

The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays*) seedlings

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Abstract. Low soil phosphorus availability is a primary constraint for plant growth in many terrestrial ecosystems. Lateral root initiation and elongation may play an important role in the uptake of immobile nutrients, such as phosphorus, by increasing soil exploration and phosphorus solubilisation. The overall objective of this study was to assess the value of lateral rooting for phosphorus acquisition through assessment of the ‘benefit’ of lateral rooting for phosphorus uptake and the ‘cost’ of lateral roots in terms of root respiration and phosphorus investment at low and high phosphorus availability. Five recombinant inbred lines (RILs) of maize derived from a cross between B73 and Mo17 with contrasting lateral rooting were grown in sand culture in a controlled environment. Genotypes with enhanced or sustained lateral rooting at low phosphorus availability had greater phosphorus acquisition, biomass accumulation, and relative growth rate (RGR) than genotypes with reduced lateral rooting at low phosphorus availability. The association of lateral root development and plant biomass accumulation under phosphorus stress was not caused by allometry. Genotypes varied in the phosphorus investment required for lateral root elongation, owing to genetic differences in specific root length (SRL, which was correlated with root diameter) and phosphorus concentration of lateral roots. Lateral root extension required less biomass and phosphorus investment than the extension of other root types. Relative growth rate was negatively correlated with specific root respiration, supporting the hypothesis that root carbon costs are an important aspect of adaptation to low phosphorus availability. Two distinct cost–benefit analyses, one with phosphorus acquisition rate as a benefit and root respiration as a cost, the other with plant phosphorus accumulation as a benefit and phosphorus allocation to lateral roots as a cost, both showed that lateral rooting was advantageous under conditions of low phosphorus availability. Our data suggest that enhanced lateral rooting under phosphorus stress may be harnessed as a useful trait for the selection and breeding of more phosphorus-efficient maize genotypes.

Keywords: cost–benefit analysis, lateral rooting, maize inbred lines, phosphorus efficiency, root respiration.

Introduction

Low soil phosphorus availability is a primary constraint to plant growth in terrestrial ecosystems (Lynch and Deikman 1998). Maize (*Zea mays* L.) is generally considered to have a high fertility requirement, but variation in phosphorus efficiency is known to exist among maize genotypes (Anonymous 1887; Clark and Brown 1974; Naismith *et al.* 1974; Nielsen and Barber 1978; DaSilva and Gabelman 1993; Kaeppler *et al.* 2000; Gaume *et al.* 2001). A better understanding of physiological traits that improve phosphorus acquisition would facilitate the development of phosphorus-efficient maize genotypes, which would be beneficial in low-input agroecosystems and would improve

the sustainability of high-input agroecosystems (Lynch 1998).

Lateral roots arise from pre-existing roots and develop from the pericycle layer (Macleod and Thompson 1979). Several lines of direct and indirect evidence have suggested a role for lateral roots in plant adaptation to low phosphorus availability. Lateral rooting is stimulated by low phosphorus availability in white lupin, in which lateral roots exude large amounts of citrate that increases phosphorus solubilisation (Watt and Evans 1999). However, lupin is unusual in being non-mycorrhizal but adapted to phosphorus-deficient soils. Its rooting strategy can be viewed as concentrated mineralisation of sparingly soluble phosphate pools (Watt

Abbreviations used: AVG, amino-ethoxyvinylglycine; *M*, phosphorus content per plant; *R*, root biomass; RILs, recombinant inbred lines; RGR, relative growth rate; RSR, root : shoot ratio; SPAR, specific phosphorus absorption rate; SRL, specific root length; SRR, specific root respiration.

and Evans 1999). In common bean (*Phaseolus vulgaris* L.), which is physiologically more representative of the majority of annual crop species, low phosphorus availability causes greater dispersal of lateral roots along the root axis, but in patches of soil with high phosphorus availability lateral rooting is denser, resulting in concentrated soil foraging (Lynch and Brown 1998; Borch *et al.* 1999). The gravitropic setpoint angle (Firn and Digby 1997) of basal roots, a special class of lateral roots (Zobel 1996), is also sensitive to phosphorus availability, such that roots of phosphorus-deficient plants are shallower, thereby concentrating root foraging in the topsoil, which usually contains a higher phosphorus availability than lower strata (Lynch and Brown 2001).

Lateral root development is affected by many internal and external factors, including phosphorus availability, hormones, nitrogen supply, soil oxygen and humic acids (Wightman *et al.* 1980; Visser *et al.* 1997; Zhang and Forde 1998; Borch *et al.* 1999; Mollier and Pellerin 1999; Canellas *et al.* 2002). In common bean, low phosphorus availability reduced lateral root number and sustained main root elongation, resulting in decreased lateral root density in phosphorus-deficient plants (Borch *et al.* 1999). In the study by Borch *et al.* (1999), inhibiting endogenous ethylene production with amino-ethoxyvinylglycine (AVG) increased lateral root density in phosphorus deficient plants and reduced it in phosphorus sufficient plants. These responses were reversed by exogenous ethylene, indicating ethylene involvement in the regulation of main root extension and lateral root spacing.

Lateral rooting could be considered to contribute to efficient phosphorus acquisition if the metabolic costs associated with the production and maintenance of lateral roots were exceeded by the metabolic benefits of enhanced phosphorus acquisition (Lynch and Ho 2004). One measurement of root cost is total belowground expenditure of carbon, which is used for root construction, root maintenance, and ion uptake (Lambers *et al.* 1996); one measurement of root benefit is phosphorus uptake (Bates and Lynch 2000). Another measurement of root cost is the nutrient investment in belowground organs (Koide and Elliott 1989; Snapp *et al.* 1995). In maize, the cost of lateral roots can be measured as carbon expended and the benefit as root phosphorus uptake, or, the benefit of lateral roots can be measured as plant phosphorus uptake and the cost as phosphorus invested in lateral roots. Accurate assessment of the costs and benefits associated with lateral roots would demonstrate the adaptive value of lateral roots in low phosphorus environments.

The overall objective of this study was to assess the value of lateral rooting for phosphorus acquisition in maize through measurement of the 'benefit' of lateral rooting for phosphorus uptake and 'cost' of lateral roots in terms of root respiration and phosphorus investment at low and high phosphorus availability. We employed maize RILs with a common genetic

background but contrasting lateral root development under low phosphorus availability.

Materials and methods

Plant materials

Five RILs of maize (*Zea mays* L.), numbers 14, 20, 81, 128, and 192 from the cross B73 × Mo17 were obtained from Dr Shawn Kaeppler (University of Wisconsin, Madison, USA), originally supplied by Charles Stuber and Lynn Senior at North Carolina State University (Senior *et al.* 1996; Kaeppler *et al.* 2000). The parents have contrasting adaptation to low phosphorus availability in the greenhouse (Kaeppler *et al.* 2000; Zhu 2003). Our previous screening in cigar-roll culture for lateral rooting demonstrated that RILs 14 and 20 had shorter lateral root length and a lower lateral root number at low phosphorus than at high phosphorus availability, and RILs 81, 128, and 192 had fixed lateral rooting at low and high phosphorus. These genotypes had similar root hair length and density, eliminating root hair variation (Zhu 2003) as a possible confounding factor.

Experimental design

The experimental design was a completely randomised design with a 2 × 5 factorial arrangement of treatments. The factors were two phosphorus levels (low and high), and five genotypes (RILs 14, 20, 81, 128, and 192), replicated four times.

Growth conditions

Seed of five genotypes were selected for uniform size, sterilised, and germinated in darkness at 28 ± 1°C in a germination chamber for 3 d. Before transplanting, the cotyledon was removed from the seedlings. Seedlings with similar size were transferred to 4-L plastic pots filled with acid-rinsed solid-phase-buffered sand (Lynch *et al.* 1990) providing a constant availability of low (0.2 µM), and high (30 µM) phosphorus concentration in the soil solution. Phosphorus availability in this media is diffusion limited and subject to localised depletion because of the action of activated aluminum oxide particles mixed in with the sand, which adsorb and desorb phosphate by the same reactions that occur on oxide surfaces in natural soil. Pots were irrigated twice daily (0700 and 1400 h) with nutrient solution consisting of (in µM): K (3000), NO₃ (7000), NH₄ (1000), Ca (2000), SO₄ (500), Mg (500), Cl (25), B (12.5), Mn (1), Zn (1), Cu (0.25) Mo (0.25) and EDTA-Fe (25). Plants were grown in a temperature-controlled greenhouse in University Park, PA, (40°49' N, 77°49' W), with a photoperiod of 14/10 h at 28/24°C (light/dark). Maximum midday photosynthetic flux densities reached 1200 µmol photons m⁻²s⁻¹. The relative humidity was 50%. The nutrient solution pH was adjusted daily to 5.8.

Intact root respiration

The 'head space' approach of sampling air flowing over the soil surface was employed to measure intact root respiration in this study. Extensive studies of common bean and citrus root respiration in soil indicated that root respiration was not influenced by soil moisture or soil CO₂ concentration, and there was no difference in intact root respiration when comparing 'head space' measurements to 'perfusive' measurements (Bouma *et al.* 1997a, b). At day 7 after transplanting, the root system was sealed off from the shoot by a PVC plate with a foam gasket sealed around the base of the stem. An air pump provided a stable flow of air through the 'head space' compartment of the growth container. The measurements were conducted in early morning with a portable infrared gas analyser in differential mode (Li-Cor 6250, Li-Cor, Lincoln, NE), ensuring that the 'head space' CO₂ concentration remained relatively low and that the 'head space' temperature only increased slightly. During the measurement, CO₂ changed less than 20 µL L⁻¹. Since the media did not contain carbon, any microbial respiration from the containers would

have consumed carbon derived from the roots and therefore represents root carbon cost.

Growth analysis

Dry weight (DW) and phosphorus concentration of shoots and roots of 3-day-old seedlings were collected before transplanting. Plants were harvested 7 d after transplanting. Shoots and roots were separated from the sand by submerging pots in a container filled with water and rinsing the roots carefully in deionised water. Lateral root length and number were obtained by scanning with image analysis software (WinRhizo Pro, Régent Instruments, Québec, Canada). The shoots, lateral roots of the taproot, and other roots were dried at 60°C for 72 h before dry weight determination. Relative growth rate (RGR_{DW}) was calculated as:

$$RGR_{DW} = (DW_7 - DW_0) / (DW_0), \quad (1)$$

where DW_0 is the plant dry weight (g) at the time of transplanting and DW_7 is plant dry weight (g) 7 d after transplanting.

Phosphorus accumulation

Tissue samples were ashed at 495°C for 8 h, then dissolved in 4 mL of 100 mM HCl and analysed for phosphorus concentration spectrophotometrically (Murphy and Riley 1962). Specific phosphorus absorption rate (SPAR) is a measure of the net phosphorus absorption per unit root biomass ($\mu\text{mol P g}^{-1}$ root DW min^{-1}) over the interval t_1 to t_2 , and was calculated as:

$$SPAR = (M_2 - M_1) / (t_2 - t_1) \times (\log_{10} R_2 - \log_{10} R_1) / (R_2 - R_1), \quad (2)$$

where M is the phosphorus content per plant (μmol), and R is root biomass (g DW) (Hunt 1990).

Statistical analysis

Data were analysed with the StatView statistical package (SAS Inc., Cary, NC). Differences in lateral root length and number, and plant dry weight between low and high phosphorus treatments were determined for each genotype by paired t -tests assuming equal variance ($P < 0.05$). RGR, root:shoot ratio, specific root respiration (SRR), SPAR, cost-benefit ratio, plant phosphorus uptake, lateral root phosphorus content, and benefit-cost ratio were analysed by two-way ANOVA for main effects and first-order interactions with a general linear model that included phosphorus and genotype factors. Genotype and phosphorus level were considered fixed effects, and replicates were random. Fisher's protected least significance difference (LSD) was calculated for multiple comparisons.

Results

Lateral rooting

At day 7 after transplanting, low phosphorus availability significantly reduced lateral root length of RILs 20 and 192, but did not affect lateral root length of RILs 14 and 128 and increased lateral root length of RIL 81 (Fig. 1). Low phosphorus availability significantly reduced lateral root number of RIL 20, but did not affect lateral root number of RILs 14, 128, and 192, and increased lateral root number of RIL 81 (Fig. 1).

Plant growth

Two RILs (20 and 192) with reduced lateral rooting at low phosphorus availability had significantly less plant DW than three RILs (14, 81, and 128) with sustained or enhanced

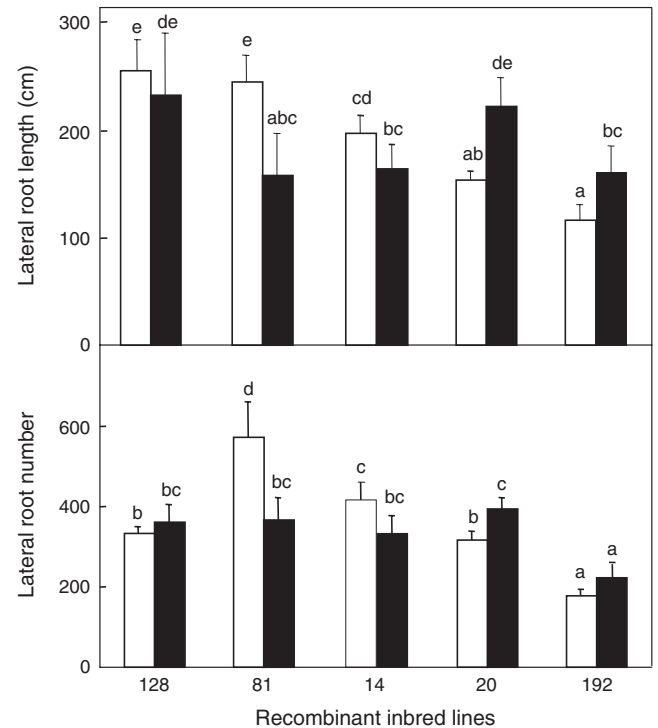


Fig. 1. Lateral root total length (top panel) and number (bottom panel) arising from the taproot 7 d after transplanting of five maize recombinant inbred lines as influenced by low-phosphorus (open bars) and high-phosphorus (closed bars) availability. Data shown are means \pm SE of mean ($n = 4$). Columns with different letters differ significantly at the 5% level by Fisher's LSD.

lateral rooting at low phosphorus availability. However, all genotypes except 81 had similar biomass at high phosphorus availability (Fig. 2). The three RILs (14, 81, and 128) with sustained or enhanced lateral rooting had similar plant DW in low and high phosphorus media (Fig. 2).

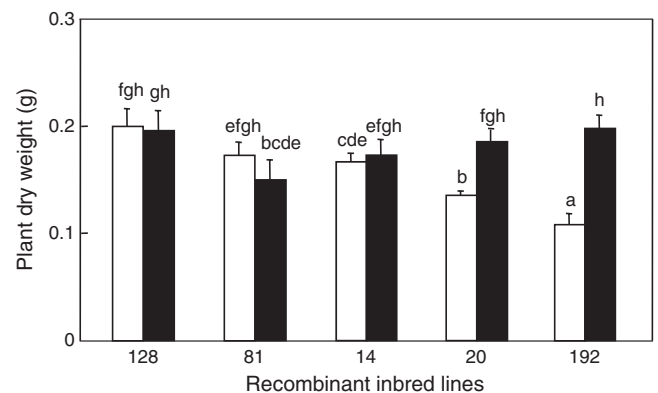


Fig. 2. Plant dry weight 7 d after transplanting of five maize recombinant inbred lines from B73 \times Mo17, as influenced by low-phosphorus (open bars) and high-phosphorus (closed bars) availability. Data shown are means \pm SE of mean ($n = 4$). Columns with different letters differ significantly at the 5% level by Fisher's LSD.

Table 1. Relative growth rate (RGR_{DW}), root : shoot ratio (RSR), and specific root length (SRL) of lateral and non-lateral roots of maize recombinant inbred lines (RILs) as influenced by phosphorus availability
Numbers with different superscript letters differ significantly at the 5% level by Fisher's LSD for means from low P or high P. *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; ns, not significant ($n = 4$)

Phosphorus level	RIL	RGR _{DW}	RSR	SRL lateral roots (m g ⁻¹)	SRL non-lateral roots (m g ⁻¹)
Low	128	5.30 ^c	0.59 ^{ab}	59.0 ^a	32.9 ^{ab}
	81	5.31 ^c	0.58 ^a	100.0 ^b	40.6 ^{ab}
	14	3.84 ^b	0.74 ^{bc}	71.3 ^a	42.3 ^b
	20	3.26 ^b	0.54 ^a	65.1 ^a	25.6 ^a
	192	2.44 ^a	0.85 ^c	48.4 ^a	43.2 ^b
High	128	5.19 ^b	0.43 ^a	59.5 ^a	27.9 ^a
	81	4.42 ^{ab}	0.56 ^b	47.6 ^a	33.5 ^{ab}
	14	4.00 ^a	0.51 ^b	55.9 ^a	36.0 ^{ab}
	20	4.86 ^{ab}	0.37 ^a	85.3 ^b	37.1 ^{ab}
	192	5.25 ^b	0.51 ^b	69.1 ^a	39.9 ^b
F from ANOVA					
Block		0.40ns	0.10ns	0.21ns	0.13ns
Genotype		2.45*	3.35**	1.54ns	1.29ns
Phosphorus		3.89**	33.7***	0.88ns	0.28ns
Genotype × phosphorus		3.33**	1.31ns	5.70***	0.80ns

At low phosphorus availability, the three RILs (14, 81, and 128) with enhanced or sustained lateral rooting had greater RGR than RILs (20 and 192) with reduced lateral rooting. Low phosphorus availability increased root : shoot ratio by approximately 39% (Table 1). In general, the SRL of lateral roots exceeded that of non-lateral roots (Table 1). Genotypes had substantially different SRL at low phosphorus, ranging from 48 to 100 m g⁻¹ (Table 1), and low phosphorus availability increased the SRL of RIL 81 (Table 1). There was a strong correlation between SRL and root diameter (Fig. 3). Phosphorus investment in lateral roots per unit root length was substantially less than in non-lateral roots (Table 2). Phosphorus content per unit length of lateral roots varied up to 175% among genotypes under phosphorus stress, which was related to significant genetic differences in phosphorus content per unit root weight as well as SRL (Table 2).

Root respiration

Low phosphorus availability significantly reduced SRR per unit of root weight and root length (Fig. 4). RIL 192, with inhibited lateral rooting at low phosphorus availability, had the greatest SRR per unit of root weight and root length among the five genotypes. Genotypes showed significant variation for root respiration per unit weight at high phosphorus availability. Specific root respiration at low phosphorus availability was negatively correlated with RGR (Fig. 5).

Cost–benefit analyses

To assess the importance of lateral rooting in phosphorus acquisition, we conducted two cost–benefit analyses; one

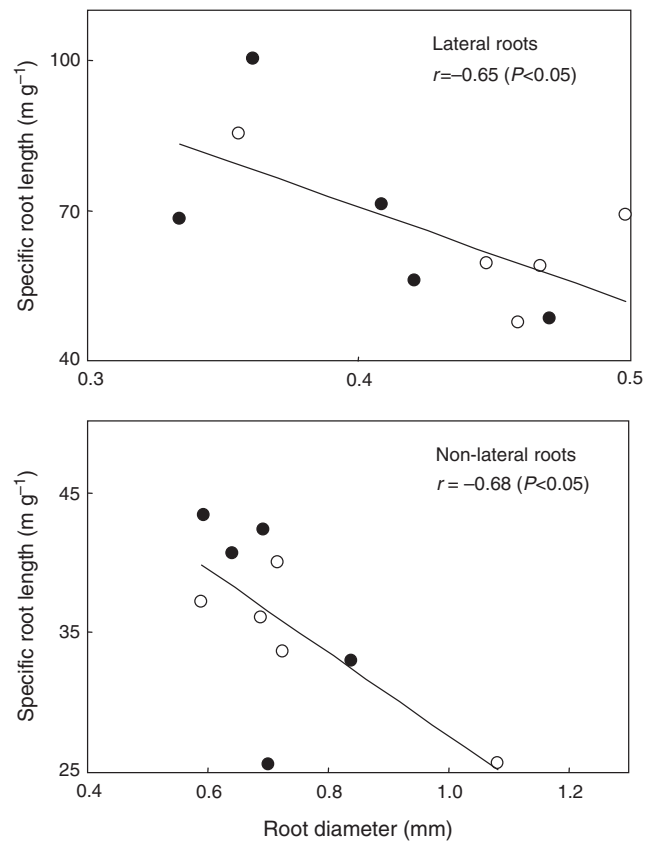


Fig. 3. Significantly negative associations between specific root length (SRL) and average root diameter of lateral and non-lateral roots of maize recombinant inbred lines grown at low- and high-phosphorus availability. ●, plants grown at low phosphorus availability; ○, plants grown at high phosphorus availability.

Table 2. Root phosphorus content of maize recombinant inbred lines (RILs) measured 7 d after transplanting, as influenced by phosphorus availability

Numbers with different superscript letters differ significantly at the 5% level by Fisher's LSD for means from low P or high P. *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; ns, not significant ($n = 4$)

Phosphorus level	RIL	P content per unit lateral root length ($\mu\text{g P m}^{-1}$)	P content per unit lateral root weight ($\mu\text{g P g}^{-1}$)	P content per unit non-lateral root length ($\mu\text{g P m}^{-1}$)	P content per unit non-lateral root weight (mg P g^{-1})
Low	128	75 ^{ab}	4.5 ^{ab}	306 ^b	11.1 ^b
	81	61 ^a	6.0 ^{bc}	103 ^a	4.1 ^a
	14	98 ^{bc}	7.0 ^c	108 ^a	4.5 ^a
	20	56 ^a	3.6 ^a	168 ^a	3.9 ^a
	192	92 ^b	4.3 ^{ab}	134 ^a	5.2 ^a
High	128	121 ^a	6.9 ^a	622 ^b	14.6 ^b
	81	275 ^b	12.2 ^c	592 ^b	18.5 ^c
	14	177 ^a	8.5 ^b	296 ^a	10.6 ^{ab}
	20	120 ^a	9.7 ^b	221 ^a	10.9 ^{ab}
	192	193 ^a	12.4 ^c	176 ^a	6.8 ^a
F from ANOVA					
Block		0.44ns	1.2ns	0.70ns	0.60ns
Genotype		2.3*	3.1**	1.0ns	4.1***
Phosphorus		26.3**	50.6***	6.5**	26.6***
Genotype \times phosphorus		2.3*	3.3**	0.62ns	3.0**

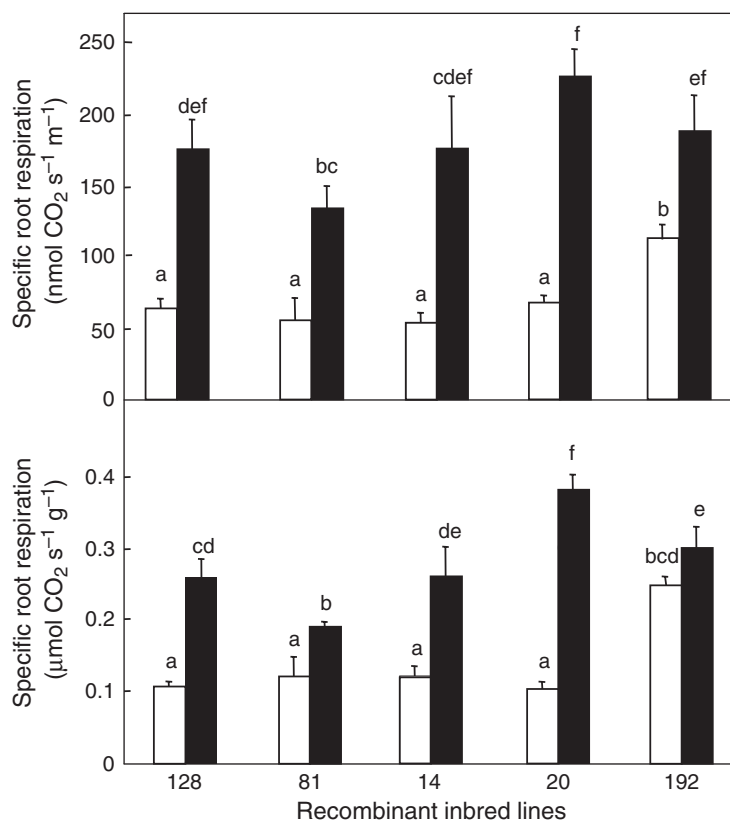


Fig. 4. Specific respiration per unit root length (top panel) and weight (bottom panel) of maize recombinant inbred lines 7 d after transplanting as influenced by low phosphorus (open bars) and high phosphorus (closed bars) availability. Data shown are means \pm SE of mean ($n = 4$). Columns with different letters differ significantly at the 5% level by Fisher's LSD.

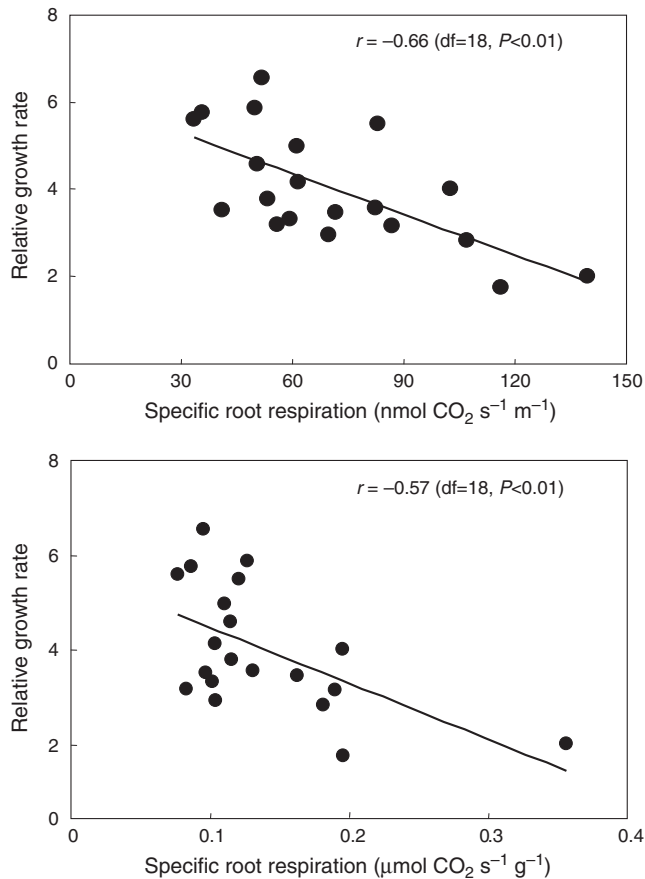


Fig. 5. Significantly negative association between RGR and SRR at low phosphorus availability in maize recombinant inbred lines 7 d after transplanting ($n = 4$).

analysis was based on carbon costs and the other on phosphorus costs of lateral rooting. In the first case, cost was defined as root respiration, measured as CO₂ evolution per minute per unit root weight, while benefit was defined as SPAR, measured as phosphorus uptake per day per unit root weight (Table 3). At low phosphorus availability, the three RILs (14, 81, and 128) with sustained or enhanced lateral rooting had greater SPARs than the RILs (20 and 192) with inhibited lateral rooting (Table 3). The RILs with sustained or enhanced lateral rooting had smaller cost–benefit values than the RILs with inhibited lateral rooting at low phosphorus availability. At high phosphorus, no significant difference was found for the cost–benefit among these five genotypes.

For the phosphorus-based analysis, benefit was defined as plant phosphorus uptake measured as nmol phosphorus per minute per plant and cost was defined as phosphorus content invested in the lateral roots. At low phosphorus, the three RILs (14, 81, and 128) with sustained or enhanced lateral rooting had plant phosphorus uptake and benefit–cost values that were greater than the RILs (20 and 192) with inhibited lateral rooting (Table 3).

Correlation analysis

Lateral root length was positively correlated with plant phosphorus content, RGR, plant DW, and SPAR at low phosphorus availability (Table 4). Lateral root length and lateral root number were negatively associated with the cost–benefit ratio. Specific root respiration, per unit of root weight or root length, was negatively correlated with RGR under low phosphorus (Table 4; Fig. 5).

Discussion

Our results support the hypothesis that increased or sustained lateral rooting promotes maize seedling growth under phosphorus limitation, as evidenced by greater phosphorus acquisition, biomass accumulation, RGR and two different estimates of cost–benefit ratios. We employed conditions designed to minimise the confounding effects of other traits related to low phosphorus adaptation as well as the autocorrelation of lateral rooting with plant growth through allometry. The use of RILs ensured that the phenotypes we compared shared the same genetic background. We specifically selected RILs with similar root hair length and density to avoid the confounding effects of root hair length and density on phosphorus acquisition, for which significant genetic variation exists in maize (Zhu 2003). The phosphorus buffered sand culture was a growth medium that provided diffusion-limited phosphorus regimes at realistic concentrations (Lynch *et al.* 1990), while eliminating possible confounding effects from genotypic differences in the ability to mobilise specific soil phosphorus pools through root exudates such as organic acids and phosphatases (Hinsinger 2001). The young seedlings sampled were still benefiting from seed phosphorus reserves, and thus, we minimised the possible confounding effects of large differences in biomass accumulation, which would confuse phenotype evaluation since growth itself is associated with increased lateral rooting through allometry (Niklas 1994). Because we used young seedlings, plants were harvested at only one age — multiple harvests over time would have permitted a more detailed analysis of growth and phosphorus dynamics. The correlation of the log of shoot DW and the log of root DW at 7 d after transplanting was weak ($r^2 = 0.2$) and non-significant (Fig. 6), indicating that differences we observed in root development were not simply determined by allometry. Therefore, we believe that the conditions we employed permit a robust evaluation of the effects of lateral rooting on phosphorus acquisition.

For the analysis of the physiological value of phenotypic traits, RILs allow for comparison of closely related genotypes with identical genetic backgrounds yet without artificially induced mutations. However, each RIL is a distinct genotype, and studying several RILs permits the analysis of a phenotype in several distinct genomes thereby reducing the risk of confounding effects from pleiotropy, epistasis, gene linkage,

Table 3. Root specific phosphorus absorption rate (SPAR), cost–benefit analysis, plant phosphorus uptake, lateral root phosphorus content, and benefit–cost analysis for maize recombinant inbred lines (RILs) measured 7 d after transplanting, as influenced by phosphorus availability

Numbers with different superscript letters differ significantly at the 5% level by Fisher's LSD for means from low P or high P. *, $P < 0.10$; **, $P < 0.05$; ***, $P < 0.01$; ns, not significant ($n = 4$)

Phosphorus level	RIL	SPAR ($\mu\text{mol P m}^{-1} \text{root}^{-1}$)	Cost–benefit [$(\mu\text{mol CO}_2 \text{ min}^{-1} \text{g root}^{-1}) / (\mu\text{mol P}^{-1} \text{min g}^{-1} \text{root})$]	Plant P uptake ($\text{nmol P min}^{-1} \text{plant}^{-1}$)	Lateral root P content (nmol P plant^{-1})	Benefit–cost [$(\text{nmol P min}^{-1} \text{plant}^{-1}) / (\text{nmol P plant}^{-1})$]
Low	128	0.394 ^b	3.762 ^a	2.56 ^c	5.77 ^{ab}	0.470 ^c
	81	0.271 ^b	5.300 ^{ab}	1.80 ^b	5.21 ^{ab}	0.375 ^{bc}
	14	0.334 ^b	3.761 ^a	1.75 ^b	6.23 ^b	0.298 ^b
	20	0.126 ^a	8.714 ^{bc}	1.06 ^a	4.55 ^a	0.236 ^a
	192	0.147 ^a	11.70 ^c	0.92 ^a	5.90 ^{ab}	0.173 ^a
High	128	0.597 ^a	5.117 ^a	3.98 ^b	10.3 ^a	0.396 ^b
	81	0.926 ^b	4.721 ^a	4.26 ^b	15.9 ^b	0.313 ^{ab}
	14	0.568 ^a	5.024 ^a	3.68 ^{ab}	8.73 ^a	0.434 ^b
	20	0.643 ^a	6.686 ^a	3.43 ^a	10.4 ^a	0.330 ^a
	192	0.704 ^a	5.066 ^a	4.47 ^b	11.5 ^{ab}	0.409 ^b
F from ANOVA						
Block		1.76ns	0.80ns	0.46ns	0.10ns	1.96ns
Genotype		2.29ns	3.26**	2.43*	2.09ns	2.94*
Phosphorus		79.9***	1.96ns	111***	54.1***	3.85*
Genotype × phosphorus		3.49**	2.42*	2.55*	2.91*	3.38**

or other genetic interactions. RILs are particularly valuable in the analysis of phenotypic traits controlled by many genes, as is the case for lateral rooting in maize (Zhu 2003).

We employed economic principles of cost–benefit analysis to quantitatively assess the adaptive value of lateral rooting under low phosphorus availability. The processes of plant growth, resource allocation, and acquisition of multiple resources have many analogous principles to optimisation theory in economics. In the present context, we were concerned with the balance between the benefits of enhanced phosphorus acquisition and the cost of the formation and maintenance of lateral roots, a problem that is well suited to economic analysis (Lynch and Ho 2004).

Economic analyses of plant resource acquisition strategies are simplified by the definition of a common currency with which to assess exchange values of diverse resources. In the case of phosphorus stress, the most direct currency is phosphorus itself, or growth parameters related to phosphorus accumulation (Koide and Elliott 1989). Carbon also has merits for this application, including direct involvement with the dynamics of both photosynthesis and respiration, whereas phosphorus is more static, i.e. the phosphorus content of an organ does not reveal the respiration or photosynthetic output of that organ. Similarly, the duration of resource commitment to a process is an important aspect of the metabolic cost of that investment, and carbon budgets

Table 4. Correlation (Pearson linear correlation coefficient, r , $df = 18$) among plant phosphorus content (PPC), relative growth ratio (RGR), plant dry weight (PDW), SRR per unit of root length (SRRL), SRR per unit of root weight (SRRW), cost–benefit ratio (CBR), specific phosphorus absorption rate (SPAR), benefit–cost ratio (BCR), lateral root number (LRN), and lateral root length (LRL) 7 d after transplanting for five maize RILs in low phosphorus media

Significant differences: *, $P < 0.05$; **, $P < 0.01$

	PPC	RGR	PDW	SRRL	SRRW	CBR	SPCR	BCR	LRN
RGR	0.81**								
PDW	0.87**	0.95**							
SRRL	−0.53*	−0.66**	−0.69**						
SRRW	−0.43	−0.57**	−0.61**	0.89**					
CBR	−0.53*	−0.47*	−0.51*	0.48*	0.48*				
SPAR	1.00**	0.82**	0.88**	−0.55*	−0.46*	−0.53*			
BCR	0.59**	0.64**	0.65**	−0.27	−0.32	−0.18	0.60**		
LRN	0.40	0.63*	0.51*	−0.68**	−0.47*	−0.51**	0.39	0.21	
LRL	0.78**	0.81**	0.78**	−0.61**	−0.43	−0.54**	0.78**	0.39	0.71**

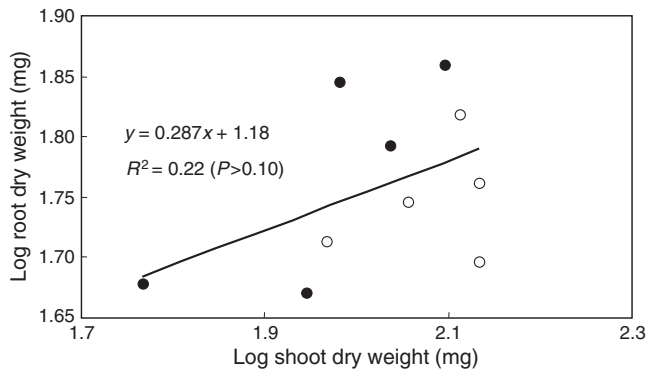


Fig. 6. Allometric relationships between the means of root DW (log mg) and shoot DW (log mg) of five maize recombinant inbred lines from B73 × Mo17, grown under low and high phosphorus conditions. ●, plants grown at low phosphorus availability; ○, plants grown at high phosphorus availability.

provide a convenient measure of that investment. Tissue phosphorus content is more static and does not readily reflect the temporal dimension of the metabolic cost of phosphorus allocation. In this study we applied both carbon- and phosphorus-based measures of cost–benefit ratios to contrasting genotypes and found that both measures provided similar results; lateral roots were worthwhile investments of plant carbon and phosphorus reserves under conditions of low phosphorus availability.

There is increasing evidence that root costs are an important component of plant growth and adaptation under low phosphorus availability. Carbon expended in root respiration is substantial and can account for 8–50% of daily net photosynthesis (Lambers *et al.* 2002). Phosphorus-deficient plants typically have greater root:shoot ratios than high phosphorus plants, either because of allometric relationships (Niklas 1994) or because of increased biomass allocation to roots (e.g. Nielsen *et al.* 2001). Increased relative allocation to root growth is obviously beneficial for phosphorus acquisition, since phosphorus is relatively immobile in soil, but may slow overall plant growth because of the increased respiratory burden of root tissue (Van der Werf *et al.* 1992; Hansen *et al.* 1998; Neilsen *et al.* 1998, 2001; Lynch and Ho 2004). Modelling exercises showed that small differences in the efficiency of phosphorus acquisition by roots can produce large changes in total plant biomass accumulation, due to the compounding effects of increased shoot growth on root growth (Wissuwa 2003). Therefore, we consider the genetic differences observed in the metabolic costs of root growth and phosphorus acquisition, such as the 300% differences observed among genotypes for the cost–benefit ratio (Table 3), to be important. Indeed, the negative correlation of SRR and RGR, as well as the correlation of root-cost parameters with plant growth under phosphorus stress after only 7 d, support the hypothesis that

root costs are important aspects of plant adaptation to low phosphorus availability in maize.

The value of lateral roots for phosphorus acquisition derives from architectural as well as physiological considerations. Architecturally, lateral roots increase the number of root apical meristems actively exploring new soil domains, and the extension of lateral roots at more or less right angles to the axial roots insures that lateral roots quickly depart from the zone of soil around their parent root already subject to phosphorus depletion (Nielsen *et al.* 1994). Physiologically, lateral roots have substantially less phosphorus investment per unit root length than non-lateral roots (Table 2). In our experimental conditions of uniformly distributed phosphorus buffer, the ability of lateral roots to elongate at reduced phosphorus cost would allow them to acquire more phosphorus from the soil per unit of plant phosphorus invested in root growth, which, under conditions of phosphorus stress, would be advantageous. In previous work with common bean, we showed that lateral root density was decreased by low phosphorus availability, a response that would be advantageous in patchy environments in which a concentration of root foraging through lateral branching in discrete microdomains would be advantageous (Borch *et al.* 1999). Therefore, the adaptive value of lateral rooting would be dependent upon the distribution of phosphorus sources in a given environment. Many low-fertility agroecosystems have substantial vertical heterogeneity of phosphorus distribution but are otherwise not very patchy because of tillage — in these environments enhanced lateral root growth under low phosphorus conditions may be advantageous. Enhanced lateral branching also promotes intensive rather than extensive soil foraging, which would be advantageous in agroecosystems, because of reduced interplant competition.

The ability of lateral roots to elongate with reduced phosphorus investment was related to the fact that lateral roots had smaller diameter and greater SRL than non-lateral roots in most genotypes (Table 1). We observed considerable genotypic variability in the SRL of lateral roots under low phosphorus, with genotypes having sustained or enhanced lateral rooting under low phosphorus generally having the largest SRL (Table 1). It appears that SRL is an important factor in the benefit of lateral rooting under low phosphorus availability, as well as the ability of some genotypes to maintain lateral root growth under phosphorus limitation. The phosphorus content (per unit weight) of lateral roots varied significantly among genotypes (Table 2). Phosphorus concentration and SRL varied independently of each other, suggesting that genotypes could be developed combining both traits. For example, a genotype that combined the largest SRL (100 mg g^{-1}) and the smallest phosphorus concentration (3.6 mg P g^{-1}) observed here would have a phosphorus investment of $36 \mu\text{g P per m}$ of root, while a genotype combining the smallest SRL (48.4 mg g^{-1}) and the

largest phosphorus concentration (7.0 mg P g^{-1}) observed here would have a phosphorus investment of $145 \mu\text{g P per m}$ of root, for a range of 400% in the phosphorus cost of root elongation. In addition to root diameter, variation in SRL and tissue phosphorus content in these genotypes may be related to aerenchyma formation, which is inducible by low phosphorus availability, varies among maize genotypes, and increases SRL while decreasing specific respiration (Fan *et al.* 2003).

Our sand culture system buffered phosphorus supply in a manner that mimics the phosphorus availability and physical impedance of natural soil. The response of lateral rooting to phosphorus availability for three of five RILs measured in sand culture was consistent with previous screening in cigar rolls. However, low phosphorus availability reduced lateral rooting in RIL 14 in cigar rolls but had no effect in sand culture, and low phosphorus availability increased lateral rooting of RIL 192 in cigar rolls but reduced it in sand culture. The sensitivity of root phenotype to growth media is not unexpected. In the present context, it appears that cigar rolls might be effective for screening a large number of genotypes, but promising genotypes must subsequently be evaluated in solid media to confirm their phenotype.

We observed significant genotypic variation for lateral rooting under phosphorus stress, even among closely related genotypes. Genotypic variation for root responses to phosphorus stress has been observed in several cases, including lateral rooting in ecotypes of *Arabidopsis* (Chevalier *et al.* 2003), basal root shallowness in common bean (Bonser *et al.* 1996; Liao *et al.* 2001), and adventitious rooting in common bean (Miller *et al.* 2003). It is becoming increasingly apparent that considerable diversity exists in root responses to phosphorus availability, both among species (see discussion in Robinson 1994) and among genotypes of the same species. This suggests that general conclusions regarding the rooting strategies of a species cannot be made without some sampling of the genetic diversity within that species. In the case of maize and other crop species, genotypic diversity in rooting strategies represents an opportunity for the selection and breeding of crops with superior adaptation to low soil phosphorus availability, an enterprise of considerable importance for low-input agriculture in developing countries (Lynch 1998). Assuming that the diversity of root responses to phosphorus availability has adaptive significance, it suggests that plasticity may not always be advantageous. A possible disadvantage of enhanced or sustained lateral rooting under conditions of low phosphorus availability is that biomass allocation to lateral roots may reduce the development or elongation of axial roots. Reduced elongation of axial roots may be disadvantageous in environments with low water availability, or where nutrient resources are discretely localised and must be found by root exploration. Lateral roots may also be more susceptible to herbivory

and pathogens than the thicker axial roots. These potential tradeoffs should be understood better to guide crop breeding programs.

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