

A. H. Price · A. D. Tomos · D. S. Virk

Genetic dissection of root growth in rice (*Oryza sativa* L.) I: a hydroponic screen

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Abstract Root growth is an important component of the adaptation of rice to drought-prone environments. A hydroponic screen was used to study root growth of 28 rice varieties. Both maximum root length and adventitious root thickness varied widely between varieties. In general, *japonica* varieties had larger root systems than *indica* varieties. Two F₂ populations involving the thick- and long-rooted upland *japonica* variety 'Azucena' and two poor-rooting varieties, namely the upland *indica* 'Bala' and the Italian *japonica* 'Maratelli', were made and screened in hydroponics. Generation means analysis revealed significant additive and dominance main effects for the root length traits with a prevalence of dominance gene effects in both crosses. The dominance × dominance type of non-allelic interactions were important for maximum root length from day 7 to day 28, root volume, root thickness and root cell length in the cross 'Bala' × 'Azucena'. The heritability (broad-sense) estimates varied from low to high for the traits and displayed differences between populations. This suggested that recombinant lines with improved root traits can be developed from the two crosses with selection methods that involve some form of progeny evaluation. In a companion paper, we report the mapping of quantitative trait loci (QTLs) for root growth traits in the 'Bala' × 'Azucena' population using restriction fragment length polymorphisms (RFLPs).

Key words Drought resistance · RFLPs · Cell division · Genetic analysis · Root growth

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A. H. Price (✉) · A. D. Tomos
School of Biological Sciences, University of Wales, Bangor,
Gwynedd, LL57 2UW, UK

D. S. Virk
Centre for Arid Zone Studies, University of Wales, Bangor,
Gwynedd, LL57 2UW, UK

Introduction

Rice produces the principal food for one-third of the world's population. Approximately 45% of the rice area relies exclusively on rainfall for water. In the rainfed lowland, deep water and upland culture types [23%, 11% and 13%, respectively, of the world's rice area (David 1991)], irregularity of water supply limits productivity. Ingram et al. (1990) suggest that about half of all rainfed lowland rice is prone to frequent drought. In upland rice, where average yields are in the order of 1 t/ha [compared to 3.5 t/ha, 1991 world average (IRRI 1993)], drought is the major abiotic constraint on production (Arraudeau and Harahap 1986; Tran Van Dat 1986).

Drought resistance is a function of drought escape, avoidance and tolerance (Levitt 1980; Jones et al. 1981). Drought avoidance, afforded by a profound root system which enhances the ability of a plant to capture water, is a fundamental adaptation to drought (Pasioura 1982; Ludlow and Muchow 1990) and is a principal component of drought resistance in rice (O'Toole 1982). Field drought resistance has been found to be related to a deep rooting system (Yoshida and Hasegawa 1982) and the force required to uproot a rice plant (O'Toole and Soemartono 1981; Ekanayake et al. 1985a). In addition, a greater ability to extract soil water has been related to improved drought recovery in rice (Lilley and Fukai 1994). Detailed measurements of the root morphology of rice plants grown in hydroponic or aeroponic culture systems have shown correlations between maximum root length and field drought resistance (Ahmadi 1983; Loresto et al. 1983; Ekanayake et al. 1985b). Loresto et al. (1983) and Ekanayake et al. (1985b) also found thicker adventitious roots to be positively related to drought resistance. Champoux et al. (1995) have shown, using recombinant inbred lines, that root thickness, root dry weight per tiller, maximum rooting depth and root/shoot ratio of plants grown in soil tubes are all

positively correlated to field drought resistance. In a recent review of the characteristics of the potential value in improving drought resistance in upland rice, Fukai and Cooper (1995) concluded that a deep root system is crucially important.

Genetic variation in rooting patterns is pronounced in rice (O'Toole and Bland 1987), and traits such as maximum root length, root thickness, root/shoot dry weight ratio and root-pulling force show quantitative inheritance (Ahmadi 1983; Loresto et al. 1983; Ekanayake et al. 1985ab). The traditional upland varieties of rice (which are generally low yielding) are those with the most pronounced rooting systems (Loresto et al. 1983).

Recent advances in molecular marker technology have led to the development of detailed RFLP (restriction fragment length polymorphism) linkage maps of rice (Kurata et al. 1994; Causse et al. 1995). In order to facilitate the rapid breeding of high-yielding rice plants with improved drought resistance achieved through the possession of an enhanced rooting system, it would be useful to locate genes that confer desirable rooting patterns on these emerging rice genetic maps (Hanson et al. 1990). This has been done very successfully in one study (Champoux et al. 1995), but comparative data from other crosses and environmental conditions will increase breeders' confidence that particular parts of the rice genome are worthwhile transferring from one variety to another.

This paper reports the identification of suitable screening methods and genetic material for mapping root characters related to drought resistance in rice using molecular markers, principally RFLPs. Firstly, a hydroponic screening system is evaluated, and parental varieties suitable for producing segregating populations identified. Then, two segregating populations are screened for hydroponic root growth, and genetic analysis conducted. A companion paper (Price and Tomos 1997) reports the results of linkage mapping with RFLPs in one of these populations and the subsequent identification of quantitative trait loci (QTLs) controlling root growth using genetic material and root growth data presented here.

Materials and methods

Screen of 26 varieties

The seeds of 26 varieties of rice (Table 1), 24 obtained from the International Rice Research Institute (IRRI), Los Baños, Philippines and 2 ('KR35' and 'KR108') from Prof. T. Flowers, University of Sussex, UK, were surface-sterilised in 1% NaClO₃, rinsed in sterile water, and germinated on wet filter paper in the dark for 4 days. Germinated seedlings were grown hydroponically in black plastic trays (Plantpack, UK) fitting into 25-l plastic tubs (520 mm long, 340 mm wide and 180 mm deep) containing aerated, half-strength Yoshida's nutrient solution (Yoshida et al. 1976), pH 5.0, made up with tap water. The trays (520 × 310 mm) contained 12 rows of seven

wells. Each well was 55 mm deep and had a 12-mm-diameter hole at the bottom. Each seedling was placed upon a 15 × 15-mm piece of 4-mm mesh netting at the bottom of a well. Each tray contained up to 84 plants. There were between 4 and 6 plants per variety (average 5.6). Evaporated water was replaced by de-ionised water daily. Nutrient was replaced weekly and after 2 weeks full-strength nutrient was used. The plants were grown in three trays under greenhouse conditions (minimum temperature 25°C) during July and August 1991. Trays were rotated between tubs daily, and the pH was adjusted every other day for the first week, then daily.

On five occasions during growth, the length of the longest root (maximum root length) of each plant was measured using a ruler while each tray was suspended above the nutrient solution. The roots were out of the solution for a maximum of 5 min before re-wetting. On the 31st day, root volume was analysed by weighing the volume of water displaced by the roots.

Final harvest was after 42 days of growth. Plants were photographed with a ruler before being divided into shoot and root for dry-weight analysis (drying for 3 days at 80°C). Maximum root length was measured from a projection of the photographic negative, and adventitious root thickness near the shoot was measured from the negative using a microscope. Information for the drought-resistance evaluation conducted by the International Rice Research Institute for each variety was provided by Dr. M. Jackson from the Genetic Resource Center, IRRI (Manila) data bank. While these data present the best available information on drought performance, the scores need to be interpreted with some caution since it is not clear whether all varieties were tested in the same year.

Eight-variety screen

The growth facility included 250-W metal halide lamps providing 600 µE PAR of supplementary lighting at the level of the tubs. The trays were painted white to reduce heat absorption, and every other well was covered with aluminium foil. Fans were fitted to prevent overheating from the lamps. Eight varieties were tested in order to conduct a detailed analysis of environmental effects in the growth conditions and to identify a poor-rooting *japonica* variety. Varieties 'Maratelli' and '63-83' were supplied by Dr. M. Jacquot, CIRAD, Montpellier. Seeds were germinated in February 1992, and nutrient was applied as described above using four trays. Only 42 plants were planted per tray. After 3 days in the trays, 2-mm-diameter expanded polystyrene balls were used to fill the wells. Trays were rotated between tubs daily, and pH adjusted every other day for the first week, then daily. Maximum root length was measured with a ruler.

Root growth in soil

Four varieties ('Azucena', 'Bala', 'IAC25' and 'Maratelli') were grown in soil tubes in August and September 1992 to provide a comparison between soil and hydroponics. A clear perspex front was attached by waterproof tape to several 1-m-long, 115-mm-diameter, black plastic half-guttering (essentially, a 1-m-long, 115-mm-diameter, plastic tube cut in half longitudinally). Each was filled with 7.5 l of John Innes No. 2 Potting Compost and covered in black card to prevent light entering the clear surface. The tubes were placed under the lights described above and leaned at an angle of 15° from vertical to encourage the growth of roots down the clear face. Each tube received 200 ml of tap water daily. At regular intervals, the length of the longest visible root was measured.

Population screens

Using the data obtained from the hydroponic screening we chose 'Azucena', 'Bala' and 'Maratelli' for a crossing programme. 'Azucena' was chosen because it had the thickest and amongst the

Table 1 Subspecies, ecosystem, country of origin, drought resistance score and hydroponic root growth results for 28 rice varieties

Variety	Subspecies	Ecosystem	Country of origin	Drought score ^a	Maximum root length ^b (mm)			Root volume ^b (ml)			Root thickness ^b (mm)		
					\bar{x}	SE	R	\bar{x}	SE	R	\bar{x}	SE	R
Sigadis	<i>Indica</i>	Lowland	Indonesia	NA	265	9	(21)	0.23	0.06	(25)	0.88	0.02	(23)
Dular	<i>Indica</i>	Lowland	India	NA	334	15	(12)	0.92	0.11	(20)	1.09	0.04	(15)
Intan	<i>Indica</i>	Lowland	Indonesia	NA	246	20	(23)	0.75	0.11	(23)	0.90	0.06	(22)
Salumpikit	<i>Indica</i>	Lowland	Unknown	4	400	41	(6)	1.38	0.28	(8)	1.13	0.04	(12)
MGL-2	<i>Indica</i>	Lowland	India	4	347	15	(11)	1.11	0.23	(13)	1.27	0.06	(5)
BK 88 BR 6	<i>Indica</i>	Lowland	India	NA	311	16	(17)	1.02	0.16	(16)	1.10	0.03	(14)
BPI 76 N.S. (Green)	<i>Indica</i>	Lowland	Philippines	NA	278	18	(19)	0.78	0.18	(22)	1.02	0.03	(18)
IR 5	<i>Indica</i>	Lowland	Philippines	5	313	16	(16)	1.10	0.18	(14)	1.12	0.05	(13)
IR 20	<i>Indica</i>	Lowland	Philippines	5	160	15	(26)	0.13	0.04	(26)	0.65	0.06	(26)
Nam sa gui 19	<i>Indica</i>	Lowland	Thailand	4	383	24	(9)	1.65	0.23	(5)	1.02	0.02	(19)
Khao Dawk Mali 105	<i>Indica</i>	Lowland	Thailand	NA	301	28	(18)	1.76	0.29	(4)	1.00	0.02	(20)
IR 36	<i>Indica</i>	Lowland	Philippines	6	218	31	(24)	1.01	0.24	(17)	0.74	0.02	(25)
IR 480-5-9-3	<i>Indica</i>	Lowland	Philippines	NA	391	36	(7)	1.54	0.30	(7)	1.15	0.02	(11)
IR 43	<i>Indica</i>	Lowland	Philippines	NA	207	24	(25)	0.79	0.26	(21)	0.77	0.03	(24)
C 22	<i>Indica</i>	Upland	Philippines	NA	270	15	(20)	0.39	0.04	(24)	1.04	0.03	(17)
Bala	<i>Indica</i>	Upland	India	3	246	31	(22)	1.33	0.59	(9)	0.97	0.05	(21)
N22	<i>Indica</i>	Upland	India	4	324	11	(14)	0.99	0.34	(18)	1.27	0.02	(6)
Azucena	<i>Japonica</i>	Upland	Philippines	4	427	31	(3)	1.06	0.11	(15)	1.42	0.06	(1)
M1-48	<i>Japonica</i>	Upland	Philippines	4	370	12	(10)	1.12	0.18	(12)	1.29	0.02	(4)
Rikuto Norin 21	<i>Japonica</i>	Upland	Japan	5	400	21	(5)	1.56	0.21	(6)	1.18	0.04	(10)
OS 4	<i>Japonica</i>	Upland	West Africa	4	418	48	(4)	1.24	0.25	(10)	1.21	0.03	(9)
Moroberekan	<i>Japonica</i>	Upland	Guinea	4	390	28	(8)	0.98	0.11	(19)	1.24	0.03	(8)
IAC 25	<i>Japonica</i>	Upland	Brazil	3	505	16	(1)	1.87	0.06	(1)	1.37	0.04	(2)
Kinandang Patong	<i>Japonica</i>	Upland	Philippines	3	448	33	(2)	1.78	0.38	(2)	1.29	0.04	(3)
KR 35	Unknown	Upland	Kenya	NA	330	20	(13)	1.77	0.43	(3)	1.26	0.04	(7)
KR 108	Unknown	Upland	Kenya	NA	314	18	(15)	1.21	0.24	(11)	1.08	0.05	(16)
63-42	<i>Japonica</i>	Upland	Cote d'Ivoire	3									
'Maratelli'	<i>Japonica</i>	Upland	Italy	6									
Average					331	5		1.19	0.04		1.09	0.01	

^a Data for early vegetative drought score (40 days after sowing) from IRRI: 3, drought resistant; 6, drought susceptible; NA, data not available

^b \bar{x} , mean; SE, standard error; R, rank

longest roots. Bala was chosen as a short-rooted parent since it appears to possess drought resistance mechanisms not related to root growth. 'Maratelli' was selected as the only poor-rooting *japonica*.

Maximum root length, root thickness and root volumes were studied in the F₂ because these traits can be easily and accurately measured non-destructively and because root length and root thickness are the traits most closely related to field drought resistance (see results). Two populations potentially segregating for root growth were produced by crossing 'Bala' with 'Azucena' and 'Azucena' with 'Maratelli' using standard hybridisation methods. Each F₂ population was derived from one self-fertilised F₁ seed.

The F₂ populations were screened alongside parental and F₁ ('Bala' × 'Azucena' only) generations in April/May 1993 ('Bala' × 'Azucena') and June/July 1993 ('Azucena' × 'Maratelli') using the hydroponic screen described in the "eight-variety screen" above, except that after 3 weeks the concentration of NH₄NO₃ was doubled. Six trays were used in each screen. The first two rows of each tray were planted with 'IR36' as a potential windbreak. One F₁ plant ('Bala' × 'Azucena' screen only) was planted in the middle of each tray. One seedling of each parental line was planted in the 5th and 9th row of each tray. One hundred and seventy-eight and 177 F₂ plants were grown in each screen. Unlike the previous experiments, maximum root length at harvest was measured with a ruler, root volume was measured at the final harvest (day 28) and adventitious root thickness near the shoot was measured directly using a binocular microscope. In the 'Bala' × 'Azucena' population screen,

five adventitious root tips were removed from each plant, placed in 1 ml of water and stored at -20°C. The length of epidermal cells in the fully expanded zone (15 mm from tip) were measured using a microscope after the roots were cleared in lactic acid for 5 min. After screening, the plants were subsequently potted in soil and grown for DNA extraction and seed collection for generation advance.

Biometrical analysis

The parental, F₁ and F₂ generations of the cross 'Bala' × 'Azucena', and the parental and F₂ generations of the cross 'Azucena' × 'Maratelli' were available for biometrical genetic analysis on generation means and variances. The analyses were performed following Mather and Jinks (1982).

For the generation means analysis, standard errors of generations were computed by performing an analysis of variance for the nested design used (Snedecor and Cochran 1989). These analyses were separately computed for segregating and non-segregating generations. The partitioning of sources of variation for the F₂ generation was: (1) between trays, (2) between rows within trays, (3) between plants within rows and within trays. The significant mean squares of the highest hierarchy divided by the total number of plants in all rows and trays gave the variance of the F₂ mean (VF₂). The square root of VF₂ was used as its standard error (SE). For the

non-segregating generations, the parents and \bar{F}_1 were considered together. This was necessary to compensate for the fewer degrees of freedom in the individual parental and F_1 generations. In doing this analysis, however, we assumed that the parental and F_1 generations show no differential response to the environmental conditions within our experiments, which is not an unreasonable assumption in view of the controlled environments of the experiment. The partitioning of sources of variation was: (1) between trays, (2) between parents or parents and F_1 , (3) within parents or parents and F_1 within trays. The item (3) divided by the total number of plants in all trays provided the variance of non-segregating generations, and its square root the SE. When mean squares for (1) was not significant, pooling was done with (3) before the computation of SE. The analysis [item (2)] also provided the opportunity to test if parents and F_1 generations differed significantly in the cross 'Bala' \times 'Azucena'. This should happen only if significant genetic variation exists in the cross. However, in the cross 'Azucena' \times 'Maratelli', in the absence of F_1 generation, only the additive parental difference could be tested, which is not a certain test of the genetic diversity between parents since the parental differences may turn out to be non-significant in the presence of complete gene dispersion.

The analysis of generation means proceeded, firstly by testing the presence of non-allelic interactions by using scaling tests, and secondly by successive model fitting in a weighted least squares procedure (Mather and Jinks 1982). The application of the scaling test was limited by the number of generations available. Therefore, the C scaling test was only possible in the cross 'Bala' \times 'Azucena'. This test was performed as;

$$C = 2\bar{F}_2 - \bar{F}_1 - (1/2)\bar{P}_1 - (1/2)\bar{P}_2$$

The variance (V) of C was computed as $4V\bar{F}_2 + V\bar{F}_1 + (1/4)V\bar{P}_1 + (1/4)V\bar{P}_2$; and the square root of VC provided its SE. Since, in the absence of non-allelic interaction, $C = 0$, its deviation from zero was tested by a t -test = $C/SE(C)$. The evidence for the presence of non-allelic interactions was confirmed by the outcome of weighted least squares analysis which involved the estimation of genetic parameters from the available types of generations followed by a comparison of the observed generation means with predicted values derived from the estimates of the parameters. The procedure provided a χ^2 test for the goodness-of-fit of the model when the number of estimated parameters was less than the number of generations. However, when it was not so, a full (perfect-fit) model was used. The successive model fitting in the present case involved the fitting of m = mean of F_2 generation inbred lines, $[d]$ = additive component, $[h]$ = dominance component and $[l]$ = dominance \times dominance interaction component parameters.

Table 2 Distribution range and F value from analysis of variance (ANOVA) for rice growth characteristics

Characteristic	Range (% of overall average)	F value for ANOVA ^a between		
		Varieties	Subspecies	Ecotypes
Root length: day 10	63–142	6.41	34.83	20.93
day 15	68–144	6.05	30.95	15.02
day 22	69–157	6.74	47.99	17.40
day 30	52–135	8.95	42.12	13.01
day 37	56–141	7.02	29.09	13.09
day 42	48–155	12.32	63.64	29.30
Root volume: day 31	11–158	4.64	14.37	7.01
Root thickness	60–130	27.48	80.41	71.08
Root dry weight	12–241	5.36	28.19	14.06
Shoot dry weight	20–223	4.77	19.88	6.24
Root: shoot dry weight ratio	75–159	14.33	9.96	26.77

^a Degrees of freedom: variety 25 (error 122), subspecies 1 (error 146), ecotype 1 (error 146); all significant at $P < 0.001$ except root: shoot ratio on subspecies ($P = 0.002$) and root volume on ecotype ($P = 0.009$)

The total variance of the F_2 generation (VP) was partitioned into genetic (VG) and environmental (VE) components. The estimate of VE was provided by the variance of non-segregating generations as: $VE = (1/2)VP_1 + (1/2)VP_2$ or $(1/2)VF_1 + (1/4)VP_1 + (1/4)VP_2$. The ratio of VG to VP was used as broad-sense heritability in percent (h^2).

Results

Screen of 26 varieties

Varietal differences in hydroponic root length after 42 days (Table 1) were within the range of 50–150% of the overall average and were highly significant (Table 2). Analysis of variance revealed highly significant differences ($P < 0.001$) between subspecies, indicating that *japonica* varieties had longer roots than *indica* varieties. The roots of upland varieties were also significantly ($P < 0.001$) longer than those of lowland varieties. Similar distinctions in root length between varieties, subspecies and ecosystem were observed at all stages of root growth ($P < 0.001$). Varieties 'IAC25', 'OS4' and 'Rikuto Norin 21' consistently displayed long roots at all ages, while 'IR20', 'IR36' and 'IR43' consistently displayed short roots. 'Azucena' and 'Kinandang Patong' initially had roots close to average in length (data not shown), but by day 22 they were amongst the longest rooting varieties. The upland *japonica* varieties ('Azucena', 'M 1-48', 'Rikuto Norin 21', 'OS 4', 'Moroberekan', 'IAC 25' and 'Kinandang Patong') displayed the longest roots, while upland *indica* varieties ('N 22', 'Bala' and 'C 22') had the shortest root systems. With the exception of 'Salumpikit', 'IR 480-5-9-3' and 'Nam sa gui', lowland *indica* varieties also had reduced root systems. Interestingly, 'Salumpikit' is an *indica* grown in rainfed lowlands and displays drought tolerance (De Datta and Seshu 1982), while 'Nam sa gui' has been used as a drought-tolerant donor in lowland breeding programmes (DeDatta et al. 1988).

Root volume on day 31 ranged from 0.13 to 1.87 ml (Table 1) and did not differentiate varieties as well as root length (Table 2); relatively large standard errors mean that statistically significant differences were only apparent between extreme ends of the distribution. However, subspecies were significantly different ($P = 0.001$), with *japonica* having higher root volumes than *indica* varieties, and upland varieties having higher volumes than lowland ones ($P = 0.009$).

Adventitious root thickness at day 42 (Table 1) ranged from 0.65 to 1.42 mm. Small standard errors led to highly significant differences between varieties (Table 2). Subspecies were highly significantly different ($P < 0.001$), with *japonica* having thicker roots than *indicas*. Upland varieties also had significantly thicker roots than lowland ones ($P < 0.001$).

Root dry weight at 42 days (data not shown) ranged from 28 mg for 'IR20' to 552 mg for 'Rikuto Norin 21'. As with root volume, root weight did not differentiate varietal groups as well as root thickness or maximum root length (Table 2). The root dry weight of *japonicas* was significantly greater than that of *indica* varieties ($P < 0.001$), and upland types were heavier than lowland varieties ($P < 0.001$). Shoot dry weight at 42 days (data not shown) followed a very similar pattern to root weight, suggesting that root weight was closely associated with total plant weight.

Root/shoot dry weight ratios (data not shown) varied considerably between varieties (0.15 for 'IR20' to 0.31 for 'N22', Table 2). This ratio differentiated both subspecies and ecotypes at a high level of significance ($P < 0.001$). Values for lowland varieties were lower than those for upland types. *Indicas* had a lower ratio than *japonicas* (average 0.22 and 0.25, respectively). Interestingly, the 3 upland *indica* varieties ('N22', 'Bala' and 'C22') had higher ratios than the other *indicas* (average 0.28, $P = 0.001$).

Amongst the varieties, maximum root length correlated positively and significantly with adventitious root thickness ($r = 0.871$), root volume ($r = 0.724$), root dry weight ($r = 0.671$) and shoot dry weight ($r = 0.657$). Root volume also correlated with root thickness ($r = 0.525$), root dry weight ($r = 0.599$) and shoot dry weight ($r = 0.677$), while root thickness also correlated with both root and shoot dry weight ($r = 0.633$ and $r = 0.569$, respectively) in addition to the root to shoot dry weight ratio ($r = 0.456$). No root growth parameters were found to be correlated significantly with the data on culm height obtained from the IRRI data bank.

Eight-variety screen and root growth in soil

When grown in hydroponics, the upland *japonica* variety '63-83' was revealed, as expected, to be a good rooting variety (Fig. 1a). Importantly, the Italian variety 'Maratelli' was revealed to be a poor rooting *japonica*.

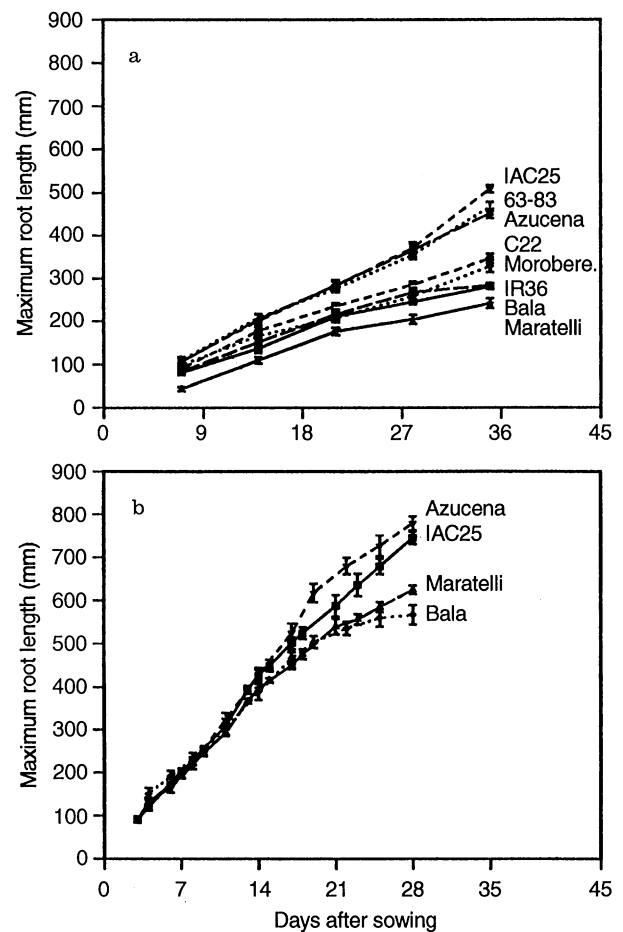


Fig. 1a,b Maximum root length plotted against age of 8 varieties grown in the modified hydroponic screening system (a) and 4 rice varieties grown in soil tubes (b). Bar: standard error

Using analysis of variance we were able to show that plants in the front two rows of each tray had shorter roots than the rest. In all subsequent hydroponic screens, the front two rows were planted with non-experimental plants ('IR36'). When 4 varieties were grown in soil, the roots grew more than two times faster than in hydroponics (Fig. 1b). As the plants developed, the roots of 'Azucena' and 'IAC25' became longer than those of 'Bala' or 'Maratelli', and the distinction between these varieties became apparent after 14 days of growth. The magnitude of the difference between the long-rooted and the short-rooted varieties was much reduced in the soil experiment.

Correlations with drought resistance scores

Using data from the Genetic Resource Centre, IRRI, we compared the field drought resistance scores of many varieties with root growth in the 26-variety screen. The two varieties '63-83' and 'Maratelli', which

were not included in the original screen, were assigned root lengths of 430 mm and 230 mm, respectively, based on extrapolation from the 8-variety screen and other data not shown (estimates of other root parameters for these varieties were not available). The most directly relevant data are the drought resistance score at the early vegetative stage (drought at 40 days after sowing), which are included in Table 1. Drought score at 40 days was negatively correlated with maximum root length ($r = -0.594$, $P = 0.003$), root volume ($r = -0.569$, $P = 0.022$) and root thickness ($r = -0.581$, $P = 0.023$). This indicates that the varieties with longer, thicker and bigger root systems are indeed the most drought resistant. Figure 2 shows a plot of maximum root length against early vegetative drought score. Only 'Bala' and 'IR20' lie substantially off a regression line linking maximum root length to drought resistance.

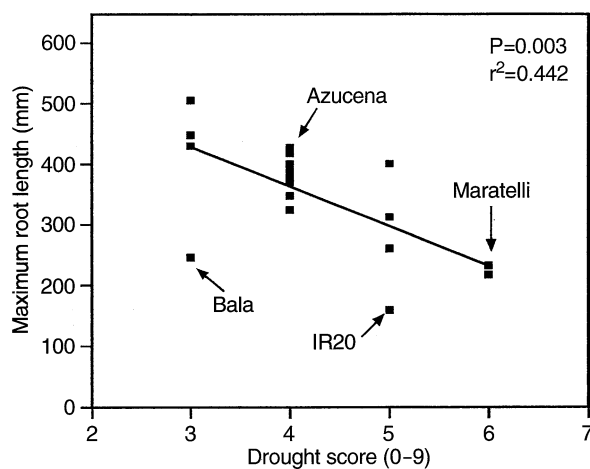


Fig. 2 Scatter plot with regression line of maximum root length of 18 hydroponically grown rice varieties versus drought score at early vegetative stage (data from IRRI). Drought score ranges from 1–9; 0 = highly drought resistant, 9 = highly drought sensitive

Drought score at the reproductive stage (data not shown) was also negatively correlated (i.e. drought resistance was positively correlated) with maximum root length ($r = -0.562$, $P = 0.012$) and root thickness ($r = -0.591$, $P = 0.008$). Data for other measures of root growth were not related to drought resistance at any stage.

Population screens

The results of F_2 population screens are presented in Table 3 and Figs. 3 and 4. Analysis of variance showed that significant differences existed between the parents and F_1 (if present) for all traits with the exception of root-cell length (measured in 'Bala' × 'Azucena' population only). However, the F_2 mean of root-cell length in the 'Bala' × 'Azucena' population was significantly higher than the parental and F_1 means, indicating the presence of complex genetic variation.

Both the 'Bala' × 'Azucena' and the 'Azucena' × 'Maratelli' populations displayed unimodal segregation for maximum root length (Fig. 3). The segregation clearly improved with time and was most pronounced at the time of harvest (day 28). The distribution of maximum root length was near normal in both populations after 14 days of growth. In the 'Bala' × 'Azucena' population, root growth was slightly skewed towards longer roots at 3 and 7 days. Transformation of the data by squaring produced a normal distribution. Early root length in the 'Azucena' × 'Maratelli' population was heavily skewed towards longer roots and could not be adequately transformed into normally distributed data. In the 'Bala' × 'Azucena' population, the F_2 mean was above the F_1 mean on all occasions except at day 28. This may be a result of an imprecise estimate of the F_1 mean from its small population ($n = 6$) or the presence of strong non-allelic interactions, particularly the dominance × dominance ($[I]$) type.

The F_2 mean of root volume was significantly smaller in the 'Azucena' × 'Maratelli' population than in the

Table 3 Generation means with standard errors (SE) of root characters in two crosses of rice

Cross	Generation	Maximum root length (mm)					Root volume (ml)	Root thickness (mm)	Root cell length (µm)
		Day 3/4 ^a	Day 7	Day 14	Day 21	Day 28			
Ba × Az	Bala	65 ± 2.4	116 ± 3.0	195 ± 5.1	208 ± 5.7	213 ± 3.9	3.58 ± 0.28	0.792 ± 0.014	54.5 ± 1.21
	Azucena	74 ± 2.4	162 ± 3.0	313 ± 5.1	401 ± 5.7	479 ± 3.9	7.09 ± 0.28	1.153 ± 0.014	54.3 ± 1.21
	F_1	52 ± 2.4	104 ± 3.0	197 ± 5.1	275 ± 5.7	377 ± 3.9	7.14 ± 0.28	0.937 ± 0.014	54.1 ± 1.21
	F_2	63 ± 1.1	137 ± 1.9	248 ± 2.8	309 ± 3.0	375 ± 4.2	6.75 ± 0.14	1.032 ± 0.008	67.4 ± 0.71
	C	1.21	5.77**	5.36**	4.14**	2.81**	2.36**	6.49**	38.50**
Az × Mar	Maratelli	25 ± 4.9	60 ± 6.0	158 ± 7.9	214 ± 6.8	274 ± 12.7	2.33 ± 0.20	0.865 ± 0.006	
	Azucena	99 ± 4.9	152 ± 6.0	282 ± 7.9	355 ± 6.8	453 ± 12.7	5.00 ± 0.20	1.019 ± 0.006	
	F_2	92 ± 1.5	144 ± 1.9	249 ± 4.2	295 ± 3.2	353 ± 3.7	4.28 ± 0.09	0.940 ± 0.005	

C = $(2\bar{F}_2 - \bar{F}_1 - (1/2)\bar{P}_1 - (1/2)\bar{P}_2)$ scaling test; * $P \leq 0.05$; ** $P \leq 0.01$.

^a Day 3, 'Bala' × 'Azucena'; day 4, 'Azucena' × 'Maratelli'

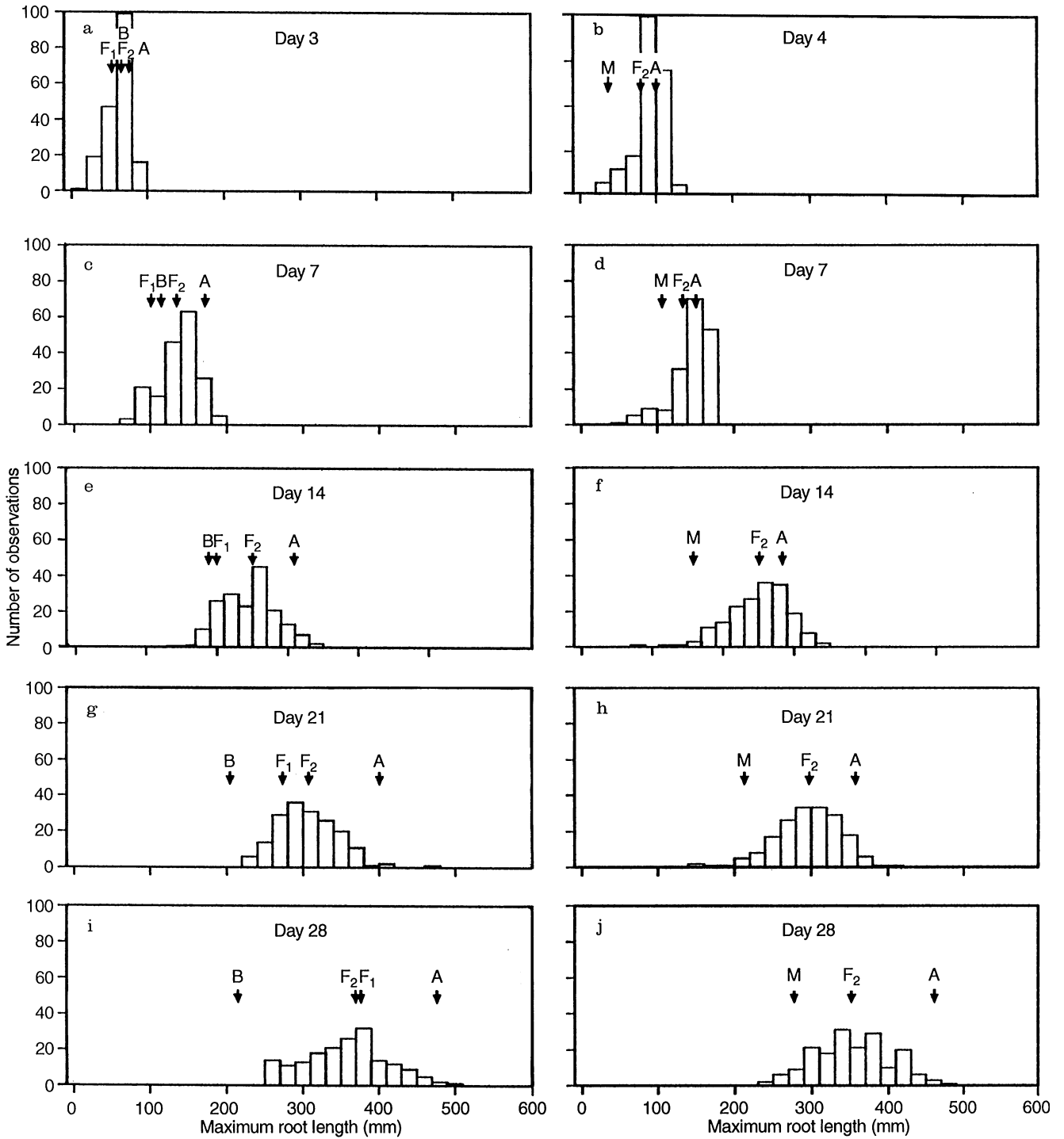


Fig. 3a-j Distribution of maximum root length measured in two hydroponically grown F_2 populations of rice, ‘Bala’ × ‘Azucena’ (a, c, e, g and i) and ‘Azucena’ × ‘Maratelli’ (b, d, f, h and j), 3 (a), 4 (b), 7 (c and d), 14 (e and f), 21 (g and h) and 28 days after sowing (i and j). Arrows indicate the mean of parents. A ‘Azucena’, B ‘Bala’, M = ‘Maratelli’, F_1 ‘Bala’ × ‘Azucena’ only

‘Bala’ × ‘Azucena’ population, but the trait segregated unimodally in both crosses, displaying a normal distribution (Fig. 4). The F_1 and F_2 means of the ‘Bala’ × ‘Azucena’ population were very close to that of the ‘Azucena’ parent, and there appeared to be substantial transgressive segregation; nearly half of the F_2 plants had root volumes higher than that of the ‘Azucena’ parent.

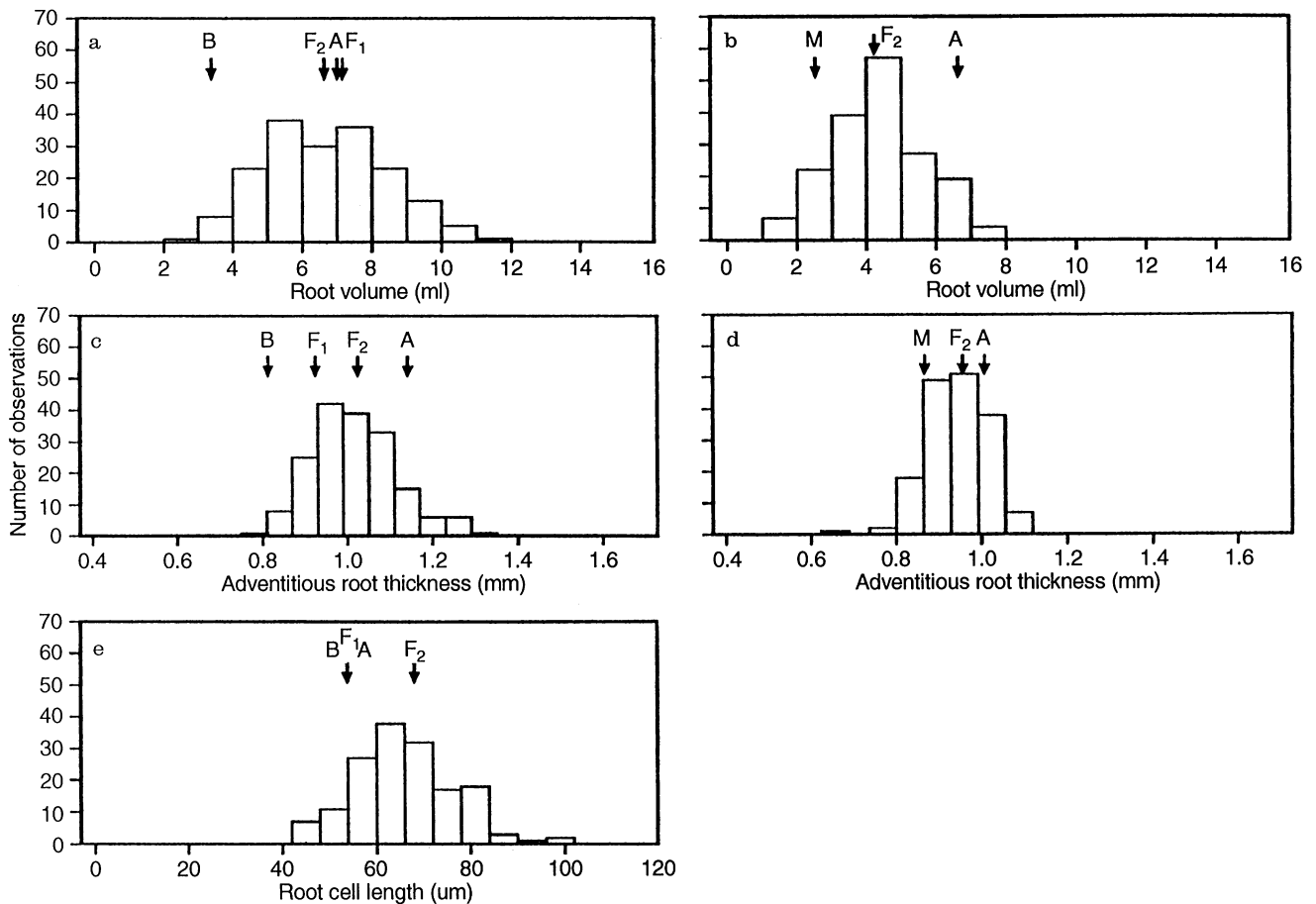


Fig. 4a–e Distribution of root volume (**a** and **b**), adventitious root thickness (**c** and **d**) and epidermal cell length in the fully expanded zone of adventitious root tips (**e**) measured in two hydroponically grown F₂ populations of rice, ‘Bala’ × ‘Azucena’ (**a**, **c** and **e**) and ‘Azucena’ × ‘Maratelli’ (**b** and **d**). Arrows indicate the mean of parents. A ‘Azucena’, B ‘Bala’, M ‘Maratelli’, F₁ ‘Bala’ × ‘Azucena’ only

Adventitious root thickness appeared to segregate unimodally in both populations (Fig. 4), although in the ‘Azucena’ × ‘Maratelli’ population the segregation was less pronounced, probably because ‘Maratelli’ had a root thickness relatively close to that of the ‘Azucena’ parent. A few individuals in both populations had thicker roots than the ‘Azucena’ parent, so both ‘Bala’ and ‘Maratelli’ may be contributing genes for thick roots to the populations. Root-cell lengths of both parents and the F₁ of the cross ‘Bala’ × ‘Azucena’ were essentially the same while those of the F₂ appeared to segregate and were, on average, significantly larger (Fig. 4 and Table 3). In the ‘Bala’ × ‘Azucena’ population it was possible to show the presence of non-allelic interactions for all traits, except root length at 3 days, since the ‘C’ scaling test (Mather and Jinks 1982) showed significant deviation from zero (Table 3); i.e. the F₂ means deviated significantly from half way between the mid-parent means and the F₁ means.

Generation means analysis

The results of generation means analysis of the data are presented in Table 4. Only a full model was adequate to explain variation for maximum root length at different growth periods in both crosses. No test of goodness-of-fit (χ^2) was, therefore, possible. A full model with m, [d], [h] and [l] parameters was fitted to the ‘Bala’ × ‘Azucena’ population, while a three-parameter model (m, [d] and [h]) was fitted to the ‘Azucena’ × ‘Maratelli’ population. With the exception of day 3 in the ‘Bala’ × ‘Azucena’ population, the signs of common parameters obtained for maximum root length were consistent throughout growth periods in both populations. Both additive and dominance components were consistently significant, while the additive component became comparatively larger as time progressed. Perhaps gene expression at different stages of growth shows differential behaviour. Except for day 3 in ‘Bala’ × ‘Azucena’, the sign of [h] and [l] are opposite, indicating that gene interactions are, on balance, mainly of the duplicate (dominant epistatic or recessive repressor) kind. The fact that [h] was positive at all growth stages (except for day 3 in ‘Bala’ × ‘Azucena’) in both populations revealed dominance for longer roots, the genes for which are largely concentrated in the ‘Azucena’ parent.

Table 4 Estimates of genetical parameters from the best-fit model for root growth traits in two crosses of rice, together with broad-sense heritability estimates (%)

Cross ^a	Parameter	Maximum root length (mm)					Root volume (ml)	Root thickness (mm)	Root cell length (μm)
		Day 3/4 ^b	Day 7	Day 14	Day 21	Day 28			
Ba \times Az	m	69.5 \pm 0.4	139.0 \pm 0.4	254.0 \pm 0.3	304.5 \pm 0.3	346.0 \pm 0.4	6.05 \pm 1.02	0.97 \pm 4.47	54.4 \pm 0.6
	[d]	4.5** \pm 0.4	23.0** \pm 0.4	59.0** \pm 0.3	96.0** \pm 0.3	133.0** \pm 0.4	—	—	—
	[h]	-8.5** \pm 4.1	27.0** \pm 3.2	33.0** \pm 2.6	47.5** \pm 2.5	85.0** \pm 2.3	—	—	52.3** \pm 5.2
	[l]	-9.5** \pm 4.1	-62.0** \pm 3.2	-90.0** \pm 2.6	-77.0** \pm 2.5	-54.0** \pm 2.3	—	—	-52.6** \pm 5.2
	χ^2 (df)	—	—	—	—	—	2.39(3)	0.001(3)	0.02(1)
	h^2	2.7	53.3	50.8	43.6	86.5	43.6	5.6	26.4
Az \times Mar	m	62.0 \pm 0.3	106.0 \pm 0.3	220.0 \pm 0.2	284.5 \pm 0.2	363.5 \pm 0.2	3.78 \pm 1.4	0.94 \pm 7.54	—
	[d]	37.0** \pm 0.3	46.0** \pm 0.3	62.0** \pm 0.2	70.5** \pm 0.2	89.5** \pm 0.2	—	—	—
	[h]	60.0** \pm 1.7	76.0** \pm 1.6	44.0** \pm 1.1	21.0** \pm 1.2	-21.0** \pm 1.2	—	—	—
	χ^2 (d.f.)	—	—	—	—	—	0.73(2)	0.000(2)	—
	h^2	15.5	—	49.2	72.1	13.4	45.8	87.9	—

^aNote: Azucena was the higher parent in both crosses; Ba, 'Bala'; Az, 'Azucena'; Mar, 'Maratelli'

* $P \leq 0.05$; ** $P \leq 0.01$

^bDay 3: 'Bala' \times 'Azucena', day 4, 'Azucena' \times 'Maratelli'

For root volume and adventitious root thickness the only model which could be adequately fitted to the data from either cross was the one with only the m parameter which amounts to the non-detection of additive and dominance gene effects in the generation means analysis. Examination of the consequence of model fitting revealed very large standard errors of the genetic parameters. Perhaps the precision of χ^2 has been affected by the smaller degrees of freedom on which the generation means standard errors were based.

With respect to root-cell length in the 'Bala' \times 'Azucena' population, the variation was adequately explained by fitting an m, [h] and [l] model, without an additive component. Again, the dominance was in the positive direction, and duplicate gene interactions were indicated. The non-significance of the additive component, [d], is apparent from the similarities of the parental means. This could be explained by the presence of dispersed genes in both parents with predominantly dominance effects (see later).

Generation means are subject to internal cancellation of gene effects. With more than two interacting genes, cancellation can arise not only from the effects of gene dispersion in the parents but also from the direction of the effects of individual genes and their interactions with pairs of interacting genes. This balancing action introduced by differences in sign is always likely to be encountered in the generation means and comparisons between them. It is partly overcome by turning to second-degree statistics. While the available number of generations did not allow model fitting on variances, the variances of the F_2 and non-segregating generations were sufficient to partition the total variance of the F_2 into genetic and non-genetic components and to compute broad-sense heritability (h^2 , Table 4).

In 'Bala' \times 'Azucena', heritability estimates increased from day 7 to day 28 for maximum root length, reaching 86.5%. However, in 'Azucena' \times 'Maratelli', day 14

and 21 had higher estimates than day 28. Non-genetic variation was detected from generation means analysis for root volume and adventitious root thickness in both crosses, but the second-degree analysis produced reasonable estimates of heritability (43.6%–87.9%) except for adventitious root thickness in the 'Bala' \times 'Azucena' population (5.6%). The presence of dispersed genes with large additive effects could give rise to the observed discrepancies between the outcome of the generation means and second degree statistical analysis. The two analyses are, therefore, complementary in studying the genetics of any trait. For root cell length, heritability was 26.4% in the 'Bala' \times 'Azucena' population where duplicate gene interactions were detected which normally result in reducing the variances.

Discussion

The work presented in this paper was initially aimed at producing a screening system and segregating populations in which the genetic control of root characters related to drought resistance could be studied in up-land rice. The hydroponic root screening system described, which can allow one person to screen up to 300 plants at a time, appears to have considerable potential for predicting root growth in the field. Maximum root length and adventitious root thickness in this hydroponic experiment agreed strongly with maximum root length ($r = 0.872$, $P < 0.001$) and root thickness ($r = 0.825$, $P < 0.001$) of 12 varieties also studied by Loresto et al. (1983). These authors found very good agreement between root growth in liquid culture and previously recorded data on root growth in both soil pots and the field. The correlations between both root length and root thickness with field drought resistance scores from the IRRI data bank reported here and by Loresto et al. (1983) provide powerful evidence that

root growth in liquid culture does reflect root growth in the field and that maximum root length and thickness are important attributes contributing to drought resistance in rice. These two conclusions are supported by the positive correlations between field drought resistance of F_3 families and data for hydroponic root growth of the F_2 plants from which they were derived as reported by Ekanayake et al. (1985b). However, since root growth in soil appeared to be much faster than in hydroponics, it could be that hydroponic systems exaggerate natural differences between varieties.

The regression of drought score on root length presented in Fig. 3 demonstrates clearly that both 'Bala' and 'IR20' have a maximum root length (and root thickness) substantially below that predicted by their drought resistance scores. Loresto et al. (1983) also found that 'IR20' was notably different from other varieties when root thickness was compared to drought resistance scores. We have found that these 2 varieties roll their leaves considerably more slowly than 11 other rice varieties when excised (unpublished data) and, in the case of 'Bala', it was found that leaf excision causes a very rapid stomatal closure (compared to 'Azucena') (unpublished data). Lilley and Ludlow (1996) report that 'Bala' has a pronounced ability to adjust osmotically, and we therefore believe that 'Bala' (and probably 'IR20') possess several leaf-related mechanisms of drought resistance which overcome its poor rooting system.

The upland varieties clearly seem to possess a more pronounced root system than lowland varieties, which may reflect adaptation to the irrigated (lowland) or rainfed (upland) conditions. In addition, *japonica* varieties possess more pronounced root systems than *indica* varieties, but the only temperate *japonica* studied, 'Maratelli', proved to be an exception to this rule.

The measurement of maximum root length, root volume and adventitious root thickness seems to be ideally suitable to the study of root growth in F_2 populations of rice, particularly if the plants are required after analysis (e.g. for seed production), since these parameters are easily and accurately measured, are non-destructive, are highly variable within the species of *Oryza sativa* and readily discriminate between subspecies and ecotypes.

The inheritance of root length, root thickness and root volume reported here is in broad agreement with results from other authors. All traits were found to segregate unimodally in F_2 populations [Chang et al. 1982; Armenta-Soto et al. 1983 (root thickness and maximum root length only) and Ekanayake et al. 1985b]. As we report, maximum root length has been shown to be controlled by a combination of additive and dominant gene effects and is moderately to highly heritable (Chang et al. 1982; Ekanayake et al. 1985b). Both Chang et al. (1982) and Ekanayake et al. (1985b) reported root thickness to be highly heritable but found

root volume or root weight to be only moderately heritable. We find low to high heritability for root thickness and moderate heritability of root volume. Chang et al. (1982) found additive and dominance gene effects for both root thickness and root weight, while Ekanayake et al. (1985b) found predominantly additive gene effects for thickness and volume. This contrasts with our failure to detect any significant additive or dominance gene effects for these traits with generation means analysis. However, the analysis of second-degree statistics and heritability supported the findings of Chang et al. (1982) and Ekanayake et al. (1985b). The contradiction between analyses within our data is perhaps due to dispersal of genes between the parents.

Transgressive segregation was not observed for maximum root length in this study, but it was for root thickness and root volume. Armenta-Soto et al. (1983) found substantial transgression for maximum root length in two out of three crosses (transgression in 'OS4' \times 'IR8' and 'Moroberekan' \times 'OS4'; no transgression in 'IR20' \times 'IR480-5-9'), while Ekanayake et al. (1985b) found no transgression for maximum root length in their cross ('MGL2' \times 'IR20'). Neither authors found transgression of root thickness and Ekanayake et al. (1985b) found no transgression for root volume either.

Taken together, studies of root growth in rice suggest that these traits are under genetic control, although reported heritabilities, transgression and the magnitude of additive and dominance effects do vary. It should, therefore, be possible to derive recombinant lines with improved root characteristics from these crosses. However, due to the presence of non-allelic interactions, the success rate will be higher if selection procedures involve progeny evaluation.

In the 'Bala' \times 'Azucena' population, root-cell length was not related to maximum root length although it does seem to be under some genetic control. Since longitudinal root growth (root extension growth) is a function of how many cells are being made and the final length to which they grow, it must be concluded that, in this population at least, the major determinant of the rate of root growth is the rate of cell division in the apical meristem.

In a companion paper (Price and Tomos 1997), the 'Bala' \times 'Azucena' population used here was mapped with 82 RFLP markers and quantitative trait loci (QTLs) for the root growth characteristics identified. Recombinant inbred lines (F_6) derived by single-seed descent from the 'Bala' \times 'Azucena' cross are being tested for drought resistance in the field (at the International Rice Research Institute, Philippines, in the first place) and for root growth in hydroponics. The segregation of shoot-related mechanisms of drought resistance mechanisms will also be investigated. Studies of this population and other populations which have been (Champoux et al. 1995) or are being developed and mapped with molecular markers will advance

knowledge of the physiology and genetics of drought resistance profoundly.

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References

- Ahmadi N (1983) Variabilité génétique et hérédité de mécanismes de tolérance à la sécheresse chez le riz *Oryza sativa* L.: I. Développement du système racinaire. *Agron Trop* 38: 110–117
- Armenta-Soto J, Chang TT, Loresto GC, O'Toole JC (1983) Genetic analysis of root characters in rice. *SABRAO J* 15: 103–118
- Arraudeau M, Harahap Z (1986) Relevant upland breeding objectives. In: Progress in upland rice research. IRRI, Manila, pp 189–197
- Causse M, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SB, Second GA, McCouch SR, Tanksley SD (1995) Saturated molecular map of the rice genome based on an inter-specific backcross population. *Genetics* 138: 1251–1274
- 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
- Chang TT, Loresto GC, O'Toole JC, Armenta-Soto JL (1982) Strategy and methodology of breeding rice for drought-prone areas. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 217–244
- David CC (1991) The world rice economy: challenges ahead. In: Khush GS, Toenniessen GH (eds) Rice biotechnology. IRRI, Manila, pp 1–18
- DeDatta SK, Seshu DV (1982) Evaluating rices for drought tolerance using field screening and multi-location testing. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 245–263
- DeDatta SK, Malabuyoc JA, Aragon EL (1988) A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. *Field Crop Res* 19: 123–134
- Ekanayake IJ, Garity DP, Masajo TM, O'Toole JC (1985a) Root pulling resistance in rice: inheritance and association with drought tolerance. *Euphytica* 34: 905–913
- Ekanayake IJ, O'Toole JC, Garrity DP, Masajo TM (1985b) Inheritance of root characters and their relations to drought resistance in rice. *Crop Sci.* 25: 927–933
- Fukai S, Cooper M (1995) Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crop Res* 40: 67–86
- Hanson AD, Peacock WJ, Evans LT, Arntzen CJ, Khush GS (1990) Drought resistance in rice. *Nature* 345: 26–27
- Ingram KT, Real JG, Maguling MA, Obien MA, Loresto GC (1990) Comparison of selection indices to screen lowland rice for drought resistance. *Euphytica* 48: 253–260
- IRRI (1993) IRRI rice almanac. IRRI, Manila
- Jones MM, Turner NC, Osmond CB (1981) Mechanisms of drought resistance. In: Paleg G, Aspinall D (eds) The physiology and biochemistry of drought resistance in plants. Academic Press, New York, pp 15–37
- Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shoura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nat Genet* 8: 365–372
- Levitt J (1980) Responses of plants to environmental stress, vol. 2: water, radiation, salt and other stresses. Academic Press, New York
- Lilley JM, Fukai S (1994) Effect of timing and severity of water deficit on four diverse rice cultivars III. Phenological development, crop growth and grain yield. *Field Crops Res* 37: 225–234
- Lilley JM, Ludlow MM (1996) Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crop Res* 48: 185–197
- Loresto GC, Zhang WX, Chaudhary D, Chang TT (1983) Aeroponic technique for screening the drought avoidance mechanism of rice genotypes by root characters. *Garcia de Orta* 10: 77–82
- Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. *Adv Agron* 43: 107–153
- Mather K, Jinks JL (1982) Biometrical genetics, 3rd edn. Chapman and Hall, London
- O'Toole JC (1982) Adaptation of rice to drought-prone environments. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 195–213
- O'Toole JC, Bland WL (1987) Genotypic variation in crop plant root systems. *Adv Agron* 41: 91–145
- O'Toole JC, Soemartono (1981) Evaluation of a simple technique for characterising rice root systems in relation to drought resistance. *Euphytica* 30: 283–290
- Passioura JB (1982) The role of root system characteristics in the drought resistance of crop plants. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 71–82
- Price AH, Tomos AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.) II: mapping quantitative trait loci using molecular markers. *Theor Appl Genet* 95: 143–152
- Snedecor GW, Cochran WG (1989) Statistical methods, 8th edn. Iowa State University Press, Ames, Iowa
- Tran Van Dat (1986) An overview of upland rice in the world. In: Progress in upland rice research. IRRI, Manila, pp 51–66
- Yoshida S, Hasegawa S (1982) The rice root system; its development and function. In: Drought resistance in crops with the emphasis on rice. IRRI, Manila, pp 83–96
- Yoshida S, Forno DA, Lock JH, Gomez KA (1976) A laboratory manual for the physiological studies of rice. IRRI, Manila