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# Physiological roles for aerenchyma in phosphorus-stressed roots

*Mingshou Fan*[A], *Jinming Zhu*[B], *Christina Richards*[B], *Kathleen M. Brown*[B] *and Jonathan P. Lynch*[B,C]

[A]Plant Nutrition Department, China Agricultural University, Haidian District, Beijing 100094, People's Republic of China.
[B]Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA.
[C]Corresponding author; email: JPL4@psu.edu

*Abstract.* Low phosphorus availability induces the formation of cortical aerenchyma in roots. The adaptive significance of this response is unknown. We hypothesized that aerenchyma may be helpful to low-phosphorus plants by reducing root respiratory and phosphorus requirements, thereby increasing the metabolic efficiency of soil exploration. To test this hypothesis we investigated aerenchyma formation, root respiration and tissue phosphorus concentration in maize and common bean genotypes in response to phosphorus availability and ethylene treatments. Genotypes differed substantially in their ability to form aerenchyma in response to low phosphorus. Aerenchyma formation was disproportionately correlated with reduced root respiration; roots with 30% cross-sectional area as aerenchyma had 70% less respiration than roots without aerenchyma. Aerenchyma formation was also proportionally correlated with reduced root phosphorus concentration. Variation in aerenchyma formation was correlated with root respiration and phosphorus concentration, regardless of whether such variation was caused genetically or by ethylene or phosphorus treatments. Results with isolated roots were confirmed by measurement of whole root respiration of intact maize plants. Our results support the hypothesis that aerenchyma formation reduces the respiratory and phosphorus requirements of soil exploration by roots, and thus, represents a useful adaptation to low phosphorus availability.

*Keywords*: genotypic variation, maize, *Phaseolus*, respiration.

## Introduction

Low phosphorus availability is a primary constraint to plant growth in many terrestrial ecosystems. Accordingly, plants have evolved a variety of mechanisms to adapt to low phosphorus availability (Raghothama 1999). Adaptations to increase phosphorus acquisition include mycorrhizal symbioses, root hair elongation and proliferation (e.g. Bates and Lynch 1996; Ma *et al.* 2001*a*, *b*) rhizosphere modification through secretion of organic acids (Gardner *et al.* 1983; Lipton *et al.* 1987), protons (Dunlop and Gardiner 1993) and phosphatases (Hayes *et al.* 1999), and modification of root architecture to maximize phosphorus acquisition efficiency (Lynch and Brown 2001). Phosphorus-deficient plants typically have higher root : shoot ratios than high-phosphorus plants, either because of allometric relationships or because of increased biomass allocation to roots (Gutschick 1993; Nielsen *et al.* 2001). Increased relative allocation to root growth is obviously beneficial for phosphorus acquisition, since phosphorus is fairly immobile in soil, but may slow overall plant growth because of the increased respiratory burden of root tissue (Hansen *et al.* 1998; Nielsen *et al.* 1998, 2001; Van der Werf *et al.* 1988).

Aerenchyma is the general term for tissue with large intercellular spaces (Esau 1977). The formation of aerenchyma in roots is associated with adaptation to hypoxia caused by flooding (reviewed in Jackson and Armstrong 1999). There is good evidence for an adaptive role for aerenchyma in flooding tolerance in many species, including maize, trees, and many wild species adapted to wetlands (reviewed in Jackson and Armstrong 1999). In flooded soils, aerenchyma improves oxygenation of root tissues by permitting oxygen flow from shoots through diffusion, or thermo-osmosis (Grosse *et al.* 1991), and protects roots from potentially toxic constituents of reduced soils by oxidizing the rhizosphere (Jackson and Armstrong 1999). In $C_3$ plants, aerenchyma may also provide a photosynthetic benefit by concentrating $CO_2$ from root respiration and channeling it to leaf intercellular spaces (Constable *et al.* 1992; Constable and Longstreth 1994). Hypoxia induces aerenchyma formation by increased ethylene biosynthesis (Atwell *et al.* 1988; Justin and Armstrong 1991), resulting in programmed death of cortical cells (Drew *et al.* 2000).

Although the overwhelming majority of research on root aerenchyma has focused on its importance in adaptation to hypoxia, root aerenchyma can also be induced by

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suboptimal nutrient availability. In well-aerated solution culture aerenchyma was observed in maize adventitious roots when nitrogen or phosphorus was omitted from the nutrient solution (Drew *et al.* 1989; Konings and Verschuren 1980). This response was also observed in common bean (Eshel *et al.* 1995) and rice (Lu *et al.* 1999). In maize the induction of aerenchyma by low P availability may be related to increased ethylene sensitivity of P-stressed roots (He *et al.* 1992). The ability of aerenchyma to facilitate gas movement within the plant has obvious implications for flooded plants, but has less obvious relevance for plants with suboptimal nutrient supply. We have proposed that the induction of aerenchyma by low phosphorus availability may be adaptive by reducing the respiratory requirement and phosphorus concentration of root tissue, thereby reducing the metabolic burden of soil exploration (Lynch and Brown 1998). In this study we test this hypothesis in contrasting genotypes of maize and common bean.

## Materials and methods

### *Plant material*

Two hybrids of maize, BH612 (Baldridge Hybrids, Cherry Fork, OH, USA) and Pioneer 3260 (abbreviated P3260; Pioneer Hi-Bred International, Des Moines, IA, USA), and two inbred lines (OH43 and W64a, supplied by Dr Shawn Kaeppler, University of Wisconsin, USA) were used in this experiment. These genotypes were selected for contrasting aerenchyma formation in a preliminary screen of multiple maize genotypes (data not shown).

For experiments with common bean, genotypes G19833 and DOR364 (CIAT, Cali, Colombia) were used. G19833 has higher yield in low-phosphorus soils than DOR364 (Beebe *et al.* 1997).

### *Nutrient solution culture*

Seeds were surface sterilized with 10% bleach for 5 min. After being rinsed with distilled water, they were placed on brown germination paper (Anchor Paper Co., St Paul, MN, USA), approximately 6 cm from the top edge, then were carefully rolled up and placed upright into a 500 mL beaker containing 200 mL of half strength nutrient solution (Epstein 1972). They were allowed to germinate at 25°C for 4 d. Uniform seedlings were transplanted to plastic boxes (24 seedlings per box) containing 30 L of aerated nutrient solution with 3 mM $KNO_3$, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 50 μM Fe-EDTA, 50 μM KCl, 25 μM $H_3BO_3$, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $(NH_4)_6Mo_7O_{24}$. The low-phosphorus treatment contained 1 μM $(NH_4)_2HPO_4$ and the high-phosphorus treatment contained 1 mM $(NH_4)_2HPO_4$. In the low-phosphorus solution, 1 mM $(NH_4)_2SO_4$ was added to make up the difference in nitrogen between high-phosphorus and low-phosphorus treatments. The pH of the nutrient solution was adjusted every 2 d, to 5.5 for maize and 6.0 for beans, with KOH, and the nutrient solutions were replaced every 6 d. The experiments were conducted in a greenhouse located at The Pennsylvania State University (40°49′ N, 77°49′ W), where the max/min temperature was 28°C/22°C. Photosynthetically active radiation (PAR) averaged 800–1000 μmol photons $m^{-2}$ $s^{-1}$, and the average humidity was 60%.

### *Harvesting and experimental design*

Maize roots were harvested at various times after initiation of the phosphorus treatments for measurements of respiration, porosity, phosphorus concentration, and collection of samples for microscopy. For experiments without ethylene and MCP (1-methylcyclopropene), there were three plants per treatment at each harvest time and the experiment was repeated three times. For experiments involving ethylene and MCP treatments, there were five plants per treatment at each harvest time and the experiment was repeated twice.

For common bean, basal roots were sampled after six weeks of growth in high- or low-phosphorus nutrient solutions. Basal roots are primary roots arising from just below the root–stem interface (Zobel 1996). Segments 2 cm in length were collected from the most basal, middle, and most apical portions of basal roots for microscopy and measurements of aerenchyma cross-sectional area. Basal root segments 12 cm in length from the most basal section were sampled for porosity measurements. Three roots were sampled per plant, and there were three plants per treatment in each of three experiments. Significant differences indicated in the text were assessed by ANOVA and *t*-tests.

### *Respiration of root segments*

Respiration of actively growing maize roots was measured as oxygen consumption by using an oxygen electrode (Oxygraph System, Hansatech Instruments Ltd, Norfolk, UK). The entire root system was washed with distilled water. Root segments (2 cm long) were sampled from basal, middle and apical parts of seminal roots, and placed in a temperature-controlled (25°C) electrode chamber containing 3–3.5 mL of 1 mM $CaSO_4$ and 5 mM Mes at pH 5.5. The buffer solution was saturated with $O_2$ by bubbling oxygen through it before respiration was measured. The chamber was sealed, and root samples were allowed to respire for 30 min at 25°C. After measurements of respiration, root segments were used for volume and length measurements (see below) with the aid of an image analysis system, then dried at 60°C for 48 h for dry weight determination. Three seminal roots were sampled from three plants per treatment and the experiment was repeated three times.

### *Sand culture*

The seeds of two genotypes (Oh43 and W64a) were selected for uniformity, sterilized, and germinated in darkness at 28 ± 1°C for 3 d. Before transplanting, the cotyledon was removed. 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 in the soil solution. Twice daily (0700 h and 1400 h), the pots were irrigated with nutrient solution consisting of (in μM): K (3000), $NO_3$ (7000), $NH_4$ (1000), Ca (2000), $SO_4$ (500), Mg (500), Cl (25), B (12.5), Mn (1), Zn (1), Cu (0.25) Mo (0.25) and Fe-EDTA (25). The plants were grown in a temperature-controlled greenhouse in University Park, Pennsylvania, USA (40°49′ N, 77°49′ W). The plants were grown under a photoperiod of 14/10 h at 28/24°C (light/darkness). Maximum midday photosynthetic flux densities reached 1200 μmol photons $m^{-2}$ $s^{-1}$. The relative humidity was 50%. The nutrient solution pH was adjusted to 5.8 daily.

### *Intact root respiration measurements*

The 'head space' approach of sampling air flowing over the soil surface was used in this study. Extensive studies of common bean and citrus root respiration in soil (Bouma *et al.* 1997*a*, *b*) indicated that root respiration was not influenced by soil moisture or soil $CO_2$ concentration, and there was no difference in intact root respiration when comparing 'head space' measurements to 'perfusive' measurements. At day 12 after transplanting, the root system was sealed off from the shoot by a PVC plate placed on top of the pot. An air pump provided a stable flow of air through the 'head space' compartment of the pot. The airflow rate was 1200 μmol $s^{-1}$. The measurements were conducted in early morning with a portable infrared gas analyser in a differential mode (Li-Cor 6250, Li-Cor,

Lincoln, NE, USA), ensuring that the 'head space' $CO_2$ concentration remained relatively low and that the 'head space' temperature only increased slightly. During the measurement, $CO_2$ changed less than 20 μmol $mol^{-1}$.

Total root length was obtained by scanning with image analysis software (WinRhizo Pro, Régent Instruments, Québec, Canada). Root samples were dried at 60°C for 72 h prior to dry weight (biomass) determination.

*Tissue sectioning*

Root segments (5 mm long) were sampled from basal (2 cm from the basal end of the root), center, and apical (2 cm from root tip) regions of seminal roots of maize or of basal roots of beans. Root samples were immediately fixed in FAA fixative (5% formaldehyde, 5% glacial acetic acid, 90% by volume of 70% ethanol) and vacuum-infiltrated for 15 min, then left in FAA for 24 h. They were then dehydrated gradually in a graded ethanol series (50%, 60%, 70%, 85%, 95% and 100%). Samples were stained with 0.1% EosinY and infiltrated in graded ethanol/histoclear (National Diagnostics, Manville, NJ, USA) (75:25, 50:50, 25:75, 0:100) followed by a graded histoclear/paraffin series (1/3, 1/2, 2/3 and all paraffin). After 3-d infiltration in paraffin, replaced twice every day, root samples were embedded in paraffin, and then sectioned transversely to a thickness of 10 μm with a microtome (model 2040 Reichert-Jung microtome, Leica Microsystems AG, Wetzlar, Germany). Finally paraffin was removed from the sections with histoclear and ethanol, for observation under a light microscope.

*Aerenchyma cross-sectional area calculation*

Nine representative sections for each treatment were selected from different roots to be photographed at 100× enlargement. Images were scanned into digital files with Desk-scan (Hewlett-Packard, Palo Alto, CA, USA), then the areas of aerenchyma, stele and whole-section were measured with the aid of Photofinish (Zsoft Corporation, Palo Alto, CA, USA) and Delta-scan software (Delta T Devices Ltd, Cambridge, UK).

*Root porosity*

For maize, whole seminal roots were sampled from three plants for each genotype and treatment. For bean, porosity was measured in the most basal 12 cm of basal roots, using three roots per plant. Lateral roots were removed from seminal and basal roots. Porosities were determined as follows. A 25-mL Pyrex pycnometer flask full of degassed water was first weighed at room temperature. Pre-weighed root tissue was added to the degassed water-filled flask and weighed at room temperature. The root tissue was moved to a scintillation vial also filled with degassed water and then vacuum-infiltrated until no more air bubbles were released from the root tissue. The vacuum-infiltrated tissue was returned to water-filled flask and reweighed. Water temperature was measured after each weighing in order to correct weight readings. Porosity of tissue was calculated as follows:

$$\text{Porosity (\%)} = 100 \times [(FA - FB)/(FW + TW - FB)].$$

FA is the weight of the pycnometer flask and water with plant tissue after vacuum-infiltration, FB is the weight of the pycnometer flask and water with plant tissue before vacuum-infiltration, FW is the weight of the pycnometer flask full of degassed water without plant tissue, and TW is the fresh weight of the plant material.

*Root volume*

Root samples, after respiration measurement, were placed in petri dishes containing 1% neutral red solution. After a staining period of 24 h, the roots were rinsed with distilled water until no more dye leached from the root. Stained root samples were placed in a plexiglass tray containing distilled water. Root images were obtained and analysed with WinRhizo (Régent Instrument Inc.). Root samples were then dried in a 60°C oven for 48 h to measure dry weight.

*Tissue phosphorus concentration*

Lateral roots were removed from seminal roots of maize, and 4-cm segments were sampled from basal, middle and tip regions for determination of phosphorus concentration. Tissue was ashed at 500°C for 24 h, the ash was dissolved in 0.1 N HCl, and then phosphorus was determined spectrophotometrically (Murphy and Riley 1962).

*Ethylene and MCP treatments*

Treatments were high phosphorus (1 mM phosphorus), high phosphorus + exogenous ethylene, low phosphorus, and low phosphorus + MCP (1-methylcyclopropene, an inhibitor of ethylene action; EthylBloc, containing 0.43% MCP, Floralife, Inc., Walterboro, SC, USA). Uniform seedlings were selected and transplanted in high-phosphorus solution as before. After 4 d of growth in high-phosphorus solution, half of the plants were transferred to low-phosphorus solution. Of those, half were treated with MCP by adding 0.01 g EthylBloc into the nutrient solution every day. The other half of the plants were kept in high-phosphorus solution, and half of the high-phosphorus plants were treated with exogenous ethylene by bubbling 5 μL $L^{-1}$ of ethylene gas (in air) through the nutrient solution for 10 min each hour. Nutrient solutions were replaced 5 d after onset of treatments. Seminal roots were harvested 2 and 10 d after the initiation of ethylene and MCP treatments. In order to sample root sections which were developmentally equivalent in all treatments, two seminal roots of each plant were marked 1 cm behind the root tip with Indian ink before the onset of low-phosphorus, MCP, and ethylene treatments. At harvest, 2-cm long root samples were taken at the marked position (1 cm above mark point and 1 cm below the point) for hand sectioning and observation of aerenchyma formation, respiration measurements, and phosphorus determination.

*Statistical analysis*

Data were analysed with the StatView statistical package (1998, SAS Inc., Cary, NC, USA) for main effects (phosphorus and genotype) and first order interactions. Fisher's least significance difference was used for multiple comparisons.

## Results

### *Aerenchyma formation and respiration in roots*

In maize, no visible cavities or collapsed cells were observed in the cortex of seminal roots of either genotype on the second day after transplanting to aerated solutions containing either high- or low-phosphorus. After 12 d of growth in high- or low-phosphorus solution, aerenchymatous tissue had formed in low-phosphorus seminal roots of both OH43 and W64a (Fig. 1), while in the roots grown in high-phosphorus solution, there were no cavities in tip and middle regions, and a few very small cavities in the base of seminal roots (Fig. 1, Table 1). An increasing percentage of whole-root cross-sectional area occupied by cavities was observed along the root axis from tip to base in low-phosphorus plants (Table 1). The high percentage of aerenchyma in the most basal sections resulted from the presence of more and larger cavities in the cortex (Fig. 1).

Aerenchyma development in bean was similar to that observed in maize (Fig. 2, Table 2). Again, aerenchyma

development was greatest in basal sections of low-phosphorus roots. The extent of aerenchyma was greater in G19833 (a relatively phosphorus-efficient genotype) than in DOR364 (a relatively phosphorus-inefficient genotype) (Table 2, Fig. 2).

In maize there were no differences among treatments, developmental stages, or genotypes in the proportion of root cross-sectional area occupied by the stele (Table 1), whereas in bean, low phosphorus greatly reduced the proportion of root cross-sectional area occupied by the stele (Table 2). The stele area was largest in the older regions of high-phosphorus roots and higher for DOR364 than for G19833, while in low-phosphorus roots the stele area was more uniform along the root axis.

Root porosity was used to quantify the volume of aerenchyma in maize and bean roots. In maize, the increase in porosity with low phosphorus availability was greater for OH43 than for W64a (Fig. 3), consistent with the data on cross-sectional area occupied by aerenchyma (Table 1). For common bean, porosity of basal roots grown under low phosphorus was also higher than that of roots grown under high phosphorus, and the difference was greater for G19833 (Fig. 4). Overall, aerenchyma development was much less in bean than in maize.

The respiration rate of root segments expressed as specific oxygen consumption (μmol $O_2$ $cm^{-3}$ root $min^{-1}$) was measured in maize seminal roots 2 and 12 d after transplanting (Fig. 4). Respiration rate decreased along the root axis from the apex to the basal region (Fig. 4). Respiration rates in root apices were about three times higher than those in the basal region. There was no significant difference in respiration rates between high-phosphorus and low-phosphorus roots at 2 days after transplanting, when aerenchyma had not yet formed. However by the 12th day, when aerenchyma were extensive in the basal parts of low-phosphorus roots (Table 1), the respiration rates in the basal and middle regions of seminal roots from plants grown in low phosphorus were significantly lower than those of high-phosphorus plants of genotype OH43 (Fig. 4).

As an alternative method to compare respiration rates in roots with and without aerenchyma, we treated maize roots grown with high phosphorus with ethylene, which has been shown to induce aerenchyma production (Drew *et al.* 1989).

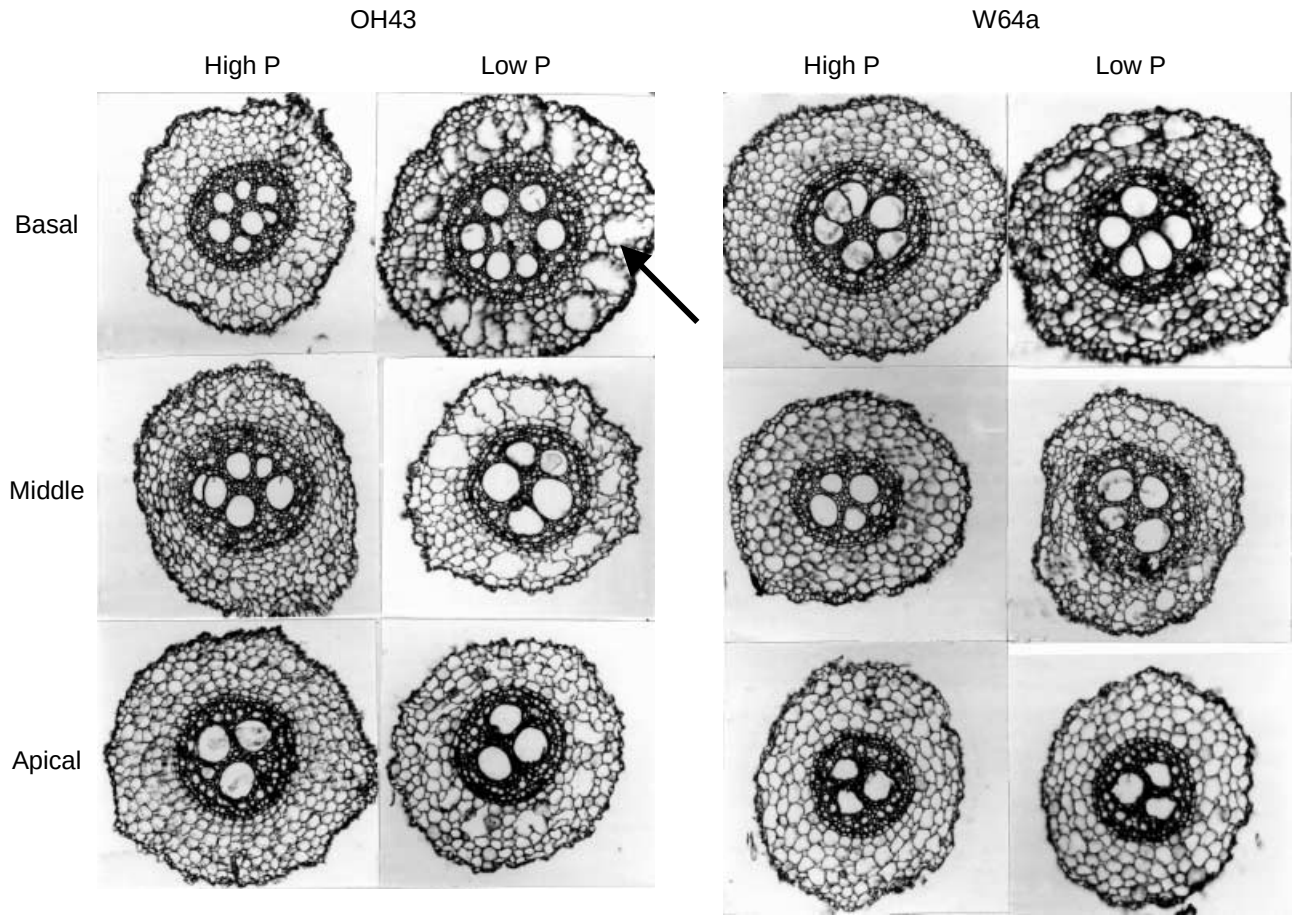


**Fig. 1.** Cross sections of seminal roots of maize genotypes OH43 and W64a grown with high P or low P. Aerenchyma are visible as large spaces in the cortex (arrow).

We also treated low-phosphorus roots with MCP to block ethylene action and aerenchyma formation in response to low phosphorus. Exogenous ethylene promoted aerenchyma formation in high phosphorus maize roots, but the extent of aerenchyma formation in response to ethylene varied among the four genotypes (Table 3). Likewise, aerenchyma formation in low-phosphorus roots was inhibited by MCP in all genotypes, the extent of inhibition ranging from 60% in W64 to 80% in P3260 (Table 3).

We observed little difference among treatments in respiration rates of maize sections at day 2, before aerenchyma formation (Fig. 5). Only in P3260 did the high-phosphorus root sections have a lower respiration rate than the other treatments. After 10 d, when extensive aerenchyma had formed in basal segments of low-phosphorus plants, there were large differences in respiration rate among treatments (Fig. 5). As in the previous study (Fig. 4), low phosphorus decreased root respiration (Fig. 5). Ethylene treatments mimicked phosphorus effects on root respiration; exogenous ethylene reduced respiration in high-phosphorus roots, whereas MCP increased respiration in low-phosphorus roots (Fig. 5). These differences were significant for all genotypes except W64a, the genotype in which aerenchyma formation was least affected by phosphorus availability (Table 3). When the results of these treatments were combined for all genotypes and treatments, there was a strong negative correlation between respiration and aerenchyma formation (Fig. 6).

*Intact root respiration*

Low phosphorus availability reduced respiration per unit of root length approximately 39% at day 12 after transplanting in intact plants (Fig. 7*a*). At low phosphorus availability, the more aerenchymatous genotype Oh43 had significantly less respiration per unit of root length than the less aerenchymatous genotype W64a (Fig. 7*a*). Intact root respiration per unit root weight is shown in Fig. 7*b*. ANOVA indicated that the effects of phosphorus and genotype × phosphorus interaction on root respiration per unit of root weight were significant ($P<0.05$). Low phosphorus availability reduced respiration per unit of root biomass significantly more in Oh43 than in W64a (43.2 ± 3.2% reduction for Oh43 *vs* 4.8 ± 7.8% reduction for W64a, a difference significant at $P=0.0041$).

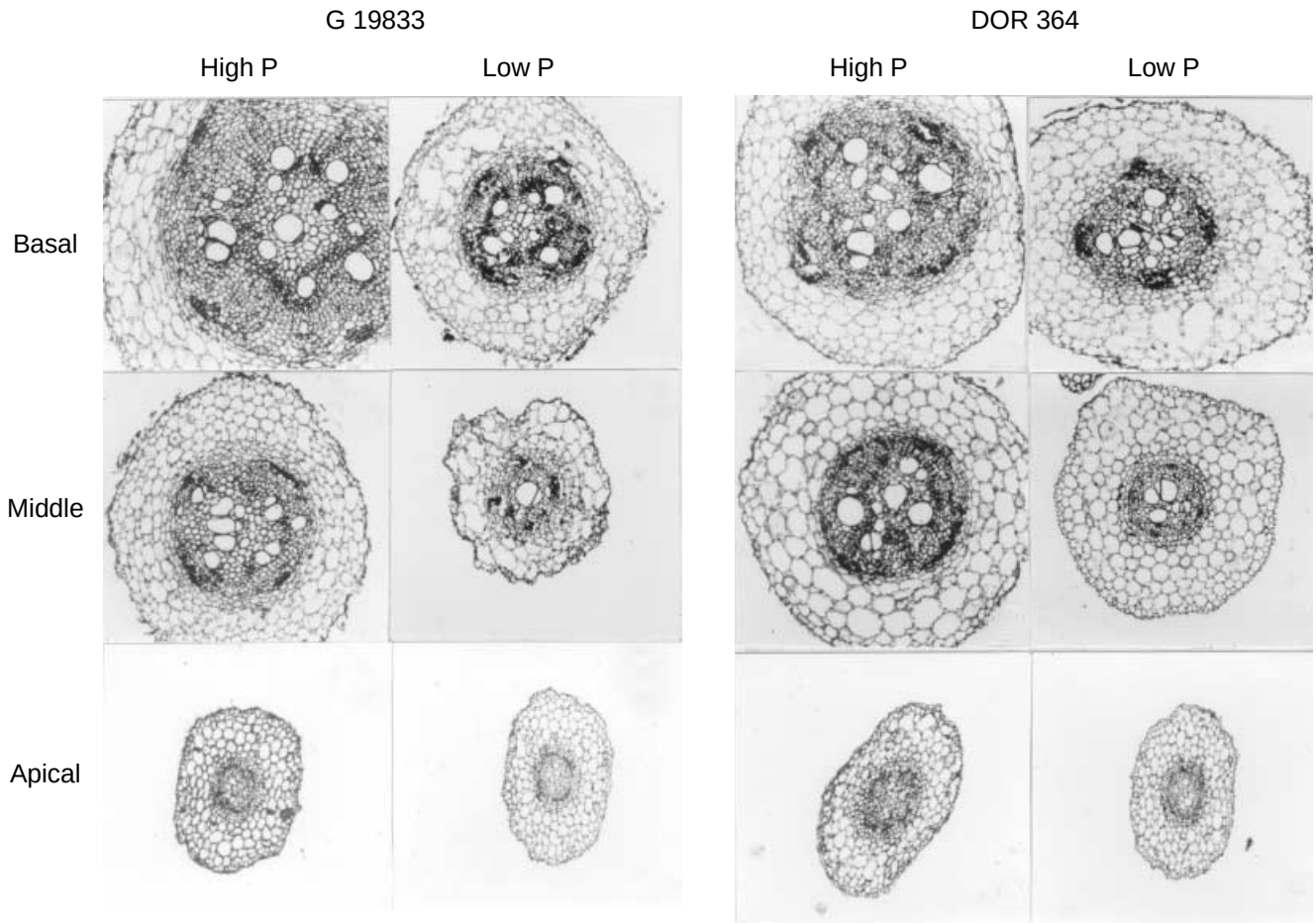


**Fig. 2.** Cross sections of basal roots of common bean genotypes G19833 and DOR 364 grown at high or low P.

**Table 1. Percentage of stele area and aerenchyma area in whole cross sections of seminal roots of two inbred genotypes of maize grown for 12 d in high-phosphorus (1 mM) or low-phosphorus (1 μM) nutrient solution**

Values are mean of six replicates with standard error (s.e.) in parentheses, *P*: * <0.05, ** <0.01, *** <0.001, ns, not significant

| Genotype | Treatment | Root region | Stele area (%) | Aerenchyma area (%) |
|---|---|---|---|---|
| OH43 | High P | Basal | 35.6 (2.9) | 4.0 (0.3) |
| | | Middle | 36.1 (1.5) | 0 |
| | | Apical | 35.2 (3.1) | 0 |
| | Low P | Basal | 36.2 (1.9) | 39.3 (2.0) |
| | | Middle | 37.1 (1.9) | 22.1 (1.3) |
| | | Apical | 36.7 (0.9) | 9.4 (1.1) |
| W64a | High P | Basal | 36.3 (1.2) | 2.0 (0.1) |
| | | Middle | 36.1 (1.1) | 0 |
| | | Apical | 36.4 (1.3) | 0 |
| | Low P | Basal | 35.3 (1.1) | 26.3 (1.1) |
| | | Middle | 37.0 (1.1) | 12.1 (0.8) |
| | | Apical | 36.1 (0.6) | 3.0 (0.2) |
| F from ANOVA | | | | |
| Genotype | | | ns | 116*** |
| P treatment | | | ns | 1327*** |
| Region | | | ns | 315*** |
| Genotype × P | | | ns | 89*** |
| Genotype × region | | | ns | 6.6** |
| P × region | | | ns | 195*** |

**Table 2. Percentage of stele area and aerenchyma area in whole cross sections of basal roots of two genotypes of common bean grown for 6 weeks in high-phosphorus (1 mM) or low-phosphorus (1 μM) nutrient solution**

Values are mean of six replicates with s.e. in parentheses, *P*: * <0.05, ** <0.01, *** <0.001, ns, not significant

| Genotype | Treatment | Root region | Stele area (%) | Aerenchyma area (%) |
|---|---|---|---|---|
| G19833 | High P | Basal | 64.9 (0.7) | 10.4 (0.6) |
| | | Middle | 57.3 (1.5) | 0 |
| | | Apical | 40.4 (1.2) | 0 |
| | Low P | Basal | 42.5 (1.1) | 26.8 (0.8) |
| | | Middle | 27.1 (1.2) | 19.8 (1.2) |
| | | Apical | 33.0 (1.0) | 4.5 (0.5) |
| DOR364 | High P | Basal | 87.8 (0.8) | 4.8 (0.4) |
| | | Middle | 46.4 (1.2) | 2.2 (0.2) |
| | | Apical | 38.2 (1.4) | 0 |
| | Low P | Basal | 36.9 (1.1) | 18.8 (0.9) |
| | | Middle | 26.3 (0.8) | 10.2 (0.8) |
| | | Apical | 32.7 (0.8) | 6.6 (0.3) |
| F from ANOVA | | | | |
| Genotype | | | ns | 83*** |
| P treatment | | | 1276*** | 1113*** |
| Region | | | 465*** | 431*** |
| Genotype × P | | | 19*** | 34*** |
| Genotype × region | | | 46*** | 43*** |
| P × region | | | 189*** | 75*** |

*Phosphorus concentration of root tissues*

Phosphorus concentration decreased basipetally along the root axes for both high-phosphorus and low-phosphorus plants (Fig. 8). In high-phosphorus roots, phosphorus concentration increased with time, but in low-phosphorus roots it decreased with time (Fig. 8). Under low phosphorus, phosphorus concentration decreased more dramatically with time for OH43 (the high-aerenchyma genotype) than for W64a (the low-aerenchyma genotype), but the difference in apical regions between the two genotypes was less than that in basal and middle regions. With high phosphorus availability, there was no significant difference in phosphorus concentration between roots of the two genotypes.

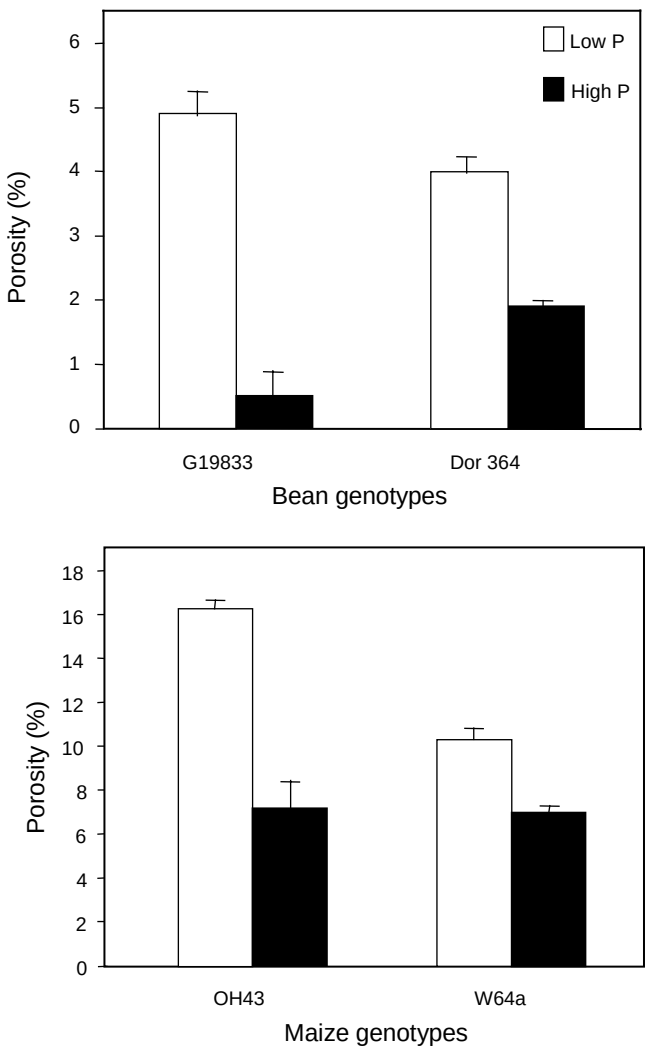


**Fig. 3.** Root porosity of genotypes of common bean and maize grown with high P (1 mM) or low P (1 μM) for 6 weeks (bean) or 12 d (maize). Values are means of six measurements, bars represent s.e. ANOVA indicated significant effects of P level and genotype × P level interaction in bean, and significant effects of genotype, P level, and genotype × P level interaction in maize.

When we examined the phosphorus concentration of roots in which aerenchyma formation was manipulated by both phosphorus availability and ethylene modulation, we again found a very strong effect of phosphorus availability on phosphorus concentration, and a variable effect of MCP and ethylene, depending on genotype (Fig. 9). Ethylene significantly reduced phosphorus concentration in high-phosphorus roots of OH43 and P3260 roots, but not in Bh612 and W64a. MCP significantly increased phosphorus concentration of low-phosphorus roots for all genotypes except OH43. In both high- and low-phosphorus roots, phosphorus concentration was negatively correlated with aerenchyma development (Fig. 10).

## Discussion

Our results confirm earlier reports that phosphorus stress induces aerenchyma formation in roots of maize plants, and also demonstrate aerenchyma induction by phosphorus stress in basal roots of common bean. We found a substantial effect of low phosphorus on aerenchyma formation in maize seedling roots (Tables 1, 3). Previous work showed that adventitious and seminal roots of maize seedlings responded to nitrogen and phosphorus deprivation with increased aerenchyma formation, particularly in the basal segments of the root, although the effect of omitting phosphorus was relatively minor in these reports (Konings and Verschuren 1980; Drew *et al.* 1989; He *et al.* 1992). The greater effect of low phosphorus on maize aerenchyma in our experiments may have been due to the maize genotypes we employed, since we found significant genetic variation for aerenchyma formation in response to low phosphorus availability (Table 1), as well as the greater age of the maize seedling roots examined (12 d in Table 1 *vs* 4 d after transplanting in previous work (Konings and Verschuren 1980; Drew *et al.*

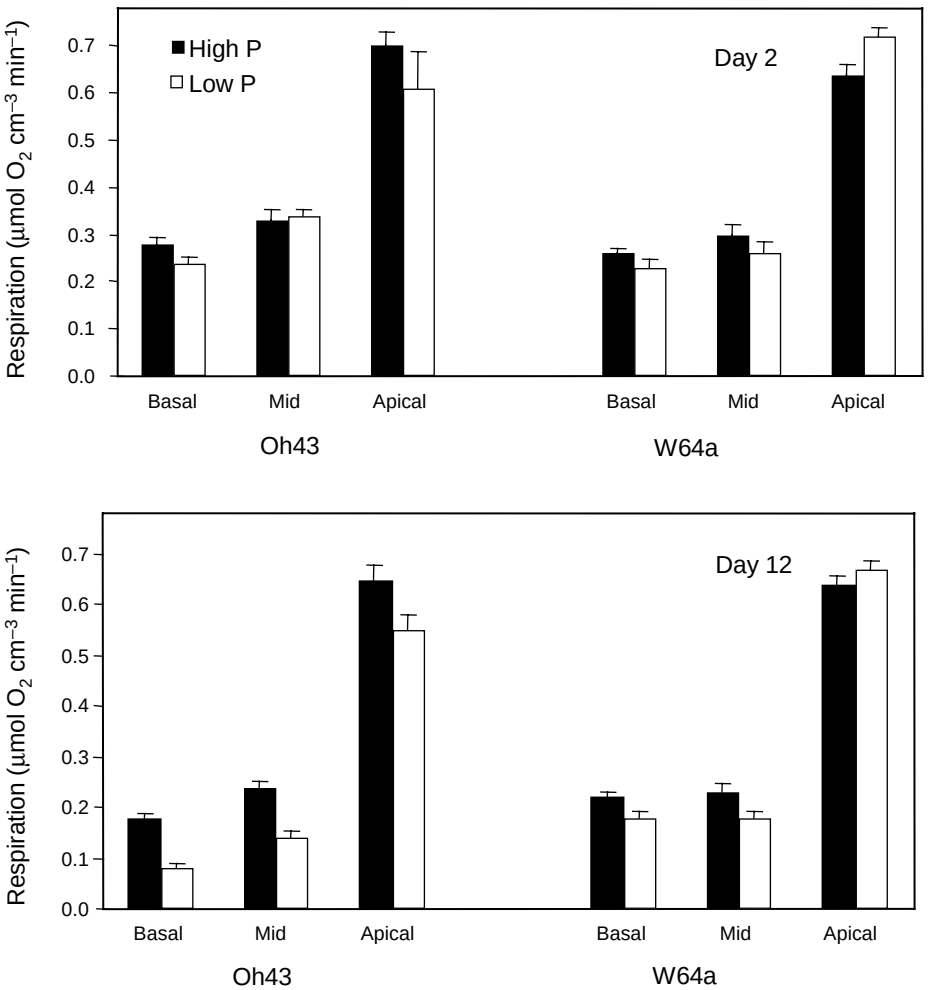


**Fig. 4.** Respiration of 2-cm segments of seminal roots of maize seedlings harvested 2 or 12 d after transplanting into nutrient solutions containing high P (1 mM) or low P (1 µM). Values are means of nine measurements, bars represent s.e. ANOVA indicated that respiration rate was significantly affected by root age, segment position, genotype, and P level, as well as all two-way factor interactions, and the three-way interaction of genotype × P level × position.

1989), since we found that the extent of aerenchyma increased with time.

Our measurements of root porosity, which quantifies the volume of air space in the root, were in agreement with our observations of aerenchyma formation from root sectioning. Root samples with greater cross-sectional area as aerenchyma (Table 1) had greater porosity (Fig. 2). This indicates that, like aerenchyma in waterlogged roots, the large intercellular spaces observed in phosphorus-stressed maize seminal root were filled with air. Therefore, the term aerenchyma applies to the cavities found in nutrient-stressed roots.

Aerenchyma had been observed previously in new tissue of maize adventitious roots formed after phosphorus starvation (Drew *et al.* 1989). One of the objectives of this research was to determine if aerenchyma forms in pre-existing tissue after nutrient stress. Aerenchyma was observed in response to low phosphorus in seminal roots that had formed before transplanting (basal root sections, Fig. 1). This shows that aerenchyma is formed not only in new tissue following phosphorus stress, but also in older tissue present before the onset of phosphorus stress.

Aerenchyma substantially reduces internal impedance to the transport of oxygen, dinitrogen and various metabolically generated gases such as carbon dioxide and ethylene, especially between roots and shoots (Jackson and Armstrong 1999). Such transport lessens the risk of asphyxiation under

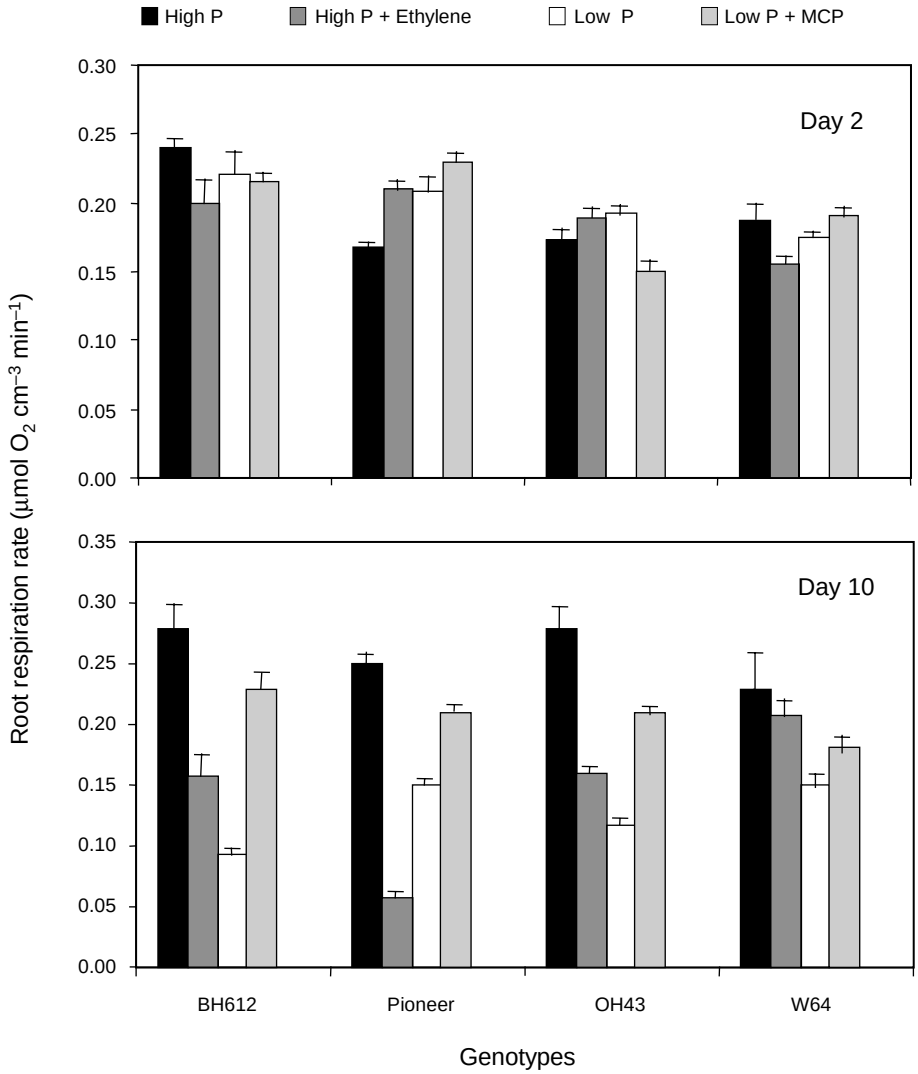


**Fig. 5.** Respiration of 2-cm segments of seminal roots of maize seedlings harvested 2 or 10 d after transplanting into nutrient solutions containing high P (1 mM) or low P (1 µM), with exogenous ethylene or the ethylene antagonist MCP. Values are means of six measurements, bars represent s.e. ANOVA indicated that respiration rate was significantly affected by root age, genotype, and treatment (phosphorus and ethylene were considered one treatment factor), as well as all factor interactions.

**Table 3. Percentage of aerenchyma area in whole cross sections from seminal roots of maize plants grown with different treatments for 10 d**

Values shown are means of 10–12 sections, with standard errors in parentheses, *P*: * <0.05, ** <0.01, *** <0.001

| Genotypes | Treatments | % of aerenchyma in root cross sections |
|---|---|---|
| Bh612 | High P control | 0 |
| | High P + ethylene | 2.4 (1.1) |
| | Low P control | 30.3 (2.3) |
| | Low P + MCP | 17.4 (2.6) |
| P3260 | High P control | 0 |
| | High P + ethylene | 19.9 (2.1) |
| | Low P control | 19.5 (1.8) |
| | Low P + MCP | 4.7 (1.1) |
| OH43 | High P control | 0 |
| | High P + ethylene | 14.7 (1.6) |
| | Low P control | 25.1 (2.1) |
| | Low P + MCP | 6.5 (1.0) |
| W64a | High P control | 0.6 (0.3) |
| | High P + ethylene | 6.7 (1.4) |
| | Low P control | 13.0 (1.2) |
| | Low P + MCP | 5.3 (0.9) |
| F from ANOVA | | |
| Genotype | | 10.2*** |
| Treatment | | 86.8*** |
| Genotype × treatment | | 15.9*** |

soil flooding. Aerenchyma also promotes radial oxygen loss from roots, leading to rhizosphere oxygenation, and increases methane loss from waterlogged sediments via plants to the atmosphere (Jackson and Armstrong 1999). The extent of lysigenous aerenchyma is linked to superior flooding tolerance among and within species. In our experiment, nutrient solutions were well oxygenated, and aerenchyma formation was minimal in plants supplied with adequate nutrition. Therefore, the function of aerenchyma under low phosphorus availability is not likely to include reduced impedance for oxygen transport between root and shoot.

We have proposed that root aerenchyma may be a useful adaptation to low phosphorus availability by reducing the metabolic costs of soil exploration (Lynch and Brown 1998). Soil exploration through root growth is critically important for the acquisition of phosphorus, which is scarcely mobile and has heterogeneous distribution in natural soils (Barber 1995). Roots may consume a significant fraction of plant available carbohydrate, especially in conditions of phosphorus limitation. Depending on the species and growth conditions, more than 50% of daily carbon fixation can be consumed by root respiration (Poorter *et al.* 1991; Lambers *et al.* 1996), a substantial portion of which is devoted to maintenance respiration (Van der Werf *et al.* 1988; Peng *et al.* 1993; Nielsen *et al.* 1998, 2001). In common bean, roots of mycorrhizal plants consume about 20% of available carbohydrate under moderate phosphorus availability, and about 35% under low phosphorus availability (Nielsen *et al.* 1998). Low phosphorus availability limits shoot photosynthesis, owing to effects on both leaf area and leaf

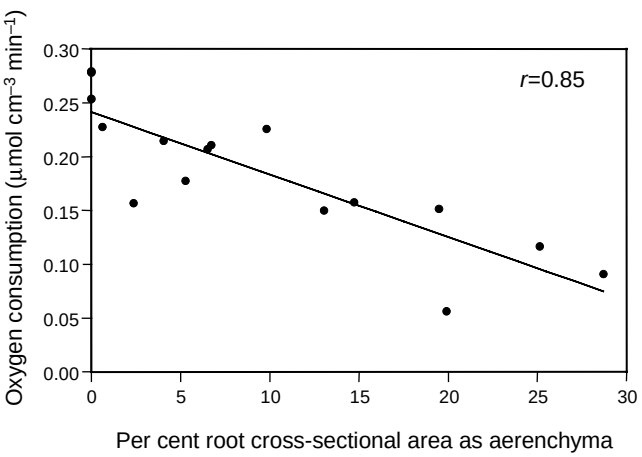


**Fig. 6.** Correlation between aerenchyma area and respiration in maize roots. Each data point is the mean of six measurements of respiration and 10–12 measurements of aerenchyma on comparable root segments. The analysis included roots treated with high and low P, MCP, and ethylene.

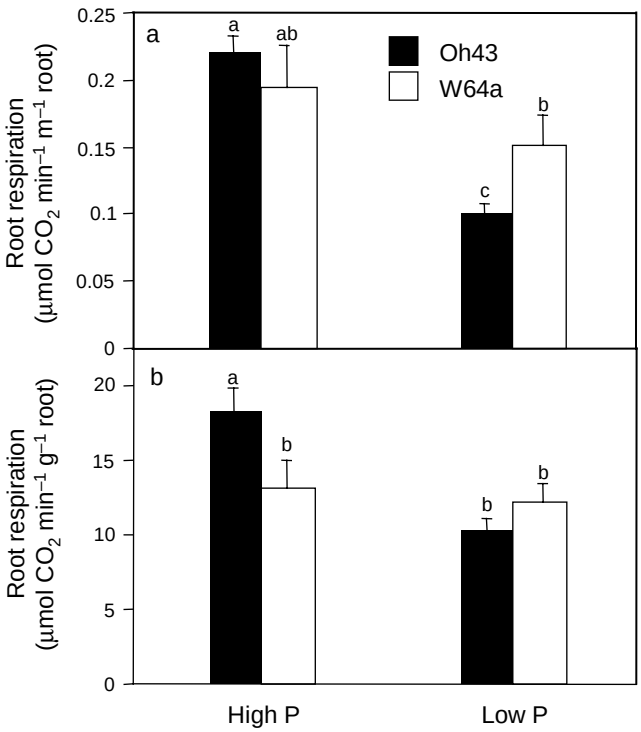


**Fig. 7.** Intact root respiration per unit of length (*a*) and per unit of weight (*b*) of maize Oh43 and W64a grown at two levels of phosphorus availability for 12 d after transplanting. Bars with different letters differ significantly at the 5% level by Fisher's PLSD (StatView, SAS Inc. 1998). Data shown are means ± standard error from four replicates.

productivity (Brooks 1986; Lynch *et al.* 1991; Theodorou *et al.* 1991). Carbon budgets of two bean genotypes well adapted to low phosphorus availability ('phosphorus-efficient' genotypes) *vs* two poorly adapted genotypes ('phosphorus-inefficient' genotypes) showed that both efficient and inefficient genotypes allocated a comparable proportion of daily photosynthate to roots, but that the efficient genotypes were able to maintain greater root growth rates per unit of carbon respired compared with inefficient genotypes (Nielsen *et al.* 2001). Several different types of root traits could alter the relationship of root growth and root carbon costs. Simulation modeling suggests that root architecture can alter the carbon cost of soil exploration by regulating the extent of root competition within (Ge *et al.* 2000) and among (Rubio *et al.* 2001) root systems. The importance of root architecture for interplant competition for phosphorus was confirmed in field studies (Rubio *et al.* 2003). Morphological traits such as root hairs could enhance phosphorus acquisition at minimal root carbon cost (Bates and Lynch 2000*a*, *b*; Ma *et al.* 2001). Anatomical traits that affect the respiratory cost of constructing and maintaining roots, or their effective lifespan, could also be important. In this regard aerenchyma is particularly interesting, since the formation of aerenchyma would be expected to dramatically reduce maintenance respiration by replacing living cortical cells with air space. Besides reducing the ongoing carbon cost of root maintenance, lysis of cortical cells may contribute pre-fixed carbon to root apices. An additional benefit from aerenchyma formation would be the reduced phosphorus requirement of root growth, which, in conditions of phosphorus limitation, can be as significant as carbon costs as an index of metabolic efficiency (Snapp *et al.* 1995; Koide *et al.* 2000). Phosphorus released from cortical tissue by aerenchyma formation would be useful in meeting the phosphorus demands of new root elongation. A similar concept has been proposed for cortical senescence in grasses [(Gillespie and Deacon 1988, Robinson 1990), although see Lascaris and Deacon (1991)].

Our results are entirely consistent with the hypothesis that aerenchyma formation reduces root respiration. We found that variation in aerenchyma formation due to genotypic variation, phosphorus availability, or ethylene

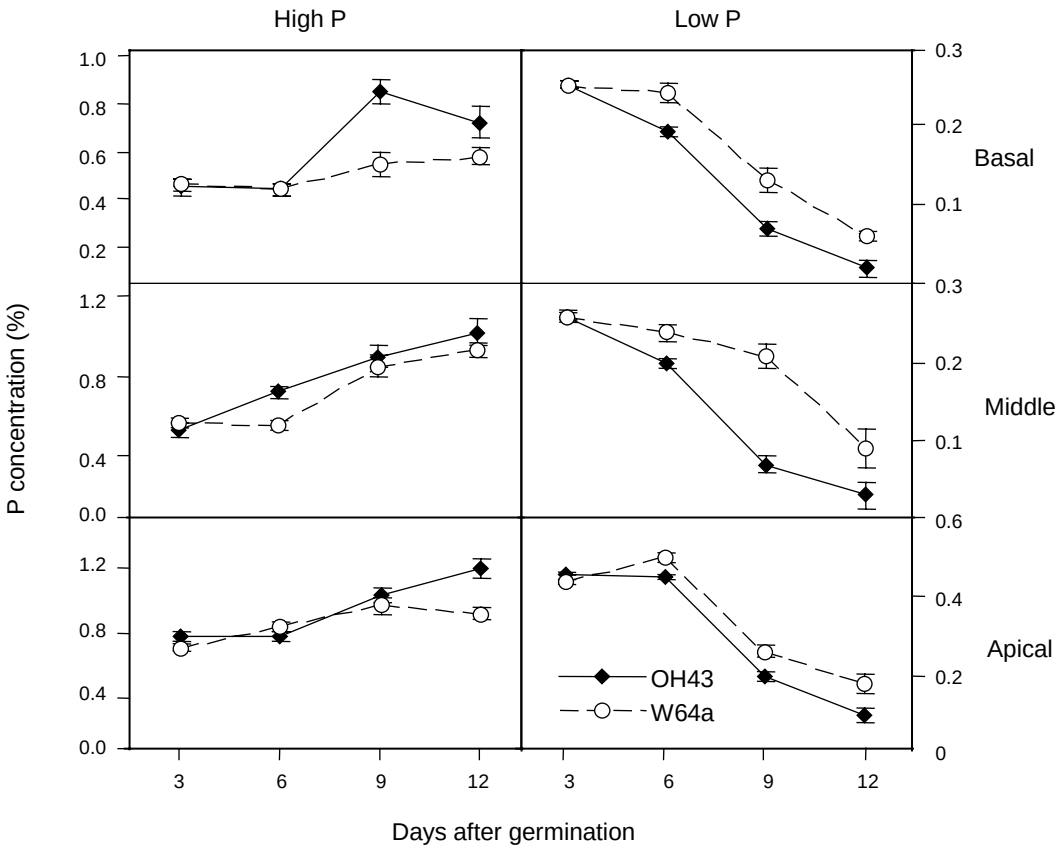


**Fig. 8.** Time course of tissue phosphorus concentration in basal, middle and apical regions of seminal roots of two maize genotypes grown in high- or low-P media. All lateral roots were removed from root segments before analysis. Each data point is the mean of three measurements ± s.e. ANOVA indicated that root phosphorus concentration was significantly affected by plant age, phosphorus treatment, genotype, segment position, as well as all factor interactions except for genotype × position and phosphorus treatment × genotype × position.

treatments, was disproportionately correlated with root respiration (Fig. 6). For example, across treatments, a root segment with 20% cross-sectional area as aerenchyma had half the respiration of a root without aerenchyma (Fig. 6). The disproportionate effect of aerenchyma formation on respiration may reflect the fact that the cortical cells lost during the formation of aerenchyma are parenchyma cells, which, although possessing a fairly large vacuole, are nonetheless metabolically active, while tissues such as sclerenchyma and xylem vessels occupy volume without contributing to maintenance respiration. Another possible factor contributing to the disproportionate effect of aerenchyma on respiration is that our methods may have underestimated actual aerenchyma volume. Our measurements of cross-sectional area may have been distorted by our tissue fixation method, and our measurements of root porosity may have underestimated aerenchyma volume, since we used fairly long root segments and subjected them to one cycle of decompression rather than several. Aerenchymal spaces are not well linked together over any distance by large openings. This, together with their hydrophobic linings, makes it very difficult to replace gas by water in all of the aerenchyma spaces (M. McCully, personal communication).

Root respiration is generally thought to be comprised of three components: one is interpreted as being related to the growth of new plant material, the second is for the maintenance of existing tissue, and the third is for ion uptake and assimilation (Lambers *et al.* 1983; Van der Werf *et al.* 1988; Johnson 1990; Peng *et al.* 1993; Bouma *et al.* 1996; Nielsen *et al.* 1998). In our study, all the lateral roots were removed from the seminal root. Therefore, total respiration in basal and middle regions of seminal roots is primarily the respiration for tissue maintenance, because the base part and middle part of a seminal root have already stopped growing. We measured root respiration in dilute calcium solutions, which would have minimized respiration due to uptake of major ions. While respiration due to tissue construction and ion uptake are important in root tips, as the root system expands and ages, maintenance respiration begins to assume a more dominant role in overall root C cost. Furthermore, the impact of aerenchyma on the respiration of the entire root system would depend on many factors such as the development and extent of aerenchyma in diverse root classes over time. For this reason our demonstration of a correlation between aerenchyma formation and the respiration of intact root systems in solid media is significant (Fig. 7). The more aerenchymatous genotype OH43 had proportionately less root respiration under low phosphorus availability than did the less aerenchymatous genotype W64a. This result shows that aerenchyma formation can significantly reduce the metabolic 'costs' of soil exploration in low-phosphorus soils.

Our results also support our hypothesis that aerenchyma formation reduces root phosphorus requirements. We found that differences in aerenchyma formation induced by ethylene treatments and genotypic variation were correlated with proportionate reductions in root phosphorus concentration in both high-phosphorus and low-phosphorus roots (Fig. 10). Obviously, reduced phosphorus requirement for soil exploration would be advantageous in conditions of low phosphorus availability. Phosphorus liberated by senescing cortical cells could presumably be used directly for continued apical growth. Previous work in bean has shown that in low phosphorus conditions, most of the phosphorus taken up by roots is retained in the root or utilized first to meet the local demand for phosphorus (Snapp and Lynch 1996).

The importance of enhanced gas transport by aerenchymatous tissue under nutrient stress is unknown. Presumably aerenchyma would allow enhanced movement of gases along the root axis and between the root and shoot, as it does in flooded plants (Jackson and Armstrong 1999). When roots are nutrient stressed but not hypoxic, oxygen

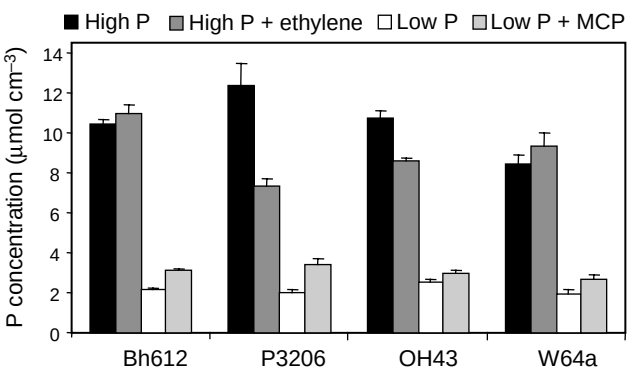


**Fig. 9.** Phosphorus concentration of segments from maize seminal roots grown with high or low P, MCP, and ethylene. Each value is the mean of four measurements ± s.e. ANOVA indicated that root phosphorus concentration was significantly affected by treatment (phosphorus and ethylene were considered one treatment factor).

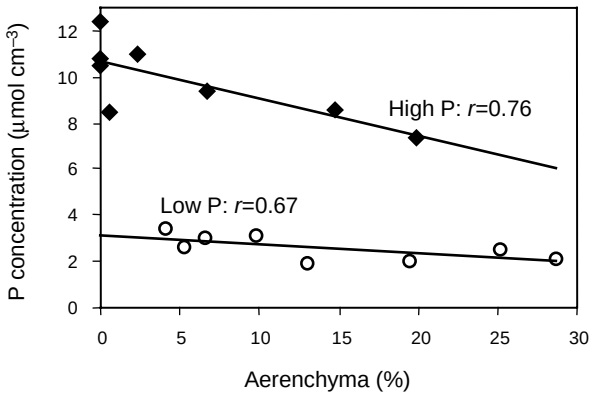


**Fig. 10.** Correlation between aerenchyma area and phosphorus concentration in maize roots grown at high or low P. Each data point is the mean of four measurements of phosphorus and 10–12 measurements of aerenchyma on comparable root segments. The analysis included roots treated with high and low P, MCP, and ethylene.

movement would probably not be a crucial factor. However movement of other gases, including ethylene, could be important for root responses to low phosphorus stress. Low phosphorus increased ethylene production in bean (Borch *et al.* 1999), and reduced it in maize, but sensitivity to ethylene increased (He *et al.* 1992). Facilitation of ethylene movement via aerenchyma could have consequences for other stress responses such as adventitious rooting (Miller *et al.* 1998; Visser *et al.* 1996).

When a phenotypic trait varies substantially among agronomically useful genotypes, the question arises: is the trait disadvantageous in some circumstances? Disadvantages of aerenchyma formation are unknown, but might include reduced opportunity for mycorrhizal colonization, decreased resistance to the longitudinal spread of pathogens, decreased capacity for metabolic storage, and decreased radial movement of water and ions. A study by Drew concluded that aerenchyma formation in maize did not substantially restrict radial transport of nutrient ions (Drew 1994), but this study was conducted in solution culture and needs to be confirmed in soil. Another possibility is that aerenchyma does not have serious drawbacks, but is only advantageous in environments with significant hypoxia or low phosphorus availability, and so may not be present in genotypes selected for adaptation to environments where these stresses are not important. This is especially likely in the case of the maize genotypes we employed, which were bred for performance in high-fertility soils. In this regard it would be interesting to compare aerenchyma formation in modern cultivars adapted to high-fertility agroecosystems with landraces adapted to low-fertility agroecosystems.

In considering the potential utility of aerenchyma in plant adaptation to low phosphorus availability, it is useful to distinguish whether this response represents a survival mechanism in extreme stress or would assist adaptation across the range of phosphorus availability encountered in typical ecosystems. Our phosphorus-stress treatments did contain phosphorus (1 μM), and we observed maize plants at 10–12 d after transplanting, which is not long after seed phosphorus reserves would have been depleted. Previous reports document aerenchyma formation in maize seedlings only 4 d after stress imposition (Konings and Verschuren 1980; Drew *et al.* 1989). Soils with very low phosphorus availability are quite common in the tropics and subtropics, which encompass many of the most densely vegetated terrestrial ecosystems, as well as agroecosystems that sustain a large fraction of the human population. In the case of common bean, one of the species used in this study, average yields in developing countries are about 10% of the yield potential, and a primary reason for low yields is low phosphorus availability (Lynch and Beebe 1995; Wortmann *et al.* 1998). Thus, adaptations to severe phosphorus stress would be useful in many ecosystems. A marginal gain in phosphorus acquisition, as would be permitted by reduced cost of soil exploration, should be advantageous whenever phosphorus availability is a limitation to plant growth, and not solely in situations of extreme stress (Lynch 1998).

In bean we observed that low phosphorus availability substantially decreases secondary growth, as seen in cross sections (Fig. 1) and in data on the proportion of the cross-sectional area occupied by the stele (Table 2). It appears that root development is delayed by low phosphorus availability. Since root secondary growth is important for root function, respiration, branching, and longevity, the adaptive consequences of this response are intriguing. Possibly it represents a strategy to concentrate resources on soil exploration through primary growth, since soil exploration is of paramount importance in conditions of phosphorus deficiency. This would be somewhat analogous to etiolation of shaded shoots.

A noteworthy feature of our findings is that we observed significant intraspecific variation for all of the principal responses and traits we observed. This has obvious relevance for crop breeding for low-fertility soils, an enterprise that is increasingly being recognized as an important component of global food security (Lynch 1998). In this regard it is interesting that the more phosphorus-efficient of the two bean genotypes we compared had greater plasticity of aerenchyma induction by low phosphorus, and greater total aerenchyma formation under low-phosphorus conditions, than the inefficient genotype (Fig. 3, Table 2). Since these are unrelated genotypes that presumably differ in many traits, this pattern could be coincidental, but it is at least consistent with the idea that cortical aerenchyma is a positive adaptation to low phosphorus availability. In the case of maize, the relative phosphorus efficiency of the four genotypes we used have not been assessed. We suggest that cortical aerenchyma deserves further attention as a potential trait for the genetic enhancement of phosphorus efficiency in crop species. Our observation of similar responses in a monocot (maize) and dicot (bean) species suggests that the trait may have utility in a variety of species.

In summary, we found that low phosphorus availability stimulates cortical aerenchyma formation in roots of maize and common bean. Genotypes differed substantially in their ability to form aerenchyma in response to low phosphorus. Aerenchyma formation was correlated with reduced root respiration and reduced root phosphorus concentration, regardless of whether such variation was caused genetically or by ethylene or phosphorus treatments. Results with isolated roots were confirmed by measurement of whole-root respiration of intact maize plants. Our results support the hypothesis that aerenchyma formation reduces the respiratory and phosphorus requirements of soil exploration by roots, and thus, represents a useful adaptation to low phosphorus availability.

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