

Root morphological traits related to phosphorus-uptake efficiency of soybean, sunflower, and maize

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Abstract

Many of the plant acquisition strategies for immobile nutrients, such as phosphorus (P), are related to the maximization of soil exploration at minimum metabolic cost. Previous studies have suggested that soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.), and maize (*Zea mays* L.) differ in their P uptake efficiency. In this investigation we employed these three species to evaluate: (1) the effect of suboptimal P conditions on root morphological traits related to root porosity and fineness and (2) how these traits are related to P-uptake efficiency. Opaque 25-L plastic containers were used to grow plants hydroponically. The three species were compared under two P availability levels (low P and high P). Most of the observed responses were in the direction to favor P uptake under low-P conditions. Compared to P-sufficient plants, P-stressed plants of the three species showed higher root-to-shoot ratio, specific root length, root porosity and root aerenchyma, and a lower root density. For example, P-stress increased root porosity by a factor of 2.0, 1.4, and 1.4 in soybean, sunflower, and maize, respectively. Soybean and sunflower were the species with the highest P-uptake efficiency, expressed as P uptake either per unit root biomass or length. The results demonstrate the central role of aerenchyma development in modifying root length per unit root biomass and, thus, reducing the root's foraging costs. Consequently, aerenchyma is suggested to be a possible mechanism for better P-uptake efficiency.

Key words: aerenchyma / mineral nutrition / plant stress / root morphology

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1 Introduction

Low soil phosphorus (P) availability is one of the most widespread constraints to agricultural productivity all over the world (Lynch, 2007). To overcome this constraint, plants have developed several strategies. Richardson et al. (2011) have classified them as “root foraging strategies”, “root mining strategies”, and “internal P utilization efficiency strategies”. The exploration of these strategies may result in crops and cropping systems more efficient in the use of soil P sources and in a reduction of the requirements of P fertilizers (Lambert et al., 2006).

Since P is very immobile in the soil, many of the “foraging strategies” are related to the maximization of soil exploration in search for P at the minimum metabolic cost (Ernst et al., 1989; Richardson et al., 2011). Several root traits may affect the metabolic cost of soil exploration, e.g., the specific root length (SRL: root length per unit root biomass) and the distribution of roots between diameter classes. The allocation of biomass to fine roots could significantly reduce the cost of soil exploration by lowering the cost to produce a unit of root length (Eissenstat, 1992).

Another root trait that can reduce the cost of root elongation is the formation of root cortical aerenchyma, in which the car-

bon-demanding cortical cells are replaced by air-filled spaces (Lynch and Brown, 2008). An additional benefit of root aerenchyma in P-deficient soils is a reduction in the P requirements of the root system, since air spaces have no P (Fan et al., 2003). It has been reported that P stress promoted aerenchyma formation in maize, bean (Fan et al., 2003), and rice (Kirk and Du, 1997; Lu et al., 1999) roots. Modeling results indicated that the benefit of root cortical aerenchyma under suboptimal P supply is larger for maize than for common bean because common bean has constitutively a more favorable root morphology for P acquisition (finer roots, more root hairs; Postma and Lynch, 2010). However, whether higher root porosity is linked to greater P acquisition remains to be experimentally established.

The “foraging strategies” may be constitutive (also present in P-sufficient plants), may appear only in response to P stress (acclimation), or may be constitutive but intensified under suboptimal P conditions (Richardson et al., 2011). The identification of root traits either as constitutive or adaptive is important to understand plant responses to P and also to find candidates amenable to breeding for P acquisition efficiency. However, this subject has received little research attention.

Soybean, sunflower and maize are three of the most important summer crops all over the world. In Argentina, they rep-



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resent more than two thirds of the planted area, mainly covered by highly fertile although P-deficient Mollisols (Rubio et al., 2008; 2012). It has been consistently reported for these (Echeverría and García, 2005) and other Mollisols in the United States (e.g., Franzen, 1999; Gerwing and Gelderman, 2005; Dodd and Mallarino, 2005) that soybean and sunflower have lower soil P critical levels and are less responsive to P fertilization than maize. This contrast makes these three crops a suitable model to assess the association between tolerance to P stress and root traits.

Previous studies have shown that the higher tolerance of soybean and sunflower to P stress may be associated to a more favorable root morphology and architecture (i.e., greater SRL, root shallowness; Fernández et al., 2009), a great ability to utilize organic P sources (Belinque et al., 2015) and, in soybean, a more effective mycorrhizal symbiosis (Fernández et al., 2011). However, it is still poorly understood whether root porosity and the distribution of roots among different diameter root classes has a role in the P-uptake efficiency of soybean and sunflower.

We designed an experiment in hydroponics under controlled conditions to evaluate (1) the effect of suboptimal P conditions on root morphological traits related to root porosity and fineness, and (2) how these traits are related to P-uptake efficiency in soybean, sunflower, and maize. Under these experimental conditions, P mobilization, and P transport to the root surface are excluded as contributing factors of nutrient acquisition.

2 Material and methods

2.1 Plant culture

Sterilized seeds of soybean (*Glycine max* L., Don Mario 4800 RR), sunflower (*Helianthus annuus* L., Paraíso 20), and maize (*Zea mays* L., DK628 RR) were germinated at 25°C in paper towels arranged in a vertical position and moistened with 0.5 mM CaCl₂. After 7 d, seedlings of similar size were assigned to the different treatments. Treatments were arranged in a randomized complete block design with two factors and four replicates. The factors were species (soybean, sunflower, and maize) and P availability (low P and high P). Opaque 25-L plastic containers (22 cm × 25 cm × 45 cm) were used to grow plants hydroponically. On the lid of each container, four equidistant holes were made to place the seedlings. A strip of cotton was wrapped around the stem to keep each seedling in the hole. The basal nutrient solution had the following composition: NH₄NO₃ (3.5 mM), K₂SO₄ (2.5 mM), CaSO₄ (1.2 mM), MgSO₄ (0.5 mM), MgCl₂ (0.025 mM), Fe-EDTA (5 μM), H₃BO₃ (7.5 μM), MnSO₄ (1.5 μM), ZnSO₄ (1.5 μM), CuSO₄ (0.5 μM), MoO₃ (0.1 μM), and CoSO₄ (0.1 μM). Low and high P levels were adjusted by the addition of 0.001 and 0.070 mM P (as KH₂PO₄), respectively. To compensate for the difference in K supplied, KOH (0.069 mM) was added to the low-P treatment. The nutrient solution was renewed every 7 d and adjusted to pH 6.5 with NaOH (0.1 mM). Each container had a system of continuous aeration to keep the nutrient solution well ventilated and make sure that aerenchyma formation was not due to the lack

of oxygen. The experiment was carried out in a greenhouse with 12 h light–dark intervals and temperature ranging from 23°C to 30°C. Three successive harvests were made 7, 14, and 21 d after transplanting the seedlings to the hydroponic system. At each harvest, the aerial components were separated from the root and placed in an oven at 60°C for 3 d to determine the dry weight. The roots were rinsed with deionized water and samples were taken to measure porosity, presence of aerenchyma, length, and P concentration. Then, the roots were placed in an oven to determine the dry weight.

2.2 Measurements of root porosity

Root porosity was measured using the pycnometric technique (Jensen et al., 1969), which is based on the changes in root weight that occurs when root air volume is replaced by water after maceration. Sections of basal roots (soybean and sunflower) or seminal roots (maize) with all lateral roots were taken for this purpose. First, the pycnometer filled with degassed water was weighed (W_w). Second, fresh weight (W_r) of the root samples was determined after gently draining them on tissue paper. Then, the sample was placed in the pycnometer and the weight of the pycnometer filled with water plus the intact root was determined (W_{r+w}). The roots were later retrieved, ground with mortar, and returned to the pycnometer. The pycnometer was then topped up with water and weighed again (W_h). The total porosity (%) of the roots was calculated by the following formula:

$$\text{Root porosity (\%)} = 100 (W_h - W_{r+w}) / (W_w + W_r - W_{r+w}). \quad (1)$$

2.3 Measurements of root aerenchyma

The presence of root aerenchyma as percent of cross-sectional area of air-filled spaces in approx. 2 mm long root segments was determined 21 d after imposing the P treatments. In soybean and sunflower measurements were done on the first-order lateral roots (the longer ones) at two positions: (a) basal region, which represents the older section of root segment, taken 2 cm from the base of the root, and (b) apical region, which represents the young section of the root, taken at 2 cm from the apex. In maize, samples from the basal and apical region of seminal roots were taken, following the same procedure described above. The root segments were immediately fixed in FAA (10% formaldehyde, 50% ethanol, 5% glacial acetic acid, and 35% distilled water). Root samples were gradually dehydrated in ethanol (70°, 80°, 90°, and 100°) and embedded in paraffin. Cross sections (12 μm thick) were cut with a rotary microtome type Minot. The sections were stained with Safranin-Fast Green and analyzed with a microscope (Nikon Eclipse 50i) connected to a digital color camera (Nikon Coolpix S10). Finally, the area occupied by aerenchyma was evaluated using the public domain software ImageJ 1.4 (National Institute of Health, Bethesda, Maryland, USA), which is available at: <http://rsb.info.nih.gov/ij/>.

2.4 Root length and class-size distribution

The total root length, the distribution of roots among the different diameter classes, and the root volume were quantified by

scanning the roots and then analyzing the images with the software WinRHIZO Pro (Regent Instruments, Québec, Canada). SRL and the root density were calculated on a dry weight basis from the software outputs root length and root volume and the weight of the analyzed sample.

2.5 Tissue P concentration

To determine the concentration of P in each plant organ (leaves, stems, and roots), subsamples of ground plant material (70 mg) were ashed at 550°C for 24 h. The ashes were dissolved in 8 mL 0.1 M HCl and P was then determined colorimetrically. All determinations were performed in duplicate. The P-uptake efficiency was calculated as the amount of P absorbed per unit length and per unit of root biomass after discounting the P content of the seed (0.66, 0.42, and 0.65 mg for soybean, sunflower and maize, respectively).

2.6 Statistical analyses

Data were statistically analyzed using analysis of variance (ANOVA). Means were compared using the Tukey test ($P < 5\%$). To achieve homogeneity of variance, aerenchyma data were transformed through the square root of arcsin of the percentage values, root length data through the square root, and the P efficiency data by a logarithmic function.

3 Results

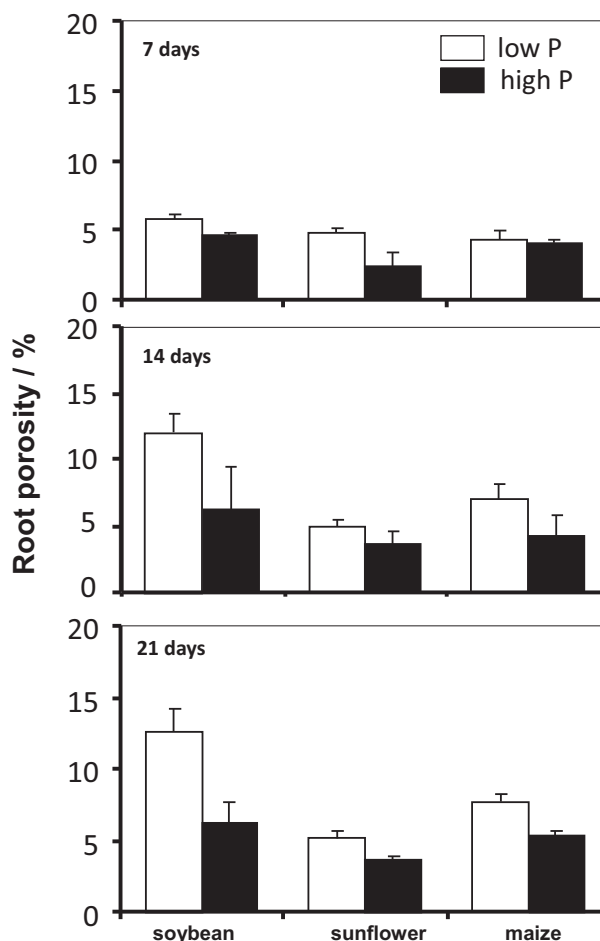
3.1 Biomass accumulation and root length

Fourteen days after transplanting, low-P sunflower and maize plants showed a significant decrease in biomass accumulation (roots and shoots) and root length compared to their high-P counterparts (Table 1). The difference between P levels increased until the following harvest date. In comparison to sunflower and maize, soybean showed a delayed response of biomass to the P treatments (only significant 21 d after transplanting) and a higher dry mass accumulation under low-P relative to high-P conditions (Table 1). This ratio was 0.24, 0.08, and 0.10 for soybean, sunflower, and maize, respectively.

The root-to-shoot ratio did not significantly differ between species but was one of the variables with the greater increase in low-P plants (Table 1). The size of the root system, either measured on a weight or a length basis, was drastically reduced under low-P conditions (Table 1). Sunflower showed the highest effect of the P treatment (6.6 and 4.5 orders of magnitude on root biomass and length, respectively).

3.2 Total root porosity and aerenchyma

Phosphorus-deficient plants exhibited higher total root porosity than P-sufficient plants (Fig. 1). Differences between P levels appeared from the beginning of the experiment (7 d after transplanting) and progressively increased until the final harvest. Differences between species began to be observed 14 d after transplanting (Fig. 1). At the end of the experiment,



ANOVA				Mean comparison			
Species	P	Sp x P	days	P level	Species		
				soybean sunflower maize			
7d	n.s.	*	n.s.	7d	-P>+P		
14d	*	*	n.s.	14d	-P>+P	a	b ab
21d	***	***	n.s.	21d	-P>+P	a	b b

Figure 1: Root porosity for soybean, sunflower and maize plants 7, 14, and 21 days after imposing the P treatments (low and high P) in the hydroponics experiment. Error bars represent the standard error of the mean. Results from ANOVA are expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$; ** $P < 1\%$; *** $P < 0.1\%$. Species in the same row followed by the same letter are not significantly different at the 5% level according to the Tukey test.

root porosity averaged 12.6, 5.1, and 7.7% for soybean, sunflower and maize P-deficient plants, respectively. Values for P-sufficient plants were 6.2, 3.7, and 5.3% for each species, respectively.

The presence of aerenchyma was evaluated at the end of the experiment in root cross sections taken of the cortex of older tissues (basal region of the root segment) and young tissues (apical region). These direct observations matched well with root porosity measurements. The proportion of aerenchyma of the basal region of the root was the variable with the highest increase in response to P stress (Table 2). The greatest P effect was observed in maize (15.2 times more aerenchyma in low-P than in high-P plants), followed

Table 1: Aerial biomass, root biomass, root-to-shoot ratio, and root length for soybean, sunflower and maize plants 7, 14, and 21 d after imposing the P treatments. Results from ANOVA expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$, ** $P < 1\%$, *** $P < 0.1\%$. Mean comparison is shown besides average values in those cases in which the interaction between the factors species and P level was statistically significant. Values of the same column and sampling date followed by the same letter are not significantly different at the 5% level using the Tukey test. In the other cases, mean comparison is provided in the ANOVA section of the table. Significant differences for the factor species in the root length at 7 d were soybean (b), sunflower (ab), and maize (a).

	P level	Aerial biomass / g	Root biomass / g	Root-to-shoot ratio	Root length / m
7 d					
Soybean	– P	0.20	0.12	0.56	9.95
	+ P	0.22	0.11	0.48	11.11
Sunflower	– P	0.15	0.20	1.44	21.54
	+ P	0.22	0.23	1.08	27.96
Maize	– P	0.19	0.21	1.08	32.62
	+ P	0.28	0.18	0.71	37.88
14 d					
Soybean	– P	0.41c	0.27b	0.64	29.84b
	+ P	0.81c	0.32b	0.39	32.24b
Sunflower	– P	0.28c	0.47b	1.63	56.85b
	+ P	2.05b	1.19a	0.59	155.89a
Maize	– P	0.39c	0.46b	1.19	57.48b
	+ P	2.83a	1.15a	0.45	120.13a
21 d					
Soybean	– P	0.55d	0.35d	0.73	33.32d
	+ P	2.76c	0.93c	0.37	104.42c
Sunflower	– P	0.44d	0.57cd	1.25	73.83c
	+ P	8.79a	3.78a	0.43	333.64a
Maize	– P	0.45d	0.53cd	1.21	86.61c
	+ P	7.33b	2.49b	0.34	259.63b
ANOVA					
7 d					
Species		n.s.	n.s.	n.s.	**
P level		n.s.	n.s.	n.s.	n.s.
Species × P		n.s.	n.s.	n.s.	n.s.
14 d					
Species		***	**	n.s.	**
P level		***	***	** –P > +P	**
Species × P		**	*	n.s.	*
21 d					
Species		***	***	n.s.	***
P level		***	***	*** –P > +P	***
Species × P		***	***	n.s.	*

Table 2: Root aerenchyma as percent of cross-sectional area of air-filled spaces in apical and basal segments of lateral roots of soybean and sunflower and seminal roots of maize 21 d after imposing P treatments. Results from ANOVA expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$, ** $P < 1\%$, *** $P < 0.1\%$. Mean comparison is shown besides average values in those cases where the interaction between the factors species and P level was statistically significant. Values in the same column followed by the same letter are not significantly different at the 5% level using the Tukey test.

Species	P level	Aerenchyma / %	
		Apical region	Basal region
Soybean	– P	4.63	16.62a
	+ P	1.77	1.42bc
Sunflower	– P	3.88	4.91bc
	+ P	1.95	0.65bc
Maize	– P	1.52	5.79b
	+ P	0.76	0.38c
ANOVA			
Species		n.s.	**
P level		n.s.	***
Species x P		n.s.	*

by soybean (11.7 times) and sunflower (7.5 times; Table 2). Soybean was the species with the highest proportion of aerenchyma in the basal region of the root. In contrast, no significant species or P effects were observed in the development of aerenchyma in the apical region (Table 2).

3.3 Phosphorus concentration and P-uptake efficiency

Soybean plants showed almost double P concentrations than sunflower and maize under high-P conditions. Phosphorus-deficient plants showed considerably lower P concentrations but no differences between species were detected at this P level (Table 3).

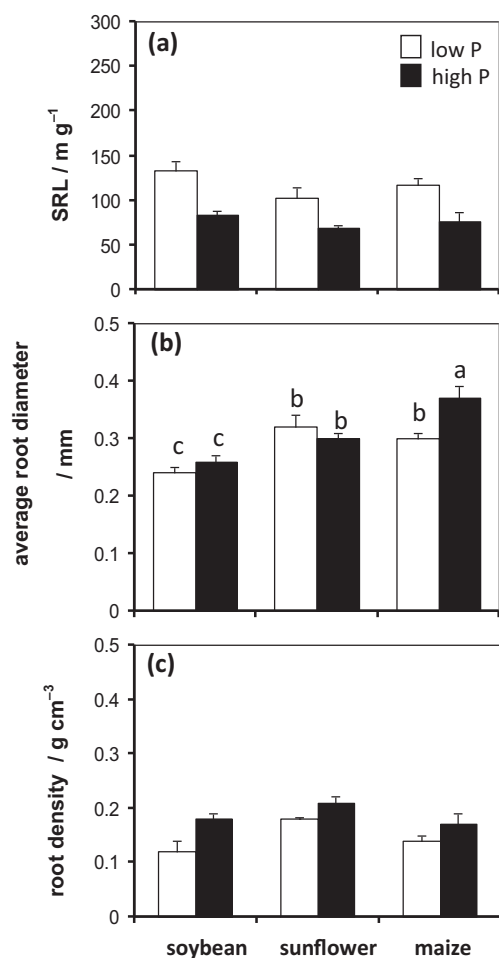
The P-uptake efficiency per unit of root biomass and per unit of root length showed a significant interaction species × P level (Table 3). As expected, the P-uptake efficiency was one of the variables most affected by P stress (Table 3). The comparison between species revealed that, under low P conditions, soybean and sunflower were consistently more efficient than maize in acquiring P either per unit of root biomass or length. Under high P conditions, the differences between species were less clear.

3.4 Specific root length, average root diameter, root density, and root distribution among diameter classes

Phosphorus deficiency increased SRL ($P < 0.1\%$) of the three species (Fig. 2). Soybean had higher SRL values than sunflower, whereas maize showed intermediate values. The average root diameter showed a significant species × P level ($P < 0.05\%$) interaction (Fig. 2). Phosphorus stress caused a 19% reduction in the average root diameter of maize but did not affect this parameter in sunflower and soybean. The latter was the species with the lowest average root diameter. On the other hand, P stress consistently reduced the root density of the three species (Fig. 2) including sunflower, the species which showed the highest values.

Table 3: Plant P concentration (dry weight basis) and P-uptake efficiency on a root weight or length basis 21 d after imposing the P treatments. Phosphorus in the seed was discounted for estimating P-uptake efficiency. Results from ANOVA expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$, ** $P < 1\%$; *** $P < 0.1\%$. Mean comparison is shown besides average values in those cases where the interaction between the factors species and P level was statistically significant. Values in the same column followed by the same letter are not significantly different at the 5% level using the Tukey test. In the other cases, mean comparison is provided in the ANOVA section of the table.

Species	P level	P concentration	P-uptake efficiency	P-uptake efficiency
		/ mg P g ⁻¹	/ mg P g root ⁻¹	/ mg P km root ⁻¹
Soybean	– P	0.91c	0.77c	8.19c
	+ P	6.97a	26.15a	235.99a
Sunflower	– P	1.02c	1.02c	8.37c
	+ P	3.29b	10.83b	124.7b
Maize	– P	0.79c	0.48d	2.90d
	+ P	3.65b	14.11b	136.08ab
ANOVA				
Species		**	**	**
P level		***	***	***
Species x P		**	**	**



	ANOVA			Means comparison			
	Species	P	Sp. x P	P level	Species		
				+P<-P	soybean	sunflower	maize
SRL	*	***	n.s.	+P<-P	a	b	ab
root diameter	***	*	*				
root density	**	**	n.s.	+P>-P	b	a	b

Figure 2: Specific root length (SRL), average root diameter, and root density for soybean, sunflower, and maize 21 d after imposing the P treatments (low and high P) in the hydroponics experiment. Error bars represent the standard error of the mean. Results from ANOVA are expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$, ** $P < 1\%$, *** $P < 0.1\%$. For average root diameter, treatments with the same letter are not significantly different ($P < 5\%$) according to the Tukey test. For SRL and root density the species \times P interaction was not statistically significant, and mean comparison test is shown in the table. Species in the same row followed by the same letter are not significantly different at the 5% level.

The three species concentrated more than 50% (range 57–76%) of their root length in the finer roots class (< 0.25 mm) and less than 25% (range 8.4–22.3%) in the coarser root class (> 0.5 mm; Fig. 3). Phosphorus treatments did not affect the proportion of fine roots in soybean but it did in sunflower and maize, although in an opposite way. Low P levels decreased the proportion of fine roots of sunflower but increased it in maize. Phosphorus treatments did not affect the proportion of thicker roots (0.25–0.50 mm and > 0.5 mm

classes) in the three species (Fig. 3). Soybean tended to have a lower proportion of thicker roots than the other two species.

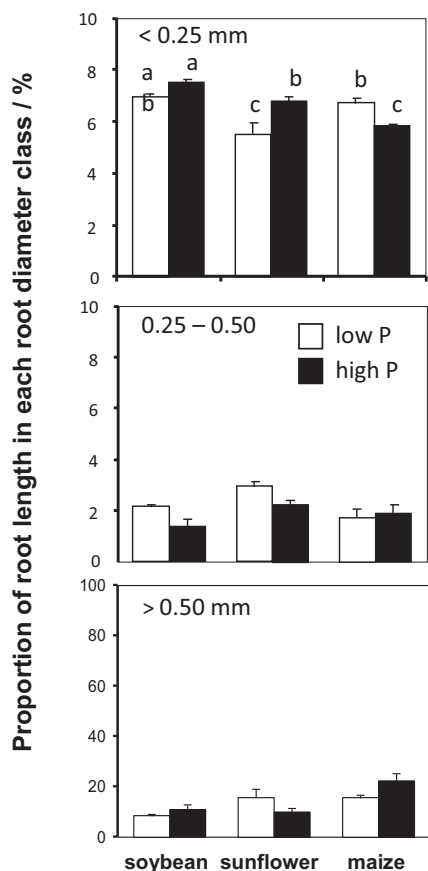
4 Discussion

4.1 Changes in root traits in response to suboptimal P conditions

Several key root traits showed a high plasticity and a similar response to P starvation in the three species. Compared to P-sufficient plants, P-stressed plants of the three species showed a higher root-to-shoot ratio, SRL, root porosity, root aerenchyma, and a lower root density (Tables 1 and 2; Figs. 1 and 2). Most of the observed responses were in favor of P uptake under low P conditions. The observed increase in the root-to-shoot ratio is a universal response to P starvation (Rubio et al., 2013), which determines that each unit of above-ground biomass is supported by a larger portion of root, increasing the specific P foraging capacity. Similarly, the higher SRL values of the P-stressed plants would be an indicator of a root system with lower metabolic demand per unit of root length (Eissenstat, 1992; Ryser, 2006). The higher SRL of low-P plants of the three species was mainly associated with a marked decrease in root porosity (Figs. 1 and 2). In line with this, root density was lower in low-P plants (Fig. 2) and, with the exception of maize, the average root diameter was not affected by P. The three species did not show a consistent pattern of response to P starvation in the proportion of fine roots (< 0.25 mm; Fig. 3). Only maize showed an increase in the proportion of this root size class. Similarly, the average root diameter did not show a consistent response to P treatments in the three species.

Phosphorus deficiency promoted a consistent increase in the root porosity and root aerenchyma of soybean, sunflower, and maize. In the case of soybean and sunflower, to our knowledge, this is the first report that demonstrates that P deficiency promotes the presence of root pore spaces. Hill et al. (2006) also found that root density decreased in response to P deficiency in some pasture species. In the case of maize, our results confirm previous evidence found by Fan et al. (2003). These authors demonstrated that the formation of new aerenchymatic tissues reduced the construction and maintenance-respiration costs in bean and maize roots. They found that roots with 20% of their area occupied with aerenchyma had 50% less respiration cost compared to roots without aerenchyma (Fan et al., 2003). These authors also observed that the formation of air spaces also may reduce the P requirements because air spaces have no P.

The increase in root porosity in response to P stress was rapid in the three species (7 d after imposing the P stress). This



	ANOVA			Means comparison		
	Species	P	Species x P	P level		
				Species		
				soybean	sunflower	maize
<0.25 mm	***	n.s.	***			
0.25 – 0.5 mm	**	n.s.	n.s.	b	a	a
>0.5 mm	***	n.s.	n.s.	b	ab	a

Figure 3: Proportion of root length in each root diameter class for soybean, sunflower, and maize 21 d after imposing the P treatments (low and high P). Error bars represent the standard error of the mean. Results from ANOVA are expressed as: n.s. (non-significant, $P > 5\%$); * $P < 5\%$, ** $P < 1\%$, *** $P < 0.1\%$. For the < 0.25 mm root class, treatments with the same letter are not significantly different ($P < 5\%$) according to the Tukey test. For the other root classes the species \times P interaction was not statistically significant, and mean comparison test is shown in the table. Species in the same row followed by the same letter are not significantly different at the 5% level.

is consistent with previous studies in maize (Drew et al., 1989; Fan et al., 2003; 2007), in which anatomical responses to P stress appeared 4–12 d from the start of the P treatment. After 3 weeks of P stress root porosity values ranged 5–13% (Fig. 1). Fan et al. (2003) found porosity values of 4–5% in beans and 10–16% in maize (both under low P conditions). In rice, Lu et al. (1999) reported a high response to P in root porosity, with values ranging from 11–69% depending on the genotype and age of the plant. In agreement with results reported by Fan et al. (2003) and Konings and Verschuren (1980), we observed that the generation of pore space was mainly concentrated in older root tissues (basal section of roots) and increased progressively through the experiment as

the P stress was more severe (Table 2). The presence of root pore space was not exclusive for P-stressed plants (Fig. 1) although this factor clearly regulated its proportion.

The replacement of cortical tissue by root air spaces presents certain "tradeoffs" which should be considered in addition to the benefits already mentioned, e.g., a decrease in the root physical resistance and the ability to penetrate compacted soils (Striker et al., 2007), a decrease in the root hospitality to mycorrhizal fungi, a reduced capacity of storage in the vacuoles, an increase in the susceptibility to herbivores pathogens (Lynch, 2007), and a decrease in the root hydraulic conductivity (Fan et al., 2007). The mechanisms of how cortical cells become aerenchymatic under P stress are still unknown.

4.2 Interspecific differences in response to suboptimal P conditions and P-uptake efficiency

Soybean, sunflower, and maize plants were above the critical P concentrations (Westermann, 2005; Sanchez, 2007) in the high-P treatments and well below those values under low-P conditions. However, the three species differed in P-uptake efficiency. Soybean and sunflower showed higher P-uptake efficiency than maize, either expressed as P uptake per unit root length or biomass (Table 3). Similar results were found under field and greenhouse conditions by Fernández et al. (2009), who observed that soybean and sunflower were able to capture more P per unit of biomass invested below-ground than maize. Interestingly, these findings are in line with the fact that soybean and sunflower have lower soil P critical levels and are less responsive to P fertilization than maize in Mollisols of the Pampas and other regions (Echeverría and García, 2005; Franzen, 1999; Gerwing and Gelderman, 2005; Dodd and Mallarino, 2005).

Soybean and sunflower appear to have followed different paths to achieve their relative high P-uptake efficiency. In the case of soybean, the high efficiency was supported by root traits associated to the fineness of the root system and the presence of root air spaces. Soybean presented thinner roots than sunflower and maize, both in terms of SRL and proportion of root length in the finer root class (Fig. 3). The fact that the P-efficient soybean had the highest root porosity and proportion of root aerenchyma suggests that the root air spaces have a relevant role in regulating its P-uptake efficiency. The link between the presence of air spaces and P efficiency had been suggested by modeling studies (Postma and Lynch, 2010), but had not been demonstrated empirically.

However, our results are not fully conclusive on this issue because sunflower had a similar P-uptake efficiency as soy-

bean but its root characteristics related to root porosity were closer to the less efficient maize. Therefore, although root porosity arises as a possible mechanism for better P efficiency, it should be considered in addition to other root-based mechanisms (Wang et al., 2010; Lynch, 2011). In the case of sunflower, the obtained results are consistent with the intense rhizospheric P depletion showed by this crop in Pampean soils (Rubio et al., 2012). However, none of the measured root traits showed more favorable values for sunflower than for the less efficient maize. Hence, the relatively higher P-uptake efficiency may be linked to other features not evaluated in this work. Because our findings are based on hydroponics, any advantages of root architecture, such as vertical distribution of roots, were not acting in our experimental approach. Also, the presence of root hairs and the capacity to modify the rhizosphere enhance the accessibility to sparingly available soil P (Richardson et al., 2011; Belinque et al., 2015). In a field study performed in Pampean soils with the same three crops, soybean showed the shallowest root system, as represented by the proportion of roots in the top 20 cm of the soil (Fernández et al., 2009). In the case of soybean, 69% of the total root length were concentrated in that layer, whereas percentages for sunflower and maize were 60% and 61%, respectively.

5 Conclusions

Our results show that P deficiency promoted root morphological modifications in the direction to favor P uptake under low P conditions in soybean, sunflower, and maize. These adaptations included a higher root-to-shoot ratio, SRL, root porosity, root aerenchyma, and a lower root density. Soybean and sunflower showed higher P-uptake efficiency than maize. In the case of soybean, this fact was related to characteristics related to the root porosity and fineness.

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