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Compensation among root classes in *Phaseolus vulgaris* L.

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Abstract The activity of soil pathogens, competition for assimilates, and the changing availability of below-ground resources make root systems subject to a continuous and dynamic process of formation and loss of both fine and coarse roots. As hypocotyl borne roots appear later than other root classes, they may serve to functionally replace basal and primary roots lost to biotic and abiotic stress. Using common bean (*Phaseolus vulgaris* L.), we conducted experiments in solution and solid media culture with treatments involving the removal of part of the root system (basal, hypocotyl borne or primary roots), phosphorus availability, and depth of seeding to test the hypothesis that there are compensation mechanisms among basal, hypocotyl borne and primary roots to cope with the loss of part of the root system. The root system was highly plastic in response to root excision, which resulted in the maintenance of below-ground biomass accumulation. In most cases, this compensation among root classes was enough to maintain plant performance in both phosphorus sufficient and phosphorus stressed plants. Removal of a specific root class induced an increase in the growth of the remaining root classes. All root classes, but especially the primary root, contributed to the compensation mechanism in some way. Primary roots represented around 10% of the root system in control plants and this proportion increased dramatically (up to 50%) when other root classes were removed. In contrast, negligible compensatory re-growth was observed following removal of the primary root. Greater planting depth increased the production of hypocotyl borne roots at the expense of basal roots. The proportion of hypocotyl borne roots increased from 25% of the whole root system when seeds were placed at a depth of 2 cm to 30% when they were placed at 5 cm and to 38% when placed at 8 cm, with corresponding decreases in the proportion represented by basal roots. The common feature of our observations is the innate ability of the root system for its own regeneration. Total root biomass maintained strict allometric relationships with total shoot biomass in all treatments. Re-stabilization of root to shoot balance after partial root loss is governed by overall plant size following allometric relationships similar to undisturbed plants. However, the pattern of this root regeneration was not uniform since the way the three root classes compensated each other after the removal of any one of them varied among the

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different growth media and phosphorus supply conditions. The resulting changes in root architecture could have functional significance for soil resource acquisition.

Keywords Root architecture · Common bean · Compensation · Phosphorus

Introduction

Root architecture, the spatial configuration of a root system over time, determines the soil domains explored and exploited by the plant in search of below-ground resources (Fitter 1991; Lynch 1995, 2005). Root architecture is especially important for diffusion-limited nutrients, such as phosphorus, since the relative immobility of these ions in soils makes their acquisition very dependent on soil exploration in time and space (Barber 1995). Root architecture is an aggregate trait resulting from the orientation, elongation, branching, and persistence of distinct root classes. In annual dicotyledonous crops, the main root classes are the primary root, basal roots, hypocotyl borne roots, and lateral roots arising from them (Fig. 1). The primary root has strong positive gravitropism and, in the absence of barriers, grows vertically downwards (Ho et al. 2005). Basal roots arise from the basal portion of the hypocotyl (Zobel 1996; Basu et al. unpublished observations). Hypocotyl borne roots arise from the subterranean portion of the hypocotyl, above the point where basal roots arise (Miller et al. 2003). Basal roots form the structural framework of the root system and their growth with respect to gravity over time determines whether this part of the root system descends rapidly into the subsoil or remains in the topsoil (Lynch and Brown 2001).

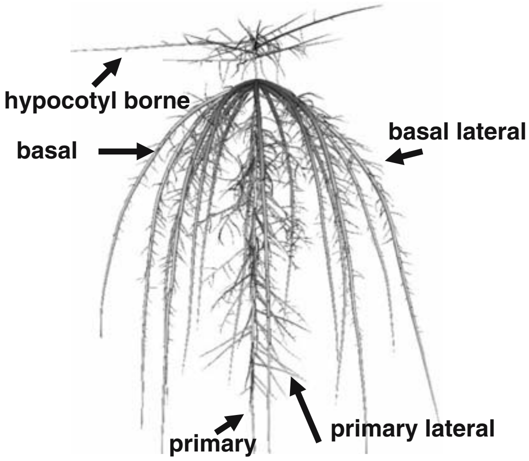

Fig. 1 Root classes of common bean. Basal, hypocotyl borne and primary roots constitute main axes from whence lateral roots arise

Although it is genetically determined, root architecture is influenced in the field by physical, chemical and biological factors (Wijesinghe et al. 2001; Passioura 2002). For example, changes in root architecture are expected whenever growth of main root axes is restricted under unfavorable physical conditions (Passioura 2002). Under these circumstances, assimilate availability increases for the remaining components of the root system (Thaler and Pages 1999). Similarly, the activity of soil pathogens and parasites, competition for assimilates, and the changing availability and localization of below-ground resources make root systems subject to a continuous and dynamic process of formation and loss of both fine and coarse roots (Gray and Steffey 1998; Eissenstat and Yanai 2002). More experimental investigation has been made for the dynamics of fine roots than of coarse roots in both herbaceous and woody plants (Eissenstat and Yanai 2002; Matamala et al. 2003). In general terms, coarse roots are those axes designed primarily to extend the root system vertically and laterally (Eissenstat 1997). It is noteworthy that 'coarse root' is a term relative to the mean diameter of the root system, but it varies between species: they are 'coarser' in woody than in herbaceous plants. Loss of coarse roots entails loss of associated fine roots and can significantly change the architecture of the whole root system. It may also influence other coarse roots through compensation mechanisms occurring after death. That is so because whenever a portion or a whole root class is eliminated, a new stage for resource competition among roots of the same root system is established (Thaler and Pages 1999). Under these circumstances, an increase in the sink strength of the remaining root classes may be expected, but the consequences of such compensation for a given root class and for organismal fitness are not obvious. If compensation mechanisms exist among root classes after partial root disappearance, the functionality of the whole root system could be maintained, but differential compensatory responses of distinct root classes would result in altered architecture and soil exploration. In our previous research with common bean (e.g. Lynch and van Beem 1993; Miller et al. 2003; Rubio et al. 2003; Ochoa et al. 2006), we have consistently observed large variation in the number of functional basal and hypocotyl borne roots and in the presence or early disappearance of the primary root. In a field study in Pennsylvania, we observed a continuous process of root disappearance that was up to 49% at pod filling (Fisher et al. 2002).

Allometric scaling relationships are a useful tool to evaluate compensation mechanisms which may affect the root to shoot ratio. They have successfully explained patterns of resources allocation in many species, including common bean (Nielsen et al. 2001; Niklas 2006). Allometric theory predicts that the biomass ratio among roots and shoots (and also among other plant parts) is regulated by the total plant size following a power function characteristic to each genotype (Hunt 1990). For most plants, the value of the power function determines that plants of reduced size because of age or stress will have a higher proportion of roots than larger plants.

Several lines of evidence show that genotypes having basal roots close to the soil surface acquire phosphorus more efficiently than other genotypes of equivalent size by enhancing topsoil foraging (Bonser et al. 1996; Liao et al. 2001; Lynch and Brown 2001; 2006; Rubio et al. 2001; Zhu et al. 2005a) and by reducing competition among roots of the same plant (Ge et al. 2000). A shallower root system may be advantageous for phosphorus acquisition, since phosphorus availability is normally greatest in the upper layers of the soil and decreases with depth (Pothuluri et al. 1986). Hypocotyl borne roots also tend to grow horizontally through the topsoil, thus they also may make an important contribution to topsoil foraging (Miller et al. 2003; Walk et al. 2005). Compared with other root classes, hypocotyl borne roots generally have greater specific root length (Miller et al. 2003), but can also have greater specific respiration rates, which means a greater metabolic demand (Lynch and Ho 2005; Walk et al. 2005). Modeling studies showed that increased hypocotyl borne rooting enhances exploration of the topsoil but can reduce the overall soil depletion volume (Walk et al. 2005). This is due to carbohydrate competition between hypocotyl borne roots and development of the primary and basal root systems in the deeper layers of the soil (Lynch and Ho 2005; Walk et al. 2005). Little is known about the factors determining the size of hypocotyl borne roots in intact plants of field crops such as cereals or legumes (Paolillo and Zobel 2002). As hypocotyl borne roots appear later than basal and primary roots, they may serve to functionally replace basal and primary roots lost to biotic and abiotic stress. In this context, hypocotyl borne roots could have an important role in functional compensation among root classes. A second factor involved in hypocotyl borne rooting could be soil phosphorus availability, as suggested by Miller et al. (2003). A third factor regulating hypocotyl borne rooting could be the depth at which the seed is placed in the soil, since hypocotyl borne roots emerge from the subterranean hypocotyl. This last factor could be easily manipulated by farmers in agricultural settings.

In this research we tested the hypotheses that there are compensation mechanisms among basal, hypocotyl borne and primary roots to cope with the loss of part of the root system, and that hypocotyl borne roots are particularly important in this capacity. A better understanding of the factors regulating root architecture would facilitate plant breeding for nutrient acquisition efficiency (Lynch in press).

Materials and methods

Seeds of common bean (*Phaseolus vulgaris* L.) genotype BAT 477 were surface sterilized for 1 min in 10% NaOCl (v/v) and germinated at 25°C for two days. BAT 477 is a medium-seeded genotype (22 g / 100 seeds) and belongs to race M of the Mesoamerican gene pool (Singh et al., 1991).

Nutrient solution experiment

To test the effects of phosphorus supply and root removal on growth and rooting patterns of bean we conducted an experiment in nutrient solution. Plastic containers (0.5 × 0.4 × 0.25 m, for 48 l solution volume) were placed in a temperature controlled (22 –30°C) greenhouse at University Park, Pennsylvania, USA (40°85′ N; 77°83′ W). Average midday photosynthetically active radiation was 1000–1600 μmol photons m^{-2} s^{-1}. Containers were filled with a solution composed of (in μM) 1500 KNO_3, 1200 $Ca(NO_3)_2$, 500 $MgSO_4$, 300 K_2SO_4, 300 $(NH_4)_2SO_4$, 400 NH_4NO_3, 25 $MgCl_2$, 5 Fe-Na-EDTA, 1.5 $MnSO_4$, 1.5 $ZnSO_4$, 0.5 $CuSO_4$, 0.5 $Na_2B_4O_7$, and 0.143 $(NH_4)_6Mo_7O_{24}$. The nutrient solution was renewed every 5 days, aerated, and its pH was maintained between 5.8 and 6.5 by additions of KOH or HCl. Plants were randomly distributed to treatments grouped in two main factors: phosphorus and root excision. The phosphorus factor included two levels, obtained by the addition of KH_2PO_4: low (0.1 μM phosphorus) and high (70 μM phosphorus). Root removal encompassed four treatments: control, hypocotyl borne, basal, and half-basal. The hypocotyl borne and basal treatments consisted of removing with blades all emerged roots of each class. In the half-basal treatment only half of the basal roots were removed. Root removal began 7 days after transplanting and was repeated every other day as needed. Each phosphorus treatment was replicated 4 times, representing a total of eight containers. In each one, three plants were assigned to each treatment of root removal (3 individuals × 4 removal treatments = 12 plants per container).

Solid media experiments

As a second test of the effects of the availability of soil phosphorus and root removal on plant performance, an experiment was conducted under similar greenhouse and environmental conditions described above but in solid media. Seedlings were placed in plastic containers (0.20 m diam, 0.25 m deep, 7.85 l) filled with (by volume) 40% perlite, 40% vermiculite, and 20% river sand. This medium permitted root excision without damage to remaining roots. The localization and availability of phosphorus were controlled with activated alumina as a solid-phase P buffer (Lynch et al. 1990), which maintained either low (0.2 μM P) or adequate (50 μM P) phosphorus availability. Pots were irrigated daily with a nutrient solution with the same composition used for the nutrient solution experiment. To minimize accumulation of nutrients in the growth media, at each irrigation sufficient nutrient solution was provided to displace the solution present in the media before the irrigation event. The experimental design was factorial with two factors (phosphorus and root removal) and five replications. The phosphorus factor included two levels as described above. Four root excision treatments consisting of removing with blades the emerged roots of each class were made: control (no root removal), hypocotyl borne, basal, and primary root. The solid media was loose enough to allow the removal of soil next to the roots to make the excisions. Root removal began 7 days after transplanting and was repeated every other day as needed.

A third experiment (called depth experiment) was conducted with plants grown in solid media to evaluate the effects of the depth of planting and heterogeneous phosphorus localization on hypocotyl borne rooting. The experimental details were as described above, including the solid-phase-buffered system and four replicates. The factors were phosphorus at three levels: low (0.2 μM phosphorus, uniform throughout the growing media); high (50 μM phosphorus, uniform throughout the growing media) and stratified (50 μM phosphorus in the top 4 cm and 0.2 μM phosphorus below 4 cm, and depth of seeding (3 levels: 2, 5 and 8 cm from soil surface).

Measurements and statistical analysis

At 28 days after transplanting, plant shoots and roots were harvested. Root material was divided into hypocotyl, hypocotyl borne, basal, and primary root classes, and stained with 0.16% neutral red dye before being scanned. Root length was quantified in the solid media experiment with image analysis (WinRhizo v. 4.0 software, Regent Systems, Quebec, Canada).

Lateral roots were kept attached to the main axes. Dry weights of shoots and roots were taken after 3 days at 60°C. Tissue phosphorus content was measured spectrophotometrically (Murphy and Riley 1962) on dry-ashed tissue. Root to shoot allometric coefficient was calculated from paired measurements of root and shoot biomass. According to Hunt (1990), the numerical formula that describes the allometric scaling relationship between roots and shoots is:

$$\log Rw = \log b + K \log Sw \qquad (1)$$

where, *Rw* is root dry weight, b is a normalization constant, *K* is the allometric coefficient and *Sw* is shoot dry weight. *K* was estimated following the reduced major axis regression analysis (Niklas 2006):

$$K_{\mathrm{RMA}} = K_{\mathrm{OLS}}\, r^{-1} \qquad (2)$$

where K_{RMA} is the reduced major axis coefficient, K_{OLS} is the slope of the log shoot biomass - log root biomass plot as in equation (1), calculated by the simple least squares regression analysis (OLS) and r is the OLS correlation coefficient.

Treatments were arranged in randomized block designs and ANOVA and regression analysis were performed using SYSTAT v. 8.0 (SPSS Inc., Evanston, IL, USA).

Results

Solution culture experiment

Phosphorus stress reduced plant biomass by more than half (Fig. 2a). Root removal treatments had less effect on total plant biomass than phosphorus treatments (Table 1). Phosphorus sufficient plants had 2.8 and and 1.4 times more shoot and root biomass, respectively, than stressed plants. Response to phosphorus was greater in shoots than in roots (Fig. 2b, c; Table 1). Removal of all basal roots had similar effects on biomass accumulation as removal of only half the basal roots (Fig. 2a–c).

Root systems of phosphorus-stressed plants had a reduced proportion of hypocotyl borne and a greater proportion of basal roots compared to phosphorus sufficient plants (Fig. 3b, d, f). The effect of root excision on the composition of the root system depended on both the root class that was eliminated and the phosphorus level (Fig. 3a–d). Elimination of hypocotyl borne roots had no direct effect on basal root biomass yet increased the proportion of root biomass represented by basal roots (Fig. 3c, d). Similarly, elimination of basal roots had no direct effect on hypocotyl borne root biomass yet increased

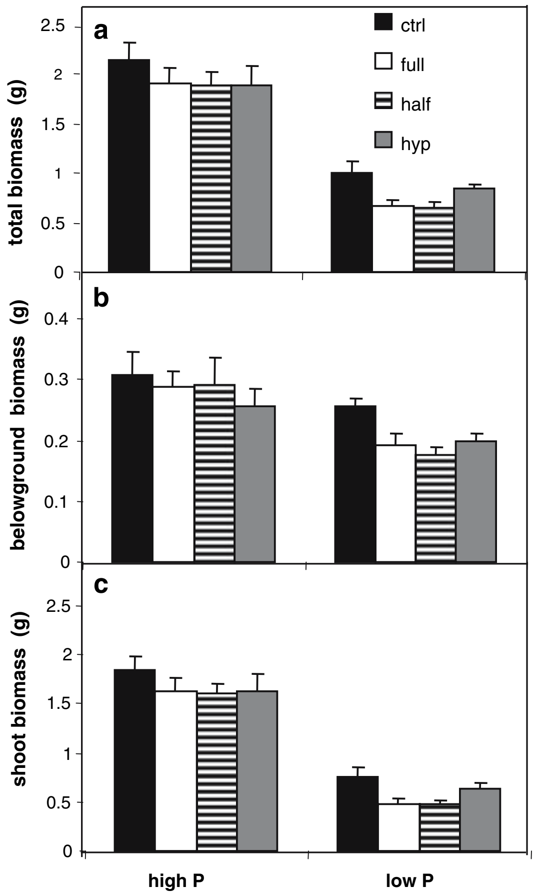

Fig. 2 Effects of root excision and phosphorus level on the total (**a**), below- (**b**) and above-ground biomass (**c**) of common bean plants grown in nutrient solution. Phosphorus treatments levels were: low (0.1 μM phosphorus) and high (70 μM phosphorus). Root excision included four levels: control (ctrl), basal (full), half-basal (half), and hypocotyl borne (hyp). The hypocotyl borne and basal treatments consisted of removing the emerged roots of each class. In the half-basal treatment only half of the basal roots were removed. Root removal began 7 days after transplanting and was repeated every other day as necessary. Plants were harvested 28 days after transplanting. Number of replicates: 4. Bars represent standard error

Table 1 *F* values, significance and mean comparison for the three experiments

	Factors				Mean comparisons				
	P level (A)	Excision (B)	A*B		P level	Excision treatments			
						ctrl	hyp	bas	half basal
Nutrient solution experiment									
Biomass									
Hypocotyl borne	64.17***	0.61NS	0.13NS		HP > LP				
Basal	2.19NS	8.46**	0.29NS			b	b		a
Primary	0.98NS	13.04***	3.50*						
Total belowground	18.35***	1.67NS	0.66NS		HP > LP				
Total aboveground	204.7***	2.50NS	0.20NS		HP > LP				
Total	160.3***	2.35NS	0.26NS		HP > LP				
Proportion of each root class									
Hypocotyl borne	78.65***	4.59*	0.83NS		HP > LP	a		b	ab
Basal	23.31***	96.91***	2.51NS		LP > HP	b	c		a
Primary	10.64**	31.82***	5.85**						
Solid media experiment						ctrl	hyp	bas	primary
Biomass									
Hypocotyl borne	9.12 ***	1.30NS	0.66NS		HP > LP				
Basal	9.12**	3.90*	1.45NS			ab	b		a
Primary	8.38**	5.13*	0.59NS		HP > LP	a	ab	b	
Total belowground	40.46***	2.29NS	0.84NS		HP > LP				
Total aboveground	158.5***	10.26**	5.32**						
Total	149.2***	9.23***	4.76**						
Proportion of each root class									
Hypocotyl borne	0.01 NS	9.11**	0.15NS			a		b	b
Basal	0.17NS	9.21**	0.59NS			a	b	a	
Primary	0.47NS	11.27***	0.38NS			a	b	c	
Specific root length									
Hypocotyl borne	0.18NS	2.22NS	0.41NS						
Basal	2.28NS	0.92NS	3.27NS						
Primary	2.47NS	2.27NS	0.62NS						
Average root diameter									
Hypocotyl borne	5.06**	6.46*	0.01NS		HP < LP	a		a	b
Basal	2.30NS	0.52NS	0.64NS						
Primary	0.11NS	0.70NS	0.48NS						
Root length									
Hypocotyl borne	3.62NS	0.09NS	0.69NS						
Basal	6.44*	0.19NS	2.82NS		HP > LP				
Primary	0.02NS	0.31NS	0.39NS						
P concentration									
Leaves	2.48NS	1.02NS	1.83NS						
Stem	5.96*	1.54NS	0.88NS		HP > LP				
Depth experiment	P level (A)	Depth (B)	A*B		P			Depth	
Biomass				HP	LP	Strat	2	5	8
Hypocotyl borne	3.27NS	3.41*	0.62NS	b	a	ab	a	ab	c
Basal	16.73***	8.77**	1.25NS	c	a	b	b	b	a
Primary	2.99NS	1.55NS	0.22NS						
Total belowground	14.51***	0.00NS	1.25NS	c	a	b			
Total aboveground	50.69***	2.02NS	1.55NS	c	a	b			
Total	44.74***	1.40NS	1.35NS	c	a	b			
Proportion of each root class									
Hypocotyl borne	0.42NS	5.65**	0.28NS				a	ab	b
Basal	1.74NS	10.81***	0.42NS				b	a	a

Table 1 continued

	Factors			Mean comparisons				
	P level (A)	Excision (B)	A*B	P level	Excision treatments			
					ctrl	hyp	bas	half basal
Primary	0.71NS	2.69NS	0.33NS					

Degrees of freedom in the nutrient solution experiment: 1, 2 and 2 for *P* level, root excision and interaction, respectively in biomass and proportion of hypocotyl borne and basal roots; 1, 3 and 3 for the other parameters. Degrees of freedom in the solid media experiment: 1, 2 and 2 for *P* level, root excision and interaction, respectively in biomass and proportion of hypocotyl borne, basal and primary roots; 1, 3 and 3 for the other parameters. Degrees of freedom in the depth experiment: 2, 2 and 4 for *P* level, depth and interaction, respectively. In the mean comparison test a < b < c < d and similar letters mean no difference between treatments at the 0.05 level using the LSD procedure

*, **, and *** significant at 0.05,0.01 and 0.001 levels, respectively. NS = non significant at 0.05 level

the proportion of root biomass represented by hypocotyl borne roots (Fig. 3a, b). Primary root biomass increased in absolute and relative terms after hypocotyl borne root removal only when plants were grown under high phosphorus conditions (Fig. 3e, f; Table 1). In contrast, under low phosphorus conditions, removal of hypocotyl borne roots decreased primary root absolute biomass almost 20%. A marked increase (more than double) in the proportion of biomass allocated to the primary root was observed at both high and low phosphorus after all basal roots were removed (Fig. 3e, f; Table 1). When only half of the basal roots were removed, primary root

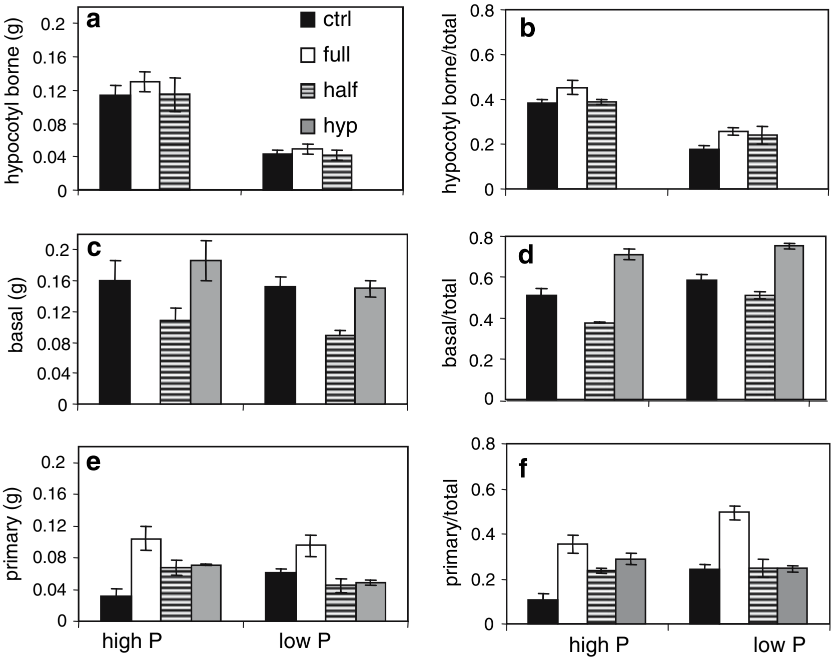

Fig. 3 Effects of root excision and phosphorus on the absolute and relative biomass of hypocotyl borne (**a, b**), basal (**c, d**) and primary (**e, f**) roots of common bean in nutrient solution. Each root class includes the main axes and laterals emerging out of it. Phosphorus availability included two levels: low (0.1 μM phosphorus) and high (70 μM phosphorus). Root excision included four levels: control (ctrl), basal (full), half-basal (half), and hypocotyl borne (hyp). The hypocotyl borne and basal treatments consisted of removing the emerged roots of each class. In the half-basal treatment only half of the basal roots were removed. Root removal began 7 days after transplanting and was repeated every other day as necessary. Plants were harvested 28 days after transplanting. Number of replicates: 4. Bars represent standard error

biomass increased slightly and only under high phosphorus conditions (Fig. 3e, f). The root to shoot allometric partitioning coefficient K_{RMA} did not differ statistically among root excision treatments ($F = 0.45$; $df = 3, 39$; $P = 0.72$). Root biomass scaled isometrically with respect to shoot biomass following a scaling component value K_{RMA} of 0.45 ± *SE* 0.05.Fig. 4.

Solid media experiment

In solid media, low phosphorus availability reduced total biomass in a similar proportion as it did in solution culture (Fig. 5a). The effect of excision treatments on above-ground and total biomass accumulation was statistically significant and regulated by the phosphorus level (Table 1). In contrast, ANOVA indicated that below-ground biomass accumulation was unaffected by root excision (Fig. 5b; Table 1). Low

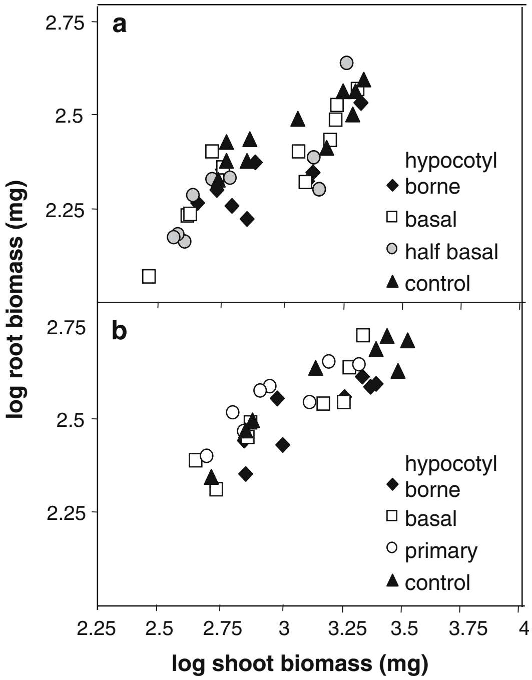

Fig. 4 Plots of $\log_{10}$ transformed data for root and shoot biomass illustrating the allometric relationships between both compartments as affected by root excision treatments. In the nutrient solution experiment (**a**) root excision included four levels: control, hypocotyl borne, basal, and half-basal. In the solid media experiment (**b**) root excision included four levels: control, hypocotyl borne, basal, and primary. The hypocotyl borne, basal and primary treatments consisted of removing the emerged roots of each class. In the half-basal treatment only half of the basal roots were removed. Root removal began 7 days after transplanting and was repeated every other day as necessary. High and low phosphorus plants were included. Plants were harvested 28 days after transplanting

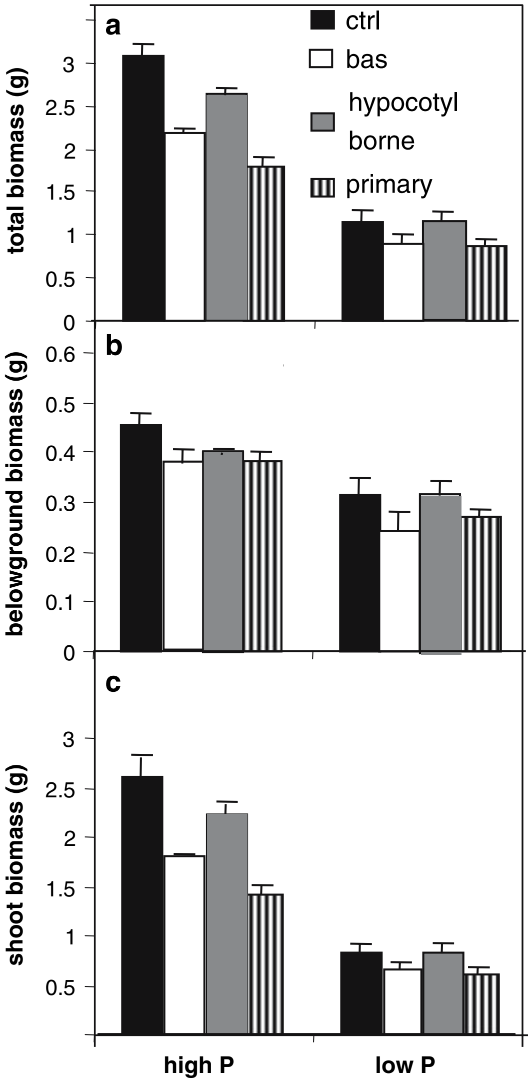

Fig. 5 Effects of root excision and phosphorus on the total (**a**), below-ground (**b**) and shoot (**c**) biomass of common bean plants grown in solid media. Phosphorus availability included two levels: low (0.2 μM P) and high (50 μM P) Root excision included four levels: control (ctrl), hypocotyl borne (hyp), basal (bas), and primary. Root removal began 7 days after transplanting and then repeated every other day when necessary. Plants were harvested 28 days after transplanting. Number of replicates: five. Bars represent standard error

phosphorus availability reduced shoot biomass by 65%, more than it reduced root biomass (30%) (Fig. 5b, c). Under high phosphorus conditions, elimination of hypocotyl borne, basal or primary roots resulted in a marked decrease in total biomass. In contrast, under low phosphorus availability, plants could compensate for the removal of the three root classes and total biomass remained unaffected by excision treatments (Fig. 5a; Table 1).

Removal of a specific root class did not necessarily imply increased biomass allocation to the remaining root classes (Fig. 6; Table 1). In most cases, removal of a particular root class led to an increase in the relative proportion of the remaining root classes, although this increase was not always associated with an increase in their absolute biomass. Excision of hypocotyl borne roots caused an increase in basal root biomass only under low phosphorus conditions (Fig. 6c, d). Primary root biomass increased after hypocotyl borne roots were removed irrespective of the phosphorus level, averaging 105%. Responses to basal root removal were consistent for both hypocotyl borne and primary roots: they increased biomass accumulation under high and low phosphorus conditions. Primary root removal caused no changes in hypocotyl borne root accumulation but increased their relative proportion. The effect of primary root removal on basal root biomass accumulation depended on phosphorus supply: no effects were observed under high phosphorus conditions but a decrease was observed in low phosphorus conditions (Fig. 6c, d; Table 1). Allometric biomass partitioning between root and shoot was very similar with that found in the nutrient solution experiment (Fig. 4). As reported for that experiment, the root to shoot allometric coefficient K did not differ statistically among root excision treatments ($F = 0.32$; $df = 3, 24$; $P = 0.81$). Root biomass scaled isometrically with respect to shoot biomass following a K_{RMA} value of $0.45 \pm SE\ 0.05$.

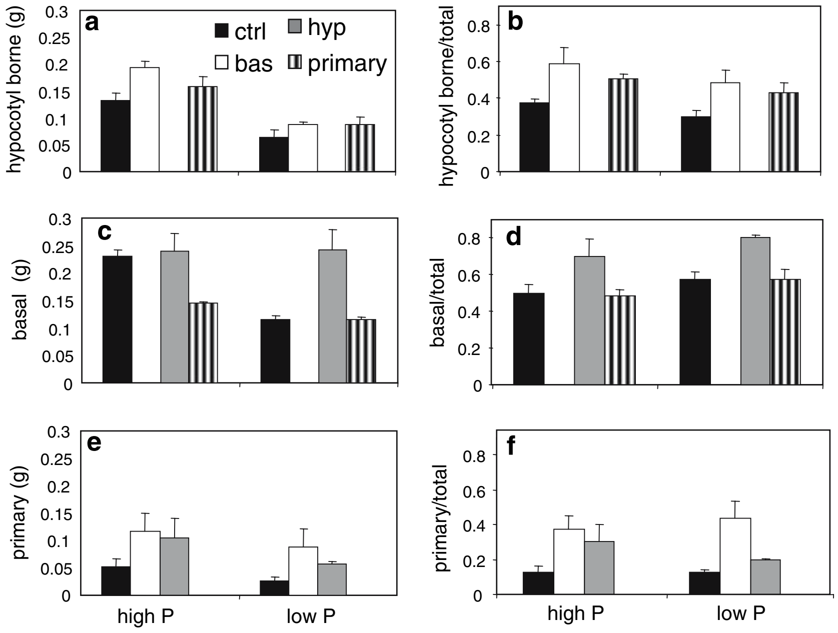

Fig. 6 Effects of root excision and phosphorus on the absolute and relative biomass of hypocotyl borne (**a, b**), basal (**c, d**) and primary (**e, f**) roots of common bean plants grown in solid media. Each root class includes the main axes and laterals emerging out of it. Phosphorus availability included two levels: low (0.2 μM P) and high (50 μM P). Root excision included four levels: control (ctrl), hypocotyl borne (hyp), basal (bas), and primary. Root removal began 7 days after transplanting and then repeated every other day when necessary. Plants were harvested 28 days after transplanting. Number of replicates: 5. Bars represent standard error

Table 2 Specific root length, average root diameter and root length of three root classes of common bean as affected by phosphorus and root excision treatments in the solid media experiment

Treatments		Target root class								
P level	Root excision	Hyp. borne Specific root length (cm mg^{-1})	Basal	Primary	Hyp. borne Average root diameter (μ)	Basal	Primary	Hyp. borne Root length (m)	Basal	Primary
High	Control	18.38 (4.32)	7.44 (1.11)	19.84 (5.60)	719 (9.3)	729 (29.4)	696 (38.7)	23.87 (4.95)	20.13 (4.05)	9.71 (4.13)
	Hyp. borne		30.47 (11.37)	11.41 (2.47)		675 (30.5)	696 (31.8)		69.87 (26.13)	11.52 (3.63)
	Basal	15.67 (4.1)		10.22 (3.73)	736 (24.1)		677 (56.8)	30.04 (8.64)		12.88 (6.52)
	Primary	12.37 (2.90)	18.74 (2.39)		799 (36.2)	678 (24.9)		24.41 (3.26)	31.62 (2.59)	
Low	Control	21.17 (3.95)	17.16 (4.71)	44.89 (21.20)	761 (28.3)	728 (19.0)	649 (37.7)	21.23 (8.41)	19.80 (4.46)	14.77 (7.69)
	Hyp. borne		8.92 (4.26)	12.48 (5.59)		723 (62.0)	730 (31.8)		13.09 (4.25)	7.15 (3.56)
	Basal	12.19 (1.63)		19.66 (3.77)	782 (19.9)		655 (54.8)	13.64 (3.41)		13.95 (4.79)
	Primary	13.97 (1.89)	12.05 (3.11)		847 (17.7)	740 (35.8)		15.91 (4.23)	13.28 (3.31)	

Phosphorus levels were: low phosphorus (0.2 μM P); and high phosphorus (50 μM P). Root excision treatments consisted in removing the emerged roots of each class: hypocotyl borne, basal, and primary root 7 days after transplanting and repeated every other day when necessary. Standard error between brackets

The effects of root excision on absolute biomass of the different root classes was not accompanied by a parallel change in root length (Tables 1, 2). The observed decrease in specific root length of hypocotyl borne roots (average 32%) and primary roots (average 59%) after the removal of the other root classes probably reflect changes in branching patterns (Table 2). In the case of hypocotyl borne roots this is supported by the significant effect of the root excision treatment on the average root diameter, suggesting a increase of root thickness after the primary root was eliminated (Table 3). No clear treatment effects on phosphorus concentration in aerial parts of the plants were observed (Table 3).

Depth experiment

No significant effects of planting depth on shoot, root and total biomass accumulation were observed (Fig. 7a; Table 1). Phosphorus availability significantly affected total, above- and belowground biomass accumulation and its subterranean and aerial components. Plants grown in media with a topsoil layer of high phosphorus concentration had intermediate growth between those under uniformly high and low phosphorus conditions.

The relative proportion of hypocotyl borne roots, basal roots and primary roots in the root system was unaffected by phosphorus level, including the stratified phosphorus treatment

Table 3 Phosphorus concentration of aerial parts of common bean plants as affected by phosphorus and root excision treatments in the solid media experiment

P level	Excision	P concentration (%)	
		Leaves	Stem
High	Control	0.09 (0.00)	0.11 (0.00)
	Hypocotyl borne	0.15 (0.04)	0.12 (0.04)
	Basal	0.11 (0.01)	0.19 (0.06)
	Primary	0.07 (0.03)	0.11 (0.00)
Low	Control	0.08 (0.00)	0.10 (0.00)
	Hypocotyl borne	0.08 (0.02)	0.07 (0.01)
	Basal	0.07 (0.01)	0.10 (0.01)
	Primary	0.09 (0.00)	0.08 (0.02)

Phosphorus levels were: low phosphorus (0.2 μM P); and high phosphorus (50 μM P). Root excision treatments consisted in removing the emerged roots of each class: hypocotyl borne, basal, and primaryprimary root 7 days after transplanting and repeated every other day when necessary. Standard error between brackets

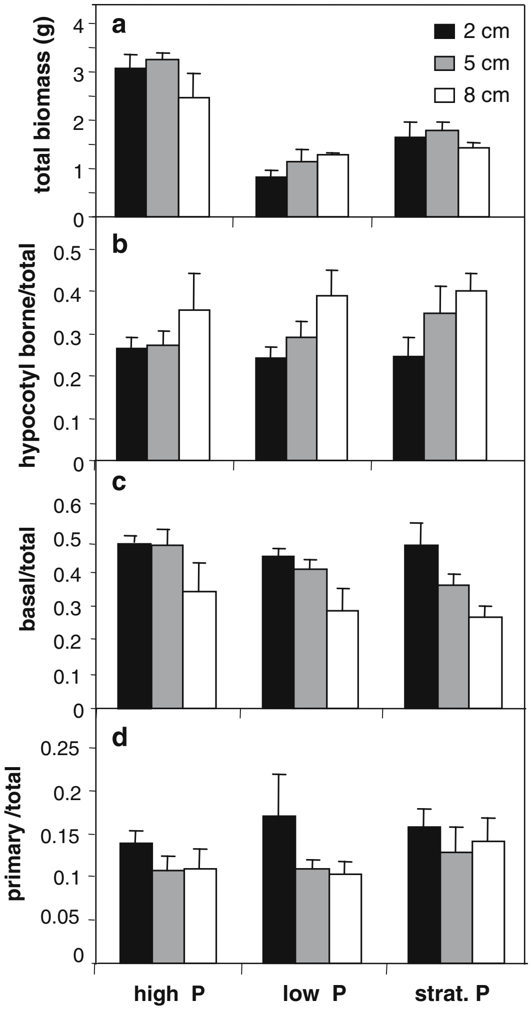

Fig. 7 Effects of depth of seeding and phosphorus on total biomass (**a**) and relative biomass of hypocotyl borne (**b**), basal (**c**) and primary (**d**) roots of common bean grown in solid media. Each root class includes the main axes and laterals emerging out of it. Phosphorus availability included three levels: low (0.2 μM phosphorus, uniform in all the growing media); high (50 μM phosphorus, uniform in all the growing media) and stratified (50 μM phosphorus in the top 4 cm and 0.2 μM phosphorus below 7 cm). Depth of seeding factor included three levels: 2, 5 and 8 cm from soil surface. Plants were harvested 28 days after transplanting. Number of replicates: 4. Bars represent standard error

(Fig. 7b ,c, d; Table 1) but was substantially affected by the depth of planting. Deeper planting of the seed resulted in an increased proportion of hypocotyl borne roots at the expense of basal roots. Relative and absolute biomass of primary roots were unaffected by the depth of seeding. No significant interactions between phosphorus levels and depth of seeding were observed for any of the analyzed parameters.

Discussion

Hypocotyl borne rooting

Hypocotyl borne roots constitute an important component of the root system of bean plants. This root class averaged 27% of root biomass in the solution culture experiment, 35% in the solid media experiment and 31% in the depth experiment. Hypocotyl borne roots may be especially important for phosphorus uptake, since the shallow growth of these enhances topsoil foraging (Lynch and Brown 2001; Miller et al. 2003; Walk et al. 2005). In common bean, hypocotyl borne rooting is under genetic control and varies substantially among genotypes (Lynch and Brown 2001; Miller et al. 2003; Rubio et al. 2003; Ochoa et al. 2006). In the present study we observed that hypocotyl borne rooting is also regulated by phosphorus availability, the removal of part of the root system and the depth of seeding. The three experiments consistently showed that phosphorus was a main factor promoting hypocotyl borne rooting. In the nutrient solution experiment, hypocotyl borne roots represented 22 and 44% of the total root system under low and high phosphorus, respectively. In solid media, hypocotyl borne roots averaged 40 and 48% for the low and high phosphorus treatments, respectively. Miller et al. (2003) found that phosphorus stress stimulated hypocotyl borne rooting in wild genotypes of common bean but this pattern was not generalized in cultivated genotypes. Among cultivated genotypes, only phosphorus-efficient genotypes manifested enhanced hypocotyl borne rooting in response to phosphorus stress. The genotype used in the present research lacks this response, but appears to have strong constitutive hypocotyl borne rooting. Removal of part of the root system promoted the formation of new hypocotyl borne roots. Elimination of half or all basal roots caused a 15 or 30%, respectively, increase in the proportion of hypocotyl borne roots in solution culture. In solid media hypocotyl borne roots increased 62% when all basal roots

Table 4 Mean difference of biomass accumulation for each root class between control plants and plants subjected to root excision treatments as affected by phosphorus level and root excision

Root excision treatments						
Grams						
Solution culture experiment						
	Hypocotyl borne		Full basal		Half basal	
	High P	Low P	High P	Low P	High P	Low P
Root class						
Hypocotyl borne	–0.11	–0.0	0.02	0.00	0.00	0.00
Basal	0.03	0.00	–0.11	–0.10	–0.05	–0.06
Primary	0.04	–0.01	0.07	0.03	0.04	–0.02
Total belowground	–0.05	–0.06	–0.02	–0.06	–0.02	–0.08
Compensatory regrowth (%)	54	NCR	82	40	60	NCR
Solid media experiment						
	Hypocotyl borne		Basal		Primary	
	High P	Low P	High P	Low P	High P	Low P
Root class						
Hypocotyl borne	–0.13	–0.06	0.06	0.02	0.03	0.02
Basal	0.01	0.13	–0.22	–0.10	–0.08	–0.00
Primary	0.05	0.03	0.06	0.03	–0.05	–0.03
Total belowground	–0.05	0.00	–0.08	–0.07	–0.08	–0.04
Compensatory regrowth (%)	62	100	64	30	NCR	NCR

Compensatory regrowth is the percentage of root biomass lost after excision of a specific root class recovered by the other root classes. Phosphorus levels were: low (0.2 μM P), and high phosphorus (50 μM P). Root excision treatments consisted in removing the emerged roots of each class: adventitious, basal and primary root 7 days after transplanting and repeated every other day when necessary. In the solution culture experiment, a treatment which included 50% of basal root excision was included. A minus sign means that the excision treatment had less biomass than the control. No preceeding sign means that the excision treatment had more biomass than the control. NCR means that no compensatory regrowth was verified for that specific treatment

were removed. Similar percentage increases were observed when the primary root was removed. These results support the importance of hypocotyl borne roots in compensating for the loss of root system biomass. This role may be effective over a long period, since hypocotyl borne roots, in contrast to basal and primary roots, emerge throughout the growing season.

Hypocotyl borne rooting was not affected by concentration of phosphorus in the topsoil but was strongly influenced by planting depth. The proportion of hypocotyl borne roots increased from 25% of the whole root system when seeds were placed at a depth of 2 cm to 30% when they were placed at 5 cm and to 38% when placed at 8 cm. The deeper the seed were planted, the more biomass was allocated to hypocotyl borne roots and the less biomass was diverted to the other root classes, mainly basal roots. The adjustment of hypocotyl borne rooting to varying planting depths did not affect the overall performance of the plant, as expressed by dry weight accumulation. Thus, compensation among root classes in response to varying planting depth was functionally neutral, since it resulted in maintenance of total and below-ground biomass.

Compensation mechanisms among different root classes after root removal

When grown in either aqueous or solid media, the root system of common bean showed substantial capacity for compensatory root re-growth in response to root removal, which resulted in maintained or only slightly reduced below-ground biomass accumulation. The compensatory abilities of the different root classes were enough to support overall plant performance in phosphorus sufficient and stressed plants grown in nutrient solution and phosphorus stressed plants grown in solid media, as indicated by the non-significant effect of root removal on total and above-ground biomass (Table 1 and 3). In contrast, when plants were grown in solid media with adequate phosphorus, a decrease in shoot biomass associated with root removal lead to lower total biomass accumulation. Our results support our working hypothesis concerning functional compensation among basal roots, hypocotyl borne roots, and primary roots. The absence of a specific root class induced an increase in the growth of the remaining root classes, as indicated by the increase in their biomass. We observed that compensatory root re-growth can be produced at a rate nearly equivalent to the loss of root biomass and may involve changes in the morphology of the remaining root classes. The strong effects of excision treatments on biomass parameters were not paralleled by root length responses. The observed decrease in specific root length of hypocotyl borne and primary roots after the removal of the other root classes may be attributable to a lower production of lateral roots arising from coarser root axes. Large sensitivity of higher-order lateral roots to assimilate competition was observed by Walk et al. (2005). They observed that increased hypocotyl borne rooting caused a diminished proliferation of fine roots of the other root classes. Regarding the effects of root removal on the intrinsic capacity for nutrient uptake, Edwards and Barber (1976) and Jungk and Barber (1975) found no statistically significant differences in phosphorus uptake kinetics between trimmed and untrimmed roots of soybean and corn.

Table 4 summarizes treatment effects on functional compensation among root classes. The common feature of our observations is the innate ability of the root system for its own regeneration. This interpretation is mainly supported by the non-significant effect of root excision in the ANOVA (Table 1). Compensatory re-growth following root excision was observed in eight of the twelve treatments compared in the solution culture and solid media experiments (Table 4). The pattern of compensatory re-growth was not uniform since the way the three root classes compensated each other after the removal of any one of them varied among the different growth media and phosphorus supply conditions. All root classes, but especially the primary root, contributed to the compensation mechanism in some way. The primary root system represented around 10% of the root system in control plants and this proportion increased dramatically (up to 50%) when other root classes were removed. The primary root was primarily responsible for the maintenance of below-ground biomass after removal of either hypocotyl borne or basal roots. In contrast, no compensatory re-growth was observed following excision of the primary root (Table 4). Increases in basal and hypocotyl borne root biomass after primary root removal had a lower absolute magnitude when compared with the effects of trimming other root classes. Observed compensation mechanisms among root classes were fairly consistent among phosphorus supply conditions, as reflected by the non-significant interactions between phosphorus level and root removal treatments in both aqueous and solid media settings (Table 1).

We observed that the allometric coefficient was not altered by root excision treatments in either solution or solid media experiments. Three weeks after root excision treatments were performed, all plants presented similar root to shoot allometry irrespective of whether a portion of the root system had been removed. Biomass allocation followed rather strict allometric rules, indicating that the re-stabilization of the root to shoot ratio after partial root loss would be governed by overall plant size, similar to undisturbed plants.

Our experiments on root removal were done in pots with homogeneous spatial distribution of phosphorus and other soil resources.. In the field, the spatial distribution of soil resources is typically quite heterogeneous. Some soil resources are located mainly in soil volumes close to the surface (i.e. most nutrients) and others mainly in the subsoil (notably water). It can be expected that the observed compensation mechanisms among root classes after root removal in field conditions are complemented with a tendency to re-establish the original pattern of soil exploration. For example, loss of hypocotyl borne roots should be compensated by shallower lateral branching from the primary root or by shallow basal roots.

The pattern of compensation among roots may ultimately vary according to the causes of the root loss. Among these causes, probably the more important ones are herbivory and diseases (Eissenstat and Yanai 2002; Fisher et al. 2002; Gray and Steffey 1998). Resistance to soil insects has been associated with larger root systems and to compensatory abilities of the plant to grow new roots after injury has occurred (Rogers et al. 1975; Riedell and Evenson 1993; Gray and Steffey 1998).

Our results are relevant to the breeding of crops with superior adaptation to low fertility soils and drought. Various components of the root system are under distinct genetic control, permitting the selection of genotypes with specific architectural patterns contributing to soil resource acquisition (Zobel 1996; Liao et al. 2004; Lynch 2005; Zhu et al. 2005b). The existence of functional and morphological complementation among root classes, as reported here, will be an important aspect of root ideotypes and selection strategies.

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