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Impact of early growth traits on further genotypic performance during the vegetative growth of maize (*Zea mays* L.) in response to phosphorus (P) availability

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Abstract
The aim of this study is to analyze the effects of early growth behaviors under conditions of P deficiency on further performances at the end of the vegetative phase of different *Zea mays* L. genotypes. The effects of soil P availability on biomass and P allocation during early growth and its effects on further performances were investigated on six maize genotypes which were chosen for their growth and development traits in response to P availability. Plants were grown under two contrasting P supplies and collected at 393°Cd and 780°Cd after emergence. Shoot and root growth, root:shoot allometric indicators and efficiencies related to P uptake and utilization, carbon (C) assimilation and allocation were determined. The results showed that the behavior of the six–leave-stage plants was a determining indicator of plant performance at the pre-anthesis phase. Total dry weight of the different maize genotypes ranged from 8.3 to 19.2 g/plant under low P supply at 780°Cd. At 393°Cd, extreme genotypes in growth were shown to have contrasting root: shoot ratios under high P supply (0.38 and 0.2) but similar ones under low P supply (0.5). We concluded that early investment in root or shoot growth in response to P availability determined the P and C partitioning at later stages. Through the screening of several maize genotypes, this study provided a quantitative analysis of plant growth and development to better understand the impact of early architectural tradeoffs and feedback effects on plant development under P deficiency.

Keywords: *Zea mays* L., genotypes, phosphorus, P-utilization efficiency, P-uptake efficiency, source: sink.
Abbrevations: °Cd_Degree Day, C_Carbon, GLA_Green Leaf Area, HP_High Phosphorus, LP_Low Phosphorus, PARa_absorbed Photosynthetically Active Radiation, PCA_Principal Component Analysis, Pi_inorganic P, P_Phosphorus, PUE_P Use Efficiency, PUpE_P Uptake Efficiency, PUtE_P utilization Efficiency, RDW_Root Dry Weight, RL_Root Length, root_PUtE-specific root PUtE, RUE_Radiation Use Efficiency, SDW_Shoot Dry Weight, shoot-PUtE_specific shoot PUtE, TDW_Total Dry Weight, TT-Thermal Time.

Introduction

Phosphorus (P) is an essential component of membranes, energetic compounds and nucleic acids. Its propensity to bind to soil particles and to form insoluble complexes leads to poor mobility even in arable lands with high absolute soil-P contents. Aside from these characteristics, the issue of the dwindling of P resources may emerge in the coming decades since at the current rate of demand for fertilizers, complete exhaustion of the world's reserves is expected in around 125 years (Gilbert, 2009).

P deficiency directly and indirectly impacts root and shoot growth. As shown in several species, (maize, Mollier and Pellerin, 1999), soybean (Fredeen et al., 1989), wheat, (Rodriguez et al., 2000), leaf growth can be reduced as a direct consequence of the impairment of major cell processes, such as cell divisions which involve the biosynthesis of nucleic acids and photosynthetic reactions (Assuero et al., 2004). P deficiency also affects indirectly organ growth through the allocation of assimilates. Indeed, P deficient plants also showed an early increase in the root:shoot biomass ratio (Stitt and Quick, 1989; Mollier and Pellerin, 1999; Hermans et al., 2006). The relative reduction in shoot growth coincided with higher carbohydrate allocation to the roots. Changes in carbohydrates allocation between roots and shoots, and modifications in root architecture were shown to be linked to the production of cytokinins and abscisic acid (Jeschke et al., 1997), the redistribution of auxin (Nacry et al., 2005) or sugar signaling (Karthikeyan, et al. 2007).

Improving the P uptake efficiency (PUpE) and the P utilization efficiency (PUtE), the two components of P Use efficiency (PUE, as defined by Gourley et al., 1993) seem to be relevant approaches to ensure sustainable high crop yields (Lynch, 2007a) since they provide a framework to dissect the morphological and physiological components that are involved in P limitation responses. First, as regards the PUpE improvement, several characteristics of the root system are targeted (i) root foraging along with modifications of architecture e.g. adventitious and lateral rooting, changing basal root gravitropism, a shallower root system (Lynch, 2007a), (ii) root uptake capacity through the development of root hairs and mycorrhizal symbioses, and enhanced expression of inorganic P (Pi) transporters (Jakobsen et al., 2005), (iii) reduction in metabolic costs per root surface unit, through the development of aerenchyma in the root cortex (Lynch 2007b; Postma and Lynch, 2011a, b) and (iv) the metabolic functions of the root system e.g. release of specific root exudates that increase the availability of P in the rhizosphere (Shen et al., 2005), or the down regulation of high affinity P transporters when the supply of P is high (Ai

Table 1. Characteristics of the analyzed genotypes reported in the literature and main research interests.

Genotype	Genetic type	General characteristics and P responses	References
B73	Inbred line	Root plasticity	Zhu et al. ,2006 Kaeppler et al., 2000 Zhu and Lynch, 2004 Zhu, et al., 2005a,b
Mo17	Inbred line	Root plasticity	
F2	Inbred line	Short plant	Bertin and Gallais, 2000, 2001
Io	Inbred line	Low density of long lateral roots	Gallais and Hirel, 2004 Quilleré, personal communication
rth3 *root hairless 3*	Mutant	Impairment of root hair elongation	Wen and Schnable 1994 Paszkowski and Boller, 2002
Oh43	Inbred line	High aerenchyma development under LP	Fan et al., 2003

The genotypes {B73, Mo17} and {F2, Io} are parents of two recombinant inbred line (RIL) populations. LP: low phosphorus; HP: high phosphorus; RHL: root hair length; LRL: lateral root length; LRN: lateral root number.

et al., 2009). Root trait plasticity and metabolic functions achieved by the root system are consequently considered to be adaptive components that impact P uptake under low P availability (Wissuwa, 2005; Yao et al., 2007). Secondly, as regards the PUtE improvement, physiological processes can be improved such as the remobilization of internal Pi, or modified to avoid P requiring steps such as alternative respiratory pathways that usually require readily available Pi (Plaxton and Carswell, 1999).

If several studies focused on the correlations between morphological traits and/or physiological processes with the components of P use efficiency by browsing genotypic diversity (Sattelmacher et al., 1994*;* Brassica: Akhtar et al., 2008; Hammond et al., 2009; barley: Brown et al., 2012; wheat: Wang et al., 2010) but to our knowledge, only a few rely on this approach for maize (Mano et al., 2005; Zhu et al., 2006; Bayuelo-Jiménez et al., 2011).

It has been shown that an early P limitation negatively impacts on leaf growth (Plenet et al., 2000a) and that P limitation between planting and the sixth leaf stage led to a reduction in grain yield (Barry and Miller, 1989). Therefore, plant traits observed before the sixth leaf growth stage would reflect the plant's responses to P stress occurring before i.e. external P supply and to a lesser extent the initial seed reserves since they support growth until two to three weeks after sowing (see White and Veneklass, 2012 for calculations; Nadeem et al., 2011, 2012a, b). In our study, we aimed to evaluate the impact of P-stressed plants at an early stage (when P deficiency symptoms were noticeable) on further performances (at the end of the vegetative phase) in several genotypes of *Zea mays* L. Therefore, the initial date of characterization was defined when plants have at least six leaves (three to four weeks after sowing) in order to ensure they would also have undergone P limitation from external P supply.

The present work aimed to test the hypothesis that early growth behaviors under P deficiency were determinant for the growth performances at the end of the vegetative phase. For this purpose, we characterized six genotypes of *Zea mays* L under two contrasting P supplies: Low Phosphorus (LP) and High Phosphorus (HP). These genotypes were initially chosen for their contrasting potential growth and their contrasting traits involved in P acquisition in response to P availability. These traits were quantified through the calculations of the following variables: root:shoot ratio (RDW:SDW ratio), relative green leaf area (GLA) and relative root length (RL) (ratios between limiting and non limiting P availabilities). They were obtained at the sixth leaf stage (393°Cd after emergence) and then compared to plant performances at the end of the vegetative growth (780°Cd after emergence). The calculated variables are related to C assimilation and partitioning and P uptake and utilization. The analysis allowed the relative early investment in root and shoot growth and its consequences on further performances to be discussed in relation with early genotypic traits.

Results

Shoot and root growths and P acquisition in response to P supply

Biomass accumulation

P deficiency affected biomass accumulation at the sixth leaf stage (393°Cd) and at the end of the vegetative growth phase (780°Cd) (P <0.01, Table 2). Under LP, the genotypic differences were more pronounced at 393°Cd than at 780°Cd since the highest value of TDW was 4 times greater than the lowest one at 393°Cd whereas it was only 2 times higher at 780°Cd (Table 2). These results highlighted that negative impacts of P deficiency tended to decrease as a consequence of the adaptive responses set up by the genotypes to face P limitation. Interestingly, although Oh43 and B73 had similar TDW at 393°Cd under LP, they had extreme values at 780°Cd. This raises the question of the origin of the discrepancy between these genotypes.

Expansion of the green leaf area

P deficiency impacted on GLA at both harvest dates (P <0.01, Table 2) was lower under LP whatever the genotype except for Oh43 (393°Cd) and Io (780°Cd) (Table 2). Like TDW under LP, the genotypic differences were greater at 393°Cd than at 780°Cd since the greatest value of GLA was 5 times greater than the lowest one at 393°Cd whereas it was only 2 times higher at 780°Cd (Table 2). These results highlight that GLA was an early indicator of genotypic responses to P deficiency.

At the end of the vegetative phase (780°Cd), a reduction in the number of fully expanded leaves under P deficiency was observed (Fig 1, from 10 to 13 leaves under LP and 11 to 15 leaves under HP, according to the genotype). With regards to the leaf area at node i (LA_i), a significant P effect occurred from leaf node i= 5 for most of the genotypes (Fig 1). Because the earliness of P deficiency effects can be evaluated by observing the lower affected nodes, these results mean that the impacts of P deficiency occurred at the same time whatever the genotype. However, the intensity of the impacts

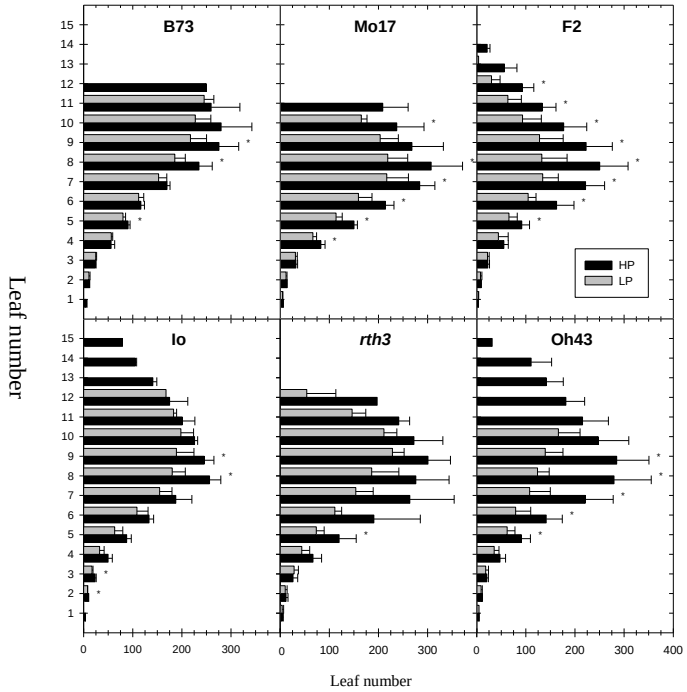

Fig 1. Measured GLA at 780°Cd according to leaf number for the six genotypes under low P (LP, grey bars) and high P (HP, black bars) treatments. The effect of the P treatment was tested at each leaf node. Levels of significance: * P<0.05. Bars denote s.d.

was different according to the genotype. The relative reduction of LA_i (ratio LP:HP) varied markedly with the genotype. For instance, at leaf node 9 which is the most affected whatever the genotype, the highest relative decrease was observed for Oh43 with a ratio of 2.2 and the lowest relative decrease was observed for B73 with a ratio of 1.3 (Fig 1).

Root growth and morphology plasticity

P limitation reduced RL at 393°Cd and 780°Cd (P <0.01, Table 2). Like TDW and GLA under LP, the greatest differences between genotypes were observed at 393°Cd since the greatest value of RL was 3.2 times greater than the lowest one at 393°Cd whereas it was only 2.5 times higher at 780°Cd (Table 2). Like GLA, RL was an early indicator of genotypic responses to P deficiency. It was also a plastic trait since for genotypes with low RL values under LP at 393°Cd (B73, Io) had the highest values at 780°Cd (Table 2). Interestingly, the hairless root mutant *rth3* had the greatest RL under HP at both harvest dates and maintained a high root growth under P limitation (Table 2).

Changes in root morphology were also evaluated by determining the percentage of total root length per root diameter (*d)* class, (RL_d). Fine roots (RL_d, d≤0.04 cm) were initially favored under LP since the proportions were similar or higher at 393°Cd (Table 2). The greatest differences between LP and HP were 8.2% (B73) and 6.3% (F2). The G x P interaction effect was significant only at 393°Cd. But differences between P-treatments tended to decrease at 780°Cd since there was no significant P-effect.

Observations of the root sections were performed at 393°Cd. They did not reveal any significant genotype, P-treatment nor interaction effects on aerenchyma area (data not shown). Mean proportions of aerenchyma area (in percentage of total cross section) were slightly higher under LP with 10.40% (se±0.73) by comparison with 9.54% (se±1.33) under HP. Consistent with these data, no significant difference in specific root length, calculated as the RDW per length unit was observed between P treatments for each genotype (values ranging from 0.008-0.01 mg RDW m^{-1} RL, data not shown), at the same sampling date. Interestingly, higher values under HP were observed for the two genotypes known for their root plasticity (B73 and Mo17). But, no genotype showed significant differences between LP and HP although the aerenchyma responsive genotype, Oh43, exhibited the highest differences between P-treatments (14% under LP and 6% under HP).

Cumulative P uptake

Values of the amount of P in the whole plant (Q_P) ranged from 0.84-4.67 mg P/plant (LP) and 7.27-19.30 mg P/plant (HP) at 393°Cd, and from 6.57-14.28 mg P/plant (LP) and 33.26-62.84 mg P/plant (HP) at 780°Cd. As expected, they were considerably reduced under LP in all the genotypes with up to a 11 times reduction at 393°Cd (Io) and 8 times reduction at 780°Cd (Oh43) (Table 2).

Responsiveness to P supply triggered contrasting investment in root or shoot growth

Responsiveness to P deficiency was analyzed through the RDW:SDW ratio at 393°Cd and 780°Cd under HP and LP (Table 2). P treatment affects RDW:SDW ratio since values under LP were higher than under at 393°Cd and 780°Cd. However, this trend was reduced at 780°Cd (P< 0.001).

Figure 2 presents the ratios between LP and HP for RL and GLA for each genotype at 393°Cd and 780°Cd. At 393 °Cd, the relative shoot growth (rGLA, ratio between LP and HP for GLA) ranged from 40 to 60% with F2 and Io being the most affected genotypes (Fig 2A; Table 2). The relative root growth (rRL, ratio between LP and HP for RL) varied between 30 to 80% in all the genotypes, except for Oh43, whose rRL under reached 110%, meaning P deficiency strongly stimulated early root growth (Fig 2B; Table 2). At

Table 2. Selected variables for the six genotypes under LP and HP, at 393° Cd and 780 °Cd after emergence.

		TDW *g/plant*		GLA *m²/plant*		RL *m/plant*		$\%R_d$<0.04cm		Q_P *mg P/plant*		$\%Q_P$ root		RDW:SDW	
		LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP
393°Cd	B73	1.48 b	4.82^{AB}*	0.021ab	0.049^{AB}*	46.3 b	111.9^{AB}*	79.0a	71.8 ^{B}*	1.71a	11.57^{AB}*	3.34a	4.03^{A}	0.51a	0.38^{A} *
	Mo17	2.29ab	4.78^{AB}*	0.026ab	0.052^{AB}*	62.4ab	98.3^{AB}	78.9a	78.0^{A}	1.88a	10.91^{AB}*	5.79a	2.00^{AB}*	0.47ab	0.27^{AB}*
	F2	1.61 b	4.05^{AB}*	0.017 b	0.045^{AB}*	45.8 b	66.6^{B}	81.2a	74.9^{AB}*	2.95a	12.72^{AB}*	2.50a	1.76^{AB}	0.27 b	0.17 ^{B}*
	Io	0.85 b	4.49^{AB}*	0.007 b	0.045^{AB}*	32.5 b	90.3^{AB}	78.0a	72.2^{AB}	0.84a	9.63^{AB}*	6.32a	3.95^{AB}	0.49ab	0.29^{AB}*
	rth3	3.69a	6.55^{A} *	0.040a	0.062^{A} *	103.1a	166.5^{A}*	76.6a	78.4^{A}	4.67a	19.30^{A}*	5.32a	2.51^{AB}	0.45ab	0.32^{A}*
	Oh43	1.48 b	2.06 ^{B}	0.018 b	0.024 ^{B}	59.1ab	54.6^{B}	81.7a	77.6^{AB}	2.58a	7.27 ^{B}	5.08a	1.22 ^{B}*	0.52a	0.20 ^{B}*
	G		*		*		*		*		*		ns		*
	P		*		*		*		*		*		*		*
	G × P		ns		ns		ns		*		*		ns		*
780°Cd	B73	19.19a	26.56^{A}*	0.123a	0.164^{A}*	343.72a	501.79^{A} *	82.9ab	82.9^{A}	13.58ab	56.36^{A}*	10.05a	3.91^{A}*	0.39ab	0.36^{A}
	Mo17	16.64ab	25.02^{A}*	0.110a	0.165^{A}*	245.64ab	401.41^{AB}*	78.9abc	79.9^{A}	12.79ab	62.84^{A}*	6.23ab	2.78^{A}*	0.34 b	0.27^{AB}*
	F2	13.68ab	23.13^{A}	0.080ab	0.137^{A}*	131.17 b	221.81 ^{B}	76.0c	78.8^{A}	14.28a	33.26^{A}	2.46 b	2.96^{A}	0.14 c	0.15 ^{B}
	Io	13.83ab	25.76^{A}*	0.104ab	0.154^{A}	188.64ab	404.06^{AB}	77.6bc	78.4^{A}	9.96ab	57.26^{A}*	4.32ab	4.22^{A}	0.27bc	0.22^{AB}
	rth3	16.50ab	28.99^{A}	0.081ab	0.151^{A}*	335.04ab	547.94^{A}	83.4a	82.9^{A}	12.04ab	62.04^{A}*	12.16a	6.01^{A}	0.57a	0.35^{A}
	Oh43	8.30 b	24.98^{A}*	0.058 b	0.171^{A}*	159.05ab	415.35^{AB}*	84.1a	82.6^{A}	6.57b	53.10^{A}*	4.55ab	3.41^{A}	0.36 b	0.26^{AB}*
	G		ns		ns		*		*		ns		*		*
	P		*		*		*		ns		*		*		*
	G × P		ns		ns		ns		ns		*		*		ns

TDW: Total Dry Weight; GLA: Green Leaf Area; RL: Root Length; Q_P : Amount of P; RDW: Root Dry Weight; SDW: Shoot Dry Weight. Levels of significance were given for genotype (G), P treatment (P) and G x P effects (ns for non significant and * *P<0.05*). Different letters (lower and upper case letters for low and high P treatments, respectively) denote significant differences between genotypes within columns at *P*<0.05 (Bonferroni's test). * after the HP values denotes significant differences between LP and HP within lines at *P*<0.05 (one factor ANOVA).

Table 3. Mean values of PUpE, PUtE, root-PUtE, shoot-PUtE and RUE under LP and HP supply for the six genotypes at 780°Cd after emergence.

	PUpE *mg P m RL^{-1}*		PUtE *g TDW mg P^{-1}*		root-PUtE *g RDW mg root P^{-1}*		shoot-PUtE *g SDW mg shoot P^{-1}*		RUE *gTDW MJQPAR^{-1}*	
	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP
B73	0.04b	0.12A *	1.46a	0.47 B *	4.26a	3.30A	1.72a	0.37B *	0.95ab	0.69A*
Mo17	0.05b	0.16A *	1.35a	0.40 B *	6.03a	3.15A*	1.07a	0.32B *	0.73b	0.61A
F2	0.10 a	0.16A	0.98a	0.90 A	4.95a	3.59A	0.89a	0.82A	0.99ab	0.71A
Io	0.06 b	0.14A	1.46a	0.43 AB	6.29a	2.44A^{NS}	1.22a	0.36B *	0.94ab	0.60A
rth3	0.04b	0.11A *	1.51a	0.49 B *	4.61a	2.43A	1.21a	0.38B*	0.77ab	0.80A
Oh43	0.04b	0.13A *	1.29a	0.47 B *	6.48a	2.89A	0.99a	0.38B *	1.31a	0.87A
G		*		ns		ns		ns		*
P		*		*		*		*		ns
G x P		ns		*		ns		*		ns

Levels of significance were given for genotype (G), P treatment (P) and G x P effects (NS for non-significant and * *P<0.05*). Different letters (lower and upper case letters for low and high P treatments, respectively) denote significant differences between genotypes within columns at *P*<0.05 (Bonferroni's test). * after the HP values denotes significant differences between LP and HP within lines at *P*<0.05 (one factor ANOVA).

780 °Cd, The rGLA was maintained or slightly increased for most of the genotypes except for Oh43 which had the lowest rGLA (ca. 30%) by contrast with B73 and Mo17 (Fig 2A). The rRL was maintained or decreased especially for Oh43 which had the lowest value in rRL (less than 40%) compared to rRL of the other genotypes (45 to 75%) (Fig 2B). The early investment in root growth for Oh43 was not sufficient to sustain whole plant growth since GLA and TDW were low compared to B73 and Mo17 under LP at 780°Cd (Fig 2; Table 2).

P uptake efficiency (PUpE) and respective P allocation to roots and shoots

P deficiency significantly affected PUpE with values ranging from 0.11-0.16 mg P m RL^{-1} under HP compared with 0.04-0.10 mg P m RL^{-1} under LP (Table 3), as a direct consequence of a low availability to the plants. Under HP, no significant genotype effect was observed (*P≥0.05*). By contrast, under LP, genotypic differences were highly significant (*P<0.05*) with F2 and {B73, Oh43, *rth3*} having the highest and lowest PUpE respectively. When comparing LP and HP values for each genotype, P effects were observed for all the genotypes (*P<0.05*) except for F2 and Io. Although Oh43 and {Mo17, B73} had contrasting early growth patterns (Fig 2; Tables 2 and 3), their PUpE did not differ under LP suggesting that PUpE was not impacted by their early growth patterns. Table 2 shows the proportion of P allocated to roots (%root Q_P) under LP and HP at 393°Cd and 780°Cd. Genotypes with contrasting PUpE also differed with respect to %root Q_P under LP at 780°C (F2 and {B73, *rth3* and to a lesser extent Oh43} having the lowest and highest values, Table 2). Therefore P uptake was directly correlated to the amount of P available for roots.

P utilization efficiency (PUtE) and organ specific, root- and shoot- P utilization efficiencies

P utilization efficiency (PUtE) was calculated to investigate whether genotypic differences at the end of the vegetative phase (780°Cd) could be explained by the ability to produce biomass according to the amount of P taken up. P limitation significantly enhanced PUtE with values ranging from 0.98-1.51 g TDW mg P^{-1} by contrast with 0.40-0.90 g TDW mg P^{-1} under HP (Table 3). Significant differences between LP and HP were observed in all the genotypes except in F2 and Io known for their low potential growth (Table 1). Suboptimal

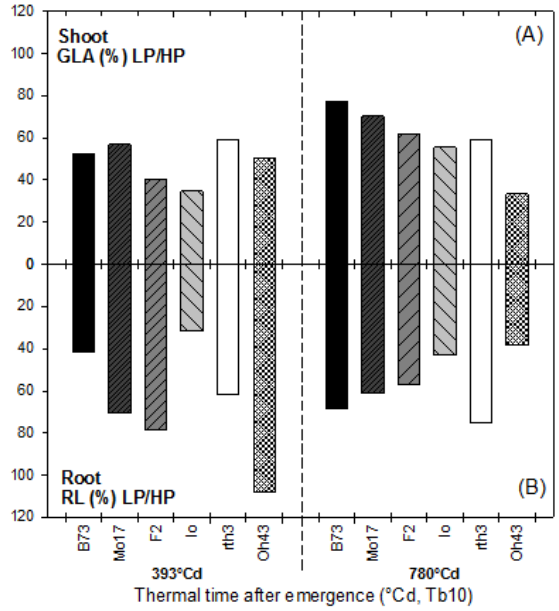

Fig 2. Variations in relative green leaf area (A) and root length (B) for the 6 genotypes analyzed at the first and last observation dates (393 and 780 °Cd after emergence). Relative growth is the ratio between low P (LP) and high P (HP) mean values.

PUtE (under LP) was similar amongst genotypes while PUtE differed under HP with F2 and Mo17 having the highest and lowest values respectively. In addition to PUtE, root-PUtE and shoot-PUtE were calculated to determine whether the differences in PUtE were explained by a greater ability of the roots or the shoot to produce root or shoot biomass with the P allocated. Similar to PUtE, root-PUtE increased under LP (*P<0.05* Table 3) with values up to twice higher than under HP. The most root-efficient genotype under LP was Oh43. Its high efficiency to produce root biomass with low P requirements was consistent with its RDW:SDW ratio and rRL observed at 393 °Cd (Fig 1; Tables 2 and 3), meaning its ability to use P allocated to the roots for root biomass production was correlated with early investment in root growth under LP. Similar to PUtE and root-PUtE, shoot-PUtE increased under LP (*P<0.05*, Table 3). All the genotypes responded to P deficiency except F2, whose shoot-PUtE did not differ between HP and LP. The most shoot-

efficient genotype under LP was B73. Its high efficiency to produce shoot biomass with low P requirements was consistent with its potential to sustain shoot growth under LP (Fig 1, Tables 2 and 3). These results showed that the early investment in root or shoot growth is associated to the increase in root- or shoot-PUtE under LP, respectively. More specifically, the early investment in root growth (Oh43) is related to a higher increase in root-PUtE under LP while an early investment in shoot growth (B73) was associated to genotypes which exhibited a higher increase in shoot-PUtE under LP.

Lower biomass production in response to low P availability was more closely linked to a lower rate of absorbed PAR than with a decrease in RUE

The production of biomass was driven by the absorbed PAR (PARa) and RUE. Since PARa is a function of photosynthetic leaf area, variation between treatments in each genotype was accounted for by differences in GLA i.e. number of green leaves and individual leaf area. Figure 3 shows differences in *Q*PARa under both P treatments. As expected, *Q*PARa was strongly affected under LP, ranging from a 2 times decrease {Mo17, B73, *rth3*} to a 6 times decrease {Oh43} in the *Q*PARa at 780 °Cd. The RUE values ranged from 0.60 to 0.87 g TDW MJ^{-1} under HP and from 0.73 to 1.31 g TDW MJ^{-1} under LP. P deficiency did not affect RUE except in B73 ($P<0.05$, Table 3) whose RUE was the highest under LP. Higher (but not significant) values of RUE under LP were the consequence of a narrower range of *Q*PARa (data not shown) which was especially pronounced in Oh43, as a consequence of its highly reduced photosynthetically active leaf area under LP (Fig 1; Table 2). Biomass production thus differed among the genotypes in response to P availability, as a consequence of the reduced green leaf area available to absorb radiation rather than the efficiency to use radiation for photosynthesis.

Relationships between early growth traits and efficiency under P deficiency

A principal components analysis (PCA) was performed on early growth traits (relative values defined as the LP:HP ratios) measured at 393 °Cd, and efficiencies under LP. The variables analyzed were (i) for early growth traits, r%RootQ_P, i.e. the LP:HP ratio of the proportion of P allocation to the roots; r%RDW i.e. the LP:HP ratio of the proportion of root biomass; and rRoot/Shoot i.e. the LP:HP ratio of the root to shoot ratio, and (ii) for efficiencies, RUE, PUpE, PUtE, shoot-PUtE and root-PUtE. We included the relative number of dried leaves (r%Dried Leaves i.e. the LP:HP ratio of the proportion of dried leaves) to account for any putative effect of C and P remobilization processes. The PCA (Fig. 4) enabled identification of the relationships among the variables and of groups of genotypes related to these variables. Using correlations between two variables, we did not observe any strong relationships (data not shown) whereas PCA could reveal correlations among several variables. The first (PC1) and second (PC2) components explained 42.8% and 37.2% of the variance, respectively. As shown in Figure 4a, three clusters of genotypes {Oh43}, {F2, Io, Mo17, B73} and {*rth3*} were identified. The variables that contributed to PC1 mainly accounted for the differences between {Oh43} and {F2, Io, Mo17, B73}-{rth3}. Figure 4b shows the variables that contributed 78% to PC1, namely r%RDW, rRoot/Shoot, r%RootQ_P and root-PUtE under LP underlining the relationships between relative C and P allocation and root-PUtE. Similarly, the variables that

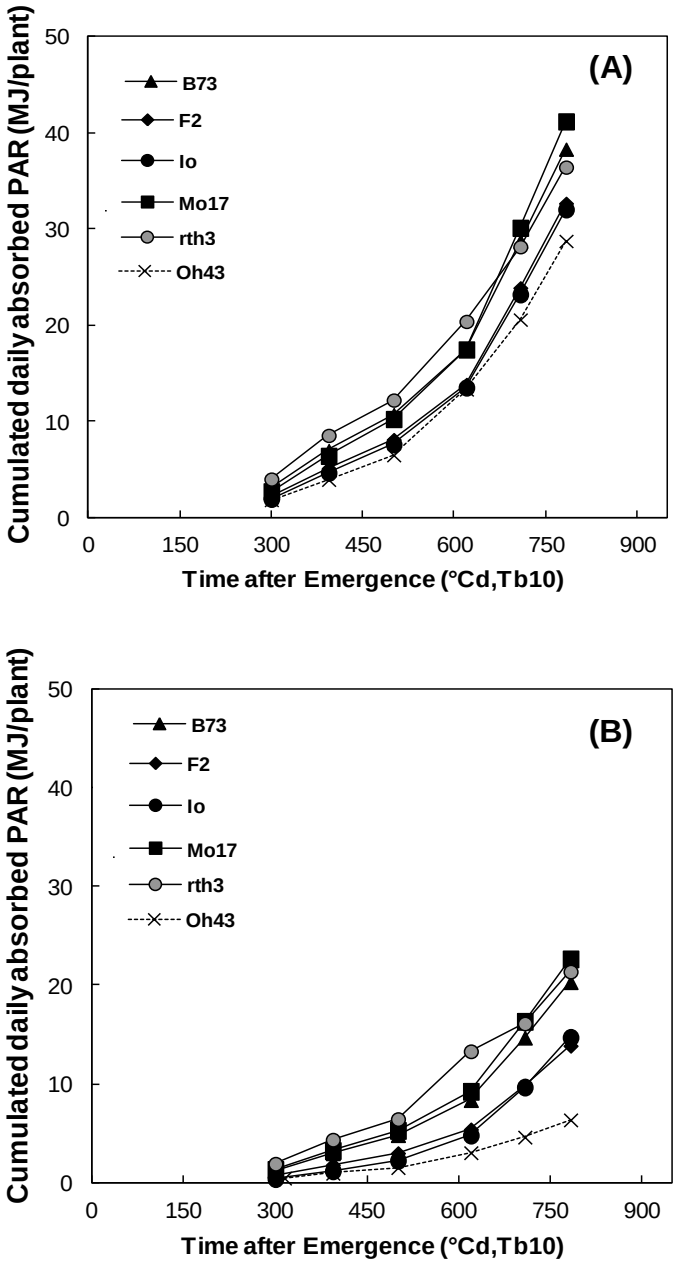

Fig 3. Cumulated daily absorbed PAR (*Q*PARa MJ $plant^{-1}$) as a function of thermal time under (A) HP and (B) LP for the genotypes analyzed.

contributed to PC2 mainly distinguished {F2, Io, Mo17, B73}-{Oh43} from {*rth3*} (Fig. 4a). PUpE, PUtE and shoot-PUtE under LP contributed 71% to PC2, meaning that at low P, these efficiencies were linked and accounted for the contrasted behavior of *rth3*. The r%Dried Leaves at 393 °Cd contributed to 70% of PC3 (which explained 13.5% of the variance, data not shown) and strongly distinguished Io and to a lesser extent B73, from F2 and Oh43 (data not shown).

Discussion

In this study, we observed contrasting plant performances at the end of the vegetative phase in response to P availability. A wide genotypic variability in TDW was highlighted in response to P limitation. Interestingly, under P limiting

conditions, the genotypes which displayed extreme performances at the end of the vegetative phase (Oh43 and B73) had similar biomass at the sixth leaf stage. By contrast, under non limiting P conditions, their biomass was similar at the end of the vegetative phase although significant differences were observed at the sixth leaf stage. These observations showed that (i) the relative traits (GLA and RL ratios between HP and LP) are important to interpret plant's responses to P limitation and that (ii) early growth patterns were determining for plant performances at the end of the vegetative phase.

Initially, our working hypotheses were based on the importance of morphological and anatomical genotypic traits in response to P limitation to explain plant performances at the end of the vegetative phase. However, our results could not reveal any strong relations between the known morphological and anatomical traits of the genotypes and their predisposition to face P limitations. Indeed, the impairment of root hairs in the hairless root mutant, *rth3*, was expected to have a negative impact on P acquisition, especially in an environment with limiting P availability (Bates and Lynch, 2001). Brown et al. (2012) showed in Barley that P limiting conditions favored the length and density of root hairs but that length was critical only for shoot P and biomass accumulation, not for yield. However, under our experimental conditions, we observed similar growth rates for the root hairless mutant *rth3* under the P-treatments, at 393°Cd and 780°Cd. Similar observations in *rth3* were previously made in field conditions and were explained by the high plasticity of the root system to compensate morphological impairment (Wen and Schnable, 1994, Paszkowski and Boller, 2002). Consistent with this, we observed an enhanced development of lateral roots in *rth3* under P limiting conditions which might be a compensatory mechanism to increase soil exploration.

In regards to the putative enhancement of aerenchyma formation under LP for Oh43, we cannot directly correlate the contrasting behavior of Oh43 to this trait since we could not significantly highlight this characteristic in our sampling conditions. Previous studies concluded to the impact of P limitation on aerenchyma development (Fan et al., 2003; Postma and Lynch, 2011a) but the conditions (P fertilization regimes) and date of characterization were different which let us assume that the discrepancies with our measurements were mainly due to less drastic conditions for aerenchyma development. In our study, we showed that Oh43 favored early root growth along with a high proportion of thinner lateral roots. This was consistent with the reduction in metabolic costs under nutrient stress conditions, since fine lateral roots require lower metabolic costs than thicker ones (Lynch, 2007a, 2011). As consequence of this increase in soil exploration by lateral roots, other nutrients and water uptake was also optimized. The coordination of these fluxes could act as signals for cellular mechanisms (cell expansion and/or division), leading to regulation of root architecture through interactions with growth regulators (López-Bucio et al., 2002; Hammond et al., 2004).

Our results showed that early biomass investment towards root or shoot growth was determining for further plant performances. The calculation of RDW:SDW ratios and relative values of GLA and RL (LP:HP) allowed these contrasting performances to be explained through quantitative calculated variables instead of basic traits measurements as initially targeted.

In line with Bayuelo-Jiménez et al. (2011), the maintenance

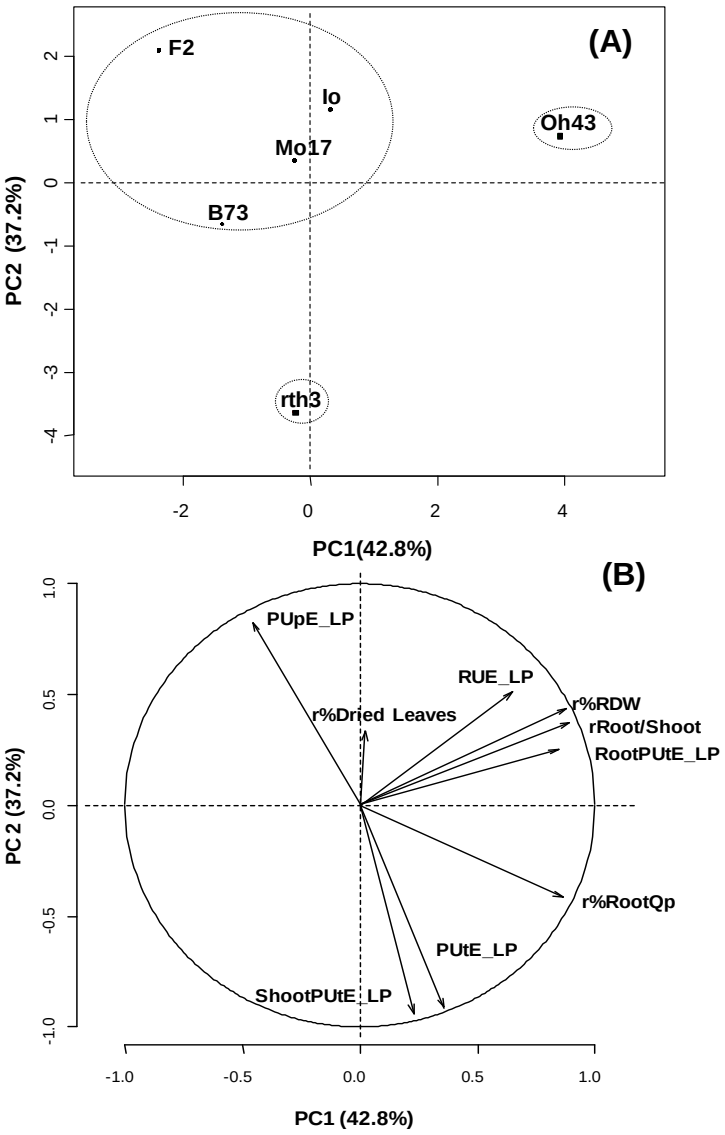

Fig 4. Principal component analysis (PCA) of the first two principal components PC1 and PC2 which accounted for 42.8% and 37.2% of the variance, respectively. (A) Plot genotypes and (B) Plot variable graphs. Clusters of genotypes are denoted by circles. Variables subjected to PCA were efficiencies calculated under LP and various relative growth traits measured at 393 °Cd after emergence (first date of destructive measurements). PUpE_LP: PUpE at LP; PUtE_LP: PUtE at LP; Shoot PUtE_LP: Shoot PUtE at LP; Root PUtE_LP: Root PUtE at LP; RUE_LP: RUE at LP; r%RDW: LP:HP ratio of %RDW, rRoot/Shoot: LP:HP ratio of Root/Shoot; r%RootQp: LP:HP ratio of $\%RootQ_P$, r%Dried Leaves: LP:HP ratio of dried leaves (as a percentage of the total number of leaves).

allocation to roots under low P availability characterized P efficient genotypes. In our study, we showed that the early investment in root growth under LP was not efficient if it is detrimental for shoot expansion as observed for Oh43. These results were consistent with the behaviors of inefficient accessions as defined in Bayuelo-Jiménez et al. (2011) for which the biomass allocation to the roots was dependant on P availability.

Our analysis showed that C assimilation and allocation to the root mainly explained the contrasting growth pattern of Oh43 which early invested in root growth under P limiting conditions. Concerning C assimilation, the genotypic differences could be explained by lower C availability for growth because of root C losses by exudates and respiratory costs (Lynch, 2011; Postma and Lynch, 2011a, b). Several studies showed that a reduction in shoot growth concomitant with stimulation of root growth were associated with a decrease in cytokinins under P starvation (Martin et al., 2000) meaning hormonal regulations are involved in the biomass ratio between root and shoot. Other studies highlighted the importance of phloem loading and translocation of sugars for root growth in response to P supply (Hammond and White 2008; Slewinski and Braun, 2010). Therefore, contrasting hormonal status and sugar transport systems might explain genotypic differences in C allocation. Among the processes that may account for the development of a specific adaptive strategy to face P deficiency, C and P remobilization through leaf senescence could also account for by the contrasting performances. But, contradictory results in maize were reported since some authors suggested a decrease in senescence rates of lower leaves under P deficiency (Colomb et al., 2000) while others reported little effect of P nutrition on the leaf senescence process (Plénet et al., 2000b). In our study, we observed more senescing leaves in genotypes that are shoot- P efficient suggesting that bottom leaves would be a source of P and C for top leaves leading to enhanced shoot expansion.

Finally, our analyses showed that adaptive strategies to face P deficiency were subjected to genotypic variability mainly because of the difference in root:shoot allometry at the six leaves stage. Under LP, genotypes that early invested in root biomass had a higher root-PUtE (e.g. Oh43 and Io) whereas genotypes that early invested in shoot biomass had a higher shoot-PUtE (B73). In addition, P acquisition was enhanced in the genotypes that early invested in root growth at the detriment of leaf expansion (Oh43).

Because the approach undertaken in this study was based on the screening of six genotypes, the extent of the variability observed on traits and efficiencies was limited. We can assume that screening a wider range of genotypes could have highlighted a wider variability on variables such as RUE, as shown in previous studies (Reynolds et al., 2000). It can also be assumed that other processes including organic acid secretion, mycorrhizae establishment, could also explain genotypic differences in efficiency variables.

Material and Methods

Plant material

Six genotypes of maize: B73, Mo17, F2, Io, *rth3* and Oh43 were initially chosen for their contrasting traits under P deficiency: (i) lateral rooting plasticity, (ii) root hair length impairment and (iii) aerenchyma formation (Table 1).

Experimental design and acquisition of environmental data

The six genotypes were grown in a greenhouse with natural day/night light from June 4 until July 27 2009, at INRA, Villenave d'Ornon, France (44° 47' N, 0° 35' W). Seeds were germinated on moistened filter paper at 20 °C before planting to ensure uniformity and permit selection of seedlings of *rth3* with no root hairs. One germinated seed per genotype and P-treatment was then transplanted into an individual 14 L container (38 cm high, 21.6 cm diameter). Each container was filled with 17.5 kg of field moist sandy soil classified as podzol (bulk density of 1.35 g cm^{-3} ±0.7) previously taken from the two field plots at Cestas-Pierroton experimental station (44° 44' N, 0° 46' W). Plots had contrasting long term P fertilization regimes, i.e. low P treatment with 9.9 kg P ha^{-1} $year^{-1}$ (LP) and high P treatment with 79.6 kg P ha^{-1} $year^{-1}$ (HP). The two P application rates represented 0.5 (LP) and 4 (HP) times the amount of the annual P exported by grains, respectively. Before collecting soil, NH_4NO_3 (150 kg ha^{-1}) and KCl (220 kg/ha) were added to prevent any other nutrient deficiency. Soil was sieved (<2 cm) to remove plant residues, stones and insects. The phosphate concentration in the soil suspension solution (C_P, mg P L^{-1}) was determined in HP and LP field plots using the method of Fardeau et al. (1991). The phosphorus soil concentration in HP and LP field plots were 2.36 mg P/l (± 0.43) and 0.04 mg P/l (± 0.01) respectively. At sowing, and 4 and 7 weeks after sowing, the soil filled containers were supplied with 1.2 g/pot of NH_4NO_3 corresponding to an application rate in the field of 150 kg/ha. Based on a control pot which was weighted daily, automatic watering was triggered when soil moisture content fell below 80% (+/- 0.5%) of the field moisture capacity (volumetric water content 0.36 cm^3 cm^{-3}) in order to prevent water stress. Five repetitions of one plant (in an individual container) per genotype, P-treatment and dates of harvest were randomly partitioned into the greenhouse.

Hourly air temperatures were recorded and stored in a data logger (CR10X, Campbell Scientific Ltd., Leicestershire, UK). Time is expressed in thermal time after emergence (*TT*, °Cd) on a daily basis using a base temperature of 10 °C (see Plénet et al., 2000a, for details). Incoming photosynthetically active radiation (PAR*i*, in µmol.m^{-2}.s^{-1}) was measured at 30 min intervals with sensors set up at the top of the plants (JYP 1000 sensor, SDEC France).

Plant measurements and calculations

Measurements were made on five plants per genotype and P treatment until the end of the vegetative phase. Non-destructive leaf measurements were performed weekly until the end of the vegetative phase. Destructive leaf and root measurements were performed at two sampling dates (4 and 8 weeks after sowing corresponding to 393°Cd and 780°Cd). Measurements of the shoot consisted in scoring total, fully expanded, and dried leaves, and in recording the length and width of each individual leaf. Leaf area (LA) was calculated as proposed by Bonhomme et al. (1982). Leaf dimensions were measured to analyse the impact of P deficiency on the phyllochron and to calculate the absorbed PAR. Root length (RL), root volume and relative root length per diameter class calculated as the length of root in the diameter class divided by total RL were measured using WinRHIZO Pro V.2005a (Regent Instruments, Quebec, Canada). Two classes were defined: fine roots (RL_d, $d \leq 0.04$ cm) and coarse roots (RL_d, $d > 0.04$ cm). At the time of the first destructive measurement, aerenchyma on the apical part of segments of lateral roots from the primary root of the 2nd phytomer was characterized. Cross sections (*ca.* 50 µm thick) of fresh tissue stained with toluidine blue were observed with a Nikon Microscope linked to with a Spot RTKE digital camera. The aerenchyma cross sectional area was determined with Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

Considering that self shading and mutual shading between plants were negligible, the daily amount of PAR absorbed by the plant (PARa, MJ/plant) was calculated as follows:

$$PARa = GLA \times PARi$$

where PARi is the daily incident PAR in MJ m^{-2} and GLA is the green leaf area per plant (m^2 $plant^{-1}$). The cumulated PARa (*Q*PARa in MJ $plant^{-1}$) is the sum of daily PARa since the emergence. Measured GLA were fitted to an expolinear function to estimate the daily GLA for each genotype and P treatment, as follows:

$$GLA = \frac{c_m}{r_m} \times \ln\left(1 + e^{r_m (T - Tb)}\right)$$

where c_m is the maximum GLA in the linear phase ($m^2.m.^{-2}.d^{-1}$), and r_m is the maximum relative GLA in the exponential phase ($m^2.m.^{-2}.\ d^{-1}$), T is the time after emergence in days, and *Tb* is the x-intercept i.e. the moment at which the linear phase actually begins (Goudriaan and Monteith, 1990). For each genotype and P treatment, plant individual values of measured GLA were pooled (5 plants dedicated to non destructive measurements plus 5 plants dedicated to destructive measurements) allowing one curve per genotype and treatment to be fitted for PARa calculation.

Determination of root and shoot dry weight and P content

Roots and shoots were weighed after drying at 60 °C for 72 hours (RDW and SDW respectively) and ground to collect a subsample (ca. 0.1-0.8 g of ground tissue depending on P treatment, date of harvest and organ) which was ashed at 550 °C for 5 hours for analysis of P content. P content was measured colorimetrically after P mineralization with HNO_3 (Van Veldhoven and Mannaerts, 1987).

Analysis of the genotypic response to P deficiency based on efficiencies calculations

Efficiencies were calculated as proposed in Gourley et al. (1993) to obtain a quantitative estimation of the genotypic responses. We determined (i) P uptake efficiency (PUpE, mg P m RL^{-1}), (ii) P utilization efficiency (PUtE, g TDW mg P^{-1}) and (iii) C assimilation efficiency via radiation use efficiency (RUE, g TDW MJ^{-1}). They were calculated as the slopes of the linear relationships obtained by taking into account data of the last harvest date (Q_P *vs.* RL for PUpE, TDW *vs.* Q_{Ptot} for PUtE and TDW *vs. Q*PARa for RUE). We also calculated organ-specific utilization efficiencies for root and shoot i.e. root-PUtE (g RDW mg roots P^{-1}) and shoot-PUtE (g SDW mg shoot P^{-1}) respectively.

Statistical analyses

Two-ways ANOVAs were performed for genotype (G), treatment (P) and G x P interactions effects analyses on the measured variables (STATGRAPHICS Plus 3.1 Software). Genotypic comparisons within a P treatment were performed with the Bonferroni's multiple comparison procedure. For a given genotype, P treatment effect was tested using a one way ANOVA. Two-way ANOVAs were also performed for efficiencies i.e. PUpE, PUtE, root- and shoot-PUtE, RUE (STATGRAPHICS Plus 3.1 Software). To perform statistical analyses of variables related to ratios and proportions, the decimal logarithm was applied for dual sided data transformed linear relations following general application methods in allometry (Niklas, 1994; Hunt, 1982) i.e. $\%RL_d$ (proportion of RL per root diameter), %Root Q_P (amount of P allocated to the roots in percentage of Q_P) and the RDW: SDW ratio. Heterogeneity among efficiencies was tested at 95% confidence intervals using the SMATR program (http://www.bio.mq.edu.au/ecology/SMATR), version 2.0 (Faslter et al., 2006; Warton et al., 2006). When significant heterogeneity was observed, a Bonferroni's multiple comparisons test was performed to identify groups of genotypes within a specific P treatment.

Principal component analysis (PCA) was performed with R language environment for statistical computing and graphics, version 2.9.1 (R Development Core Team 2009). This allowed the relationships between the measured early traits (at 393°Cd) and the efficiency variables to be correlated.

Conclusion

These findings are of interest for further modeling analyses with the model of soil-plant P transfer (Mollier et al., 2008) which initially allowed putative adaptive traits to be focused on. In the perspective of designing tolerant varieties to P deficiency, the impact of early growth patterns could be simulated to determine the most adapted ones under different environmental scenarii.

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References

Ai PH, Sun SB, Zhao JN, Fan XR, Xin WJ, Guo Q, Yu L, Shen QR, Wu P, Miller AJ, Xu G.H (2009) Two rice phosphate transporters, OsPht1;2 and OsPht1;6, have different functions and kinetic properties in uptake and translocation. Plant J. 57 (5):798-809.

Akhtar Z, Oki Y, Adachi T (2008) Genetic variability in phosphorus acquisition and utilization efficiency from paringly soluble P-sources by Brassica cultivars under P-stress environment. J Agron Crop Sci. 194: 380-392.

Assuero SG, Mollier A, Pellerin S (2004) The decrease in growth of phosphorus-deficient maize leaves is related to a lower cell production. Plant Cell Environ 27: 887-895.

Barry DAJ, Miller MH (1989) Phosphorus nutritional requirement of maize seedlings for maximum yield. Agron J. 81: 95-99.

Bates TR, Lynch JP (2001) Root hairs confer a competitive advantage under low phosphorus availability. Plant Soil 236: 243-250.

Bayuelo-Jiménez JS, Gallardo-Valdéz M, Pérez-Decelis VA, Magdaleno-Armas L, Ochoa I, Lynch JP (2011) Genotypic variation for root traits of maize (*Zea mays* L.) from the Purhepecha Plateau under contrasting phosphorus availability. Field Crop Res. 121 (3):350-362.

Bertin P, Gallais A (2000) Genetic variation for nitrogen use efficiency in a set of recombinant maize inbred lines I. Agrophysiological results. Maydica 45: 53-66.

Bertin P, Gallais A (2001) Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines II - QTL detection and coincidences. Maydica 46: 53-68.

Bonhomme R, Ruget F, Derieux M, Vincourt P (1982) Relationship between aerial dry matter production and intercepted solar radiation for various maize genotypes. C R Acad Sci III-Vie 294: 393-398.

Brown LK, George TS, Thompson JA, Wright G, Lyon J, Dupuy L, Hubbard SF, White PJ, (2012) What are the implications of variation in root hair length on tolerance to phosphorus deficiency in combination with water stress in barley (*Hordeum vulgare*)? Ann Bot. 110 (2):319-328.

Colomb B, Kiniry JR, Debaeke P (2000) Effect of soil Phosphorus on leaf development and senescence dynamics of field-grown maize. Agron J. 92: 428-435.

Falster DS, Warton DI, Wright IJ (2006) SMATR: Standardised major axis tests and routines, version 2.0; http://www.bio.mq.edu.au/ecology/SMATR/

Fan MS, Zhu JM, Richards C, Brown KM, Lynch JP (2003) Physiological roles for aerenchyma in phosphorus-stressed roots. Funct Plant Biol. 30: 493-506.

Fardeau, JC, Morel C, Boniface R (1991) Phosphate ion transfer from soil to soil solution: kinetic parameters. Agronomie 11: 787-797.

Fredeen AL, Rao IM, Terry N (1989) Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. L. Plant Physiol. 89: 225-230.

Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize. J Exp Bot. 55: 295-306.

Gilbert N (2009) The disappearing nutrient. Nature 461: 716-718.

Goudriaan J, Monteith JL (1990) A mathematical function for crop growth based on light interception and leaf area expansion. Ann Bot. 66: 695–701.

Gourley CJP, Allan DL, Russelle MP (1993) Defining phosphorus efficiency in plants. Plant Soil 156: 289-292.

Hammond JP, Broadley, MR, White PJ (2004) Genetic responses to Phosphorus deficiency. Ann Bot. 94: 323-332.

Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. J Exp Bot. 59: 93-109.

Hammond JP, Broadley MR, White PJ, King GJ, Bowen HC, Hayden R, Meacham MC, Mead A, Overs T, Spracklen WP, Greenwood DJ (2009) Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. J Exp Bot. 60: 1953-1968.

Hermans C, Hammond JP, White PJ, Verbruggen N (2006) How do plants respond to nutrient shortage by biomass allocation? Trends Plant Sci. 11: 610-617.

Hunt R (1982) Plant growth curves: the functional approach to plant growth analysis. In: Edward Arnold (ed). London, UK.

Jakobsen I, Leggett ME, Richardson AE (2005) Rhizosphere microorganisms and plant phosphorus uptake. In: Sims JT and Sharpley AN (eds) Phosphorus, agriculture and environment, American Society for Agronomy, Madison, USA.

Jeschke WD, Peuke AD, Pate JS, Hartung W (1997) Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. J Exp Bot. 48: 1737-1747.

Kaeppler SM, Parke JL, Mueller S, Senior L, Stuber C, Tracy WF (2000) Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. Crop Sci. 40: 358-364.

Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG (2007) Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. Planta 225: 907-918.

López-Bucio J, Hernandez-Abreu E, Sanchez-Calderon L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. Plant Physiol. 129: 244-256.

Lynch JP (2007a) Roots of the second green revolution. Aust J Bot. 55, 493-512.

Lynch JP (2007b) Rhizoeconomics: the roots of shoot growth limitations. Hortic Sci. 42 (5): 1107-1109.

Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol. 156 (3): 1041-1049.

Martin AC, del Pozo C, Iglesias J, Rubio V, Solano R, de la Peña A, Leyva A, Paz-Ares, J (2000) Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. Plant J. 24: 559-567.

Mano YM, Muraki M, Fujimori M, Kindiger B (2005) Identification of QTL controlling adventitious root formation during flooding conditions in teosintle (*Zea mays* spp. Huehuetenangensis) seedling. Euphytica 142: 33-42.

Mollier A, Pellerin S (1999) Maize root system growth and development as influenced by phosphorus deficiency. J Exp Bot. 50: 487-497.

Mollier A, De Willigen P, Heinen M, Morel C, Schneider A, Pellerin S (2008) A two dimensional simulation model of phosphorus uptake including crop growth and P response. Ecol Model. 210: 453-464.

Nacry P, Canivenc G, Muller B, Azmi A, Van Onckelen H, Rossignol M, Doumas P (2005) A Role for auxin redistribution in the responses of the root system architecture to phosphate starvation in Arabidopsis. Plant Physiol. 138: 2061-2074.

Nadeem M, Mollier A, Morel C, Vives A, Prud'homme L, Pellerin S (2011) Relative contribution of seed phosphorus reserves and exogenous phosphorus uptake to maize (*Zea mays* L.) nutrition during early growth stages. Plant Soil 349: 231-244.

Nadeem M, Mollier A, Morel C, Vives A, Prud'homme L, Pellerin S (2012a) Maize (*Zea mays* L.) endogenous seed phosphorus remobilization is not influenced by exogenous phosphorus availability during germination and early growth stages. Plant Soil 357: 13-24.

Nadeem M, Mollier A, Morel C, Vives A, Prud'homme L, Pellerin S (2012b) Seed phosphorus remobilization is not a major limiting step for phosphorus nutrition during early growth of maize. J Plant Nutr Soil Sc. 175: 805-809.

Niklas KJ (1994) Plant allometry: the scaling of form and process. In: Niklas (ed). The University of Chicago Press, Chicago.

Paszkowski U, Boller T (2002) The growth defect of Irt1, a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. Planta 214: 584-590.

Plaxton WC, Carswell MC (1999) Metabolic aspects of the phosphate starvation response in plants. In: Lerner HR (ed) Plant responses to environmental stress: from phytohormones to genome reorganization, Marcel-Dekker, New York, USA.

Plénet D, Etchebest S, Mollier A, Pellerin S (2000a) Growth analysis of field maize crops under phosphorus deficiency. I. Leaf growth. Plant Soil 223: 117-130.

Plénet D, Mollier A, Pellerin S (2000b) Growth analysis of field maize crops under phosphorus deficiency. II. Radiation-use efficiency, biomass accumulation and yield components. Plant Soil 224: 259-272.

Postma JA, Lynch JP (2011a) Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. Plant Physiol. 156: 1190-1201.

Postma JA, Lynch JP (2011b) Theoretical evidence for the functional benefit of root cortical aerenchyma in soils with low phosphorus availability. Ann Bot. 107: 829-841.

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Reynolds MP, Van Ginkel M, Ribaut JM (2000) Avenues for genetic modification of radiation use efficiency in wheat. J Exp Bot. 51: 459–473.

Rodriguez D, Andrade FH, Goudriaan J (2000) Does assimilate supply limit leaf expansion in wheat grown in the field under low phosphorus availability? Field Crop Res. 67: 227-238.

Shen J, Li H, Neumann G, Zhang F (2005) Nutrient uptake, cluster root formation and exudation of protons and citrate in *Lupinus albus* as affected by localized supply of phosphorus in a split-root system. Plant Sci. 168 (3):837-845.

Slewinski TL, Braun DM (2010) Current perspectives on the regulation of whole-plant carbohydrate partitioning. Plant Sci. 178: 341-349.

Stitt M, Quick WP (1989) Photosynthetic carbon partitioning: its regulation and possibilities for manipulation. Physiol Plantarum 77: 633-641.

Van Veldhoven PP, Mannaerts GP (1987) Inorganic and organic phosphate measurements in the nanomolar range. Anal Biochem. 161: 45-48.

Wang L, Chen F, Zhang F, Mi G (2010) Two strategies for achieving higher yield under phosphorus deficiency in winter wheat grown in field conditions. Field Crop Res. 118 (1):36-42.

Warton DI, Wright IJ, Falster DS, Westoby M (2006) Bivariate line fitting methods for allometry. Biol Rev Camb Philos Soc. 81: 259-291.

Wen TJ, Schnable PS (1994) Analyses of mutants of three genes that influence root hair development in *Zea mays* (Graminae) suggest that root hairs are dispensable. Am J Bot. 81: 833-842.

White PJ, Veneklaas EJ (2012) Nature and nurture: the importance of seed phosphorus content. Plant Soil 357, 1-8

Wissuwa M, Gamat G, Ismail AM (2005) Is root growth under phosphorus deficiency affected by source or sink limitations? J Exp Bot. 56: 1943-1950.

Yao Q, Yang K, Pan G, Rong T (2007) The effects of low phosphorus stress on morphological and physiological characteristics of maize (*Zea mays* L.) landraces. Agric Sci China 6: 559-566.

Zhu J, Mickelson S, Kaeppler S, Lynch JP (2006) Detection of quantitative trait loci for seminal root traits in maize (*Zea mays* L.) seedlings grown under differential phosphorus levels. Theor Appl Genet. 113: 1-10.

Zhu JM, Kaeppler S, Lynch JP (2005a) Mapping of QTL for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. Theor Appl Genet. 111: 688–695.

Zhu JM, Kaeppler SM, Lynch JP (2005b) Mapping of QTL controlling root hair length in maize (*Zea mays* L.) under phosphorus deficiency. Plant Soil 270: 299–310.

Zhu JM, Lynch JP (2004) The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays* L.) seedlings. Funct Plant Biol. 31: 949–958.