medium. The wax layer screen was considered to be more realistic in terms of reflecting the situation in the field, where strong soil tends to be concentrated in discrete hard pans.

5.2 Screen results related to plant performance in controlled environments

The results were difficult to interpret and are not shown in detail. Azucena, Bala and Moroberekan appeared to take up *more* water from the compacted soil than from the control. In addition, the plants produced more tillers when grown in compacted soil. This contrasted with the behaviour in the sand cores, where the high impedance treatment decreased tiller number.

5.3 Screen results related to field performance

Comparison between field and the wax layer screen results was difficult owing to the small differences between lines in root length density in the field and the effect of the field hydrology on ranking. However, IR58821 tended have greater rooting at depth in the field than the others and IR58821 had the greatest number of penetrated axes in the flooded wax layer screen.

5.4 Effect of combined water and mechanical stresses elucidated

This work was planned to be carried out in the seminal root axis of non-transpiring seedlings so that water potential and mechanical impedance could be controlled independently. However, this approach was not continued with when it became clear from the sand core work that the behaviour of the seminal root axis was different to that of the nodal root axes (Table 4), which form most of the root system in rice.

5.5 QTLs for root penetration ability in upland rice identified

Analysis of the root penetration data by Dr. Adam Price at Aberdeen revealed that there were seven QTLs controlling root penetration ability in this population (Fig. 1). These results were very different to those reported in a previous study by Ray *et al.*(1996, Theoretical and Applied Genetics 92, 627-636), which presumably reflects the carefully developed screen that we used to phenotype the lines.

6. Contribution of Outputs

The two main outputs from the project were the improved wax layer screening method and (together with project R6673) the identification of QTLs for root penetration ability. The other outputs were to ensure that these main outputs were soundly based. IRRI are using wax layer screens in their breeding programme and the method of the improved wax layer screen has already been sent to interested parties at IRRI in order that they may take up the technology. The outputs have the potential to improve the effectiveness of breeding programmes. More reliable identification of alleles that lead to better root penetration and hence drought resistance will help breeders develop better rice varieties for rainfed environments. A QTL for good root penetration is linked to a target for marker-assisted selection in project R6673. There will therefore be a need to test new material arising from that project for its root penetration ability towards the end of 1999.

A paper on the wax layer method was submitted for publication in 1998 and a paper on the identification of QTLs for root penetration will be submitted in January 1999. Further papers will be written on the sand core method and on the comparison of field and wax layer screen results.

Fig. 1. *Quantitative trait loci (QTLs) for characters related to root penetration. Boxes to the left of each chromosome identify QTLs where Azucena alleles have a positive effect, whereas boxes to the right identify QTLs where Bala alleles have the positive effect.*