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Original Article

Understanding phosphorus dynamics in wheat plant and growth response in a split-root system in acidic soil

Rubya Shabnam,[a, *] Mehedi Hasan Tarek,[b] Md Toufiq Iqbal[c]

[a] Institute of Biological Sciences, Rajshahi University, Rajshahi 6205, Bangladesh
[b] Streams Tech, Inc., 4031 University Drive, Suite 100, Fairfax, VA, USA
[c] Department of Agronomy and Agriculture Extension, Faculty of Agriculture, Rajshahi University, Rajshahi 6205, Bangladesh

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ABSTRACT

Phosphorus (P) is an essential nutrient for plant growth since it is involved in cellular energy transfer, respiration, and photosynthesis. The P status and P distribution were examined in different parts of the wheat (*Triticum aestivum*) plant and root and shoot growth response in a split-root soil culture in acidic soil (pH 5.2), collected from the acidic region of Bangladesh. KH_2PO_4 was used as the source of P for the different levels of P application. Two recently developed wheat varieties (BARI-GOM 25 and BARI-GOM 26) were used as testing plants with three replications. The results showed that growth parameters like plant biomass increased by up to 80% over the control P application. Likewise, P uptake by wheat seedlings also increased by up to more than 8 times compared with the control P application. However, no significant differences were observed between wheat varieties irrespective of growth and P uptake by the wheat seedlings. Moreover, elevated P concentrations in the shoot of wheat plants probably provided more P for shoot unloading of P and for P assimilation in the control roots, resulting in increased P concentrations in the roots of wheat plants that indicated the translocation of P in the roots. These findings indicated that added soluble P increased the absorption of nutrients under acidic soil conditions. However, application of elevated P is efficient for both increasing shoot development and root growth and plays a significant role in the phosphorus dynamics within wheat plants in a split-root system.

Introduction

Phosphorous (P) plays a key role in plant growth and is the major plant growth-limiting nutrient despite its abundance in soils in both inorganic and organic forms (Gyaneshwar et al., 1999). It is absorbed by plants in orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) forms (Hinsinger, 2001). Phosphorus is a structural component of many co-enzymes, phosphoproteins, phospholipids (Ozanne, 1980) and a part of the DNA genetic memory of all living things. It is involved in the transfer and storage of energy which is used for growth and reproduction (Griffith, 1999). Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism and membrane formation (Wu et al., 2005). Low P availability is one of the major factors limiting crop production in acidic soils. However, the concentration of inorganic P in soil solution is typically very low, due to the propensity of inorganic P to bind strongly to soil surfaces or form insoluble complexes with cations (Talboys et al., 2014). This means that inorganic P is often a limiting factor in plant growth and development and this has resulted in a large number of developmental traits amongst plant species that can enhance inorganic P uptake; physiologically these include the modulation of root elongation (Sánchez-Calderón et al., 2005), branching (Linkohr et al., 2002; López-Bucio et al., 2002) and root hair density (Ma et al., 2001). The root system may also act to enhance inorganic P uptake by exuding protons (Hinsinger, 2001), organic acid anions (Ryan et al., 2001) and phosphatases (Tadano and Sakai, 1991) into the rhizosphere, or by the formation of symbioses with arbuscular mycorrhizas or ectomycorrhizas (Péret et al., 2011; Smith et al., 2011). Kirkham and Erickson (1997) studied a split-root system with wheat by applying different nutrient solution in different root compartments. They reported that wheat grown with roots between controlled and nutrient solution was taller than wheat with roots both compartments are in nutrient solution. Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms

* Corresponding author.
E-mail address: rubya_ru@yahoo.com (R. Shabnam).

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cells and develops roots, stems and leaves (Schachtman et al., 1998). Moreover, in inorganic *P*-deficient plants, the restricted supply of P to the shoots from the roots via the xylem is supplemented by increased mobilization of stored P in the older leaves and retranslocation to both the younger leaves and growing roots (Jeschke et al., 1997). Therefore, understanding the mechanisms controlling these traits is of great importance in the pursuit of improved crop inorganic P uptake, and the root can be split into two different compartments in pot experiments to determine the nutrient dynamics, especially for P.

The objectives of the present study were to understand the mechanisms involved in the utilization of inorganic P by wheat plants under various split-root systems and to quantify how translocated P affects the wheat plant within a split-root system under different *P*-efficient conditions.

Materials and methods

Experimental design

Soil and plants

Acidic soil (pH 5.2) collected from the Thakurgaon district, Bangladesh, was used as the experimental soil. The basic properties of soil are provided in Table 1. BARI-GOM 25 and BARI-GOM 26 wheat varieties were used as testing plants.

The split-root experiment was conducted with the treatments described in Table 2. The BARI-GOM 25 and BARI-GOM 26 varieties were compared. The treatments were replicated three times. KH_2PO_4 was used as the P fertilizer. To avoid the interactions between soil nutrients and added P, no basal nutrients were added. The plants were grown for 28 d and they had to depend on the reserve food in seeds and the added P for their growth.

The soil was incubated at 30°C for 7 d then KH_2PO_4 as per P doses was applied directly to the soil in each cup and mixed thoroughly before sowing. The total experiment was conducted in the Research Laboratories, Department of Agronomy andAgricultural Extension, Rajshahi University, Rajshahi, Bangladesh.

Construction of a split-root system

Pots having two compartments or chambers with a fixed partition-wall at the middle of the pot were used for the treatment (Fig. 1). Each compartment was filled with 500 g of experimental soil. The soil was compacted. The whole split-root system with soil and plant was monitored for 28 d.

Table 1
Properties of soils used in different experiments.

Soil pH	Total N (%)	Available P (ppm)	Exchangeable K (Cmol/kg)	Available S (ppm)	Available Zn (ppm)	Organic matter (%)
5.2	0.05	10.2	0.2	19.5	0.59	0.85

ppm = parts per million.

Crop management

Seed germination and seedling preparation

Seeds of uniform size were selected for germination. The seeds of BARI-GOM 25 and BARI-GOM 26 were germinated in moist sand in two separate trays in the dark at 25 °C for 70 h. The germinated seeds were grown for 5 d in the separate trays to produce young seedlings.

Cultivation of plants

Five slots were made on each side of the partition wall of the pot to support the transplanted seedlings. Five-day-old healthy seedlings were transplanted. Each seedling had four seminal roots, (6–7 cm long) after cutting off one-to-three uneven roots. A single-seedling was put into each slot keeping two seminal roots in each compartment. Then, the roots were covered with the same treated soil and watered immediately after planting and 20 mL of water was added to each compartment every day and watering was stopped 3 d before harvesting.

Harvesting

The experimental plants were harvested 27 d after transplanting. The shoots were cut uniformly at 0.5 cm above the base part of the stem. Then, the roots were cut 0.5 cm below the base part and separated carefully into two halves as previously marked. Soil from the two root halves was removed carefully so that roots were not torn or left in the soil. Then the collected bulk soil was air-dried and stored in a controlled room temperature (25 °C) until analysis. The roots were washed with deionized (DI) water to remove the adhered soil from roots. The washed roots were oven-dried at 70 °C for 3 d. Shoots were also oven-dried at the same temperature for the same time. After drying, the root and shoot samples were weighed and stored for analytical experiments.

Laboratory analysis

Measurements of soil physical and chemical properties

Soil textural analyses were conducted using an abbreviated version of the international pipette method (Olmstead et al., 1990). The clay content was determined using a pipette method after pretreatment with H_2O_2 to remove organic matter (Gee and Bauder, 1986). The pH of the soil was determined before incubation in DI water using a soil-to-solution ratio of 1:2.5. Organic carbon in the soil samples was determined using the wet oxidation method (Walkley and Black, 1934). Soil organic matter content was determined by multiplying the percentage of organic carbon by the conventional Van-Bemmelen's factor of 1.724 (Piper, 1950). The nitrogen content of the soil sample was determined by distilling soil with alkaline potassium permanganate solution (Subbiah and Asija, 1956). The distillate was collected in 20 mL of 2% boric acid solution with methyl red and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4) (Podder et al., 2012). Soil available S (measured as parts per million) was determined using the calcium phosphate extraction method with a

Table 2
The split-root system with different treatments.

Treatment	Symbol	Treatment		P level	
		Compartment 1	Compartment 2	Compartment 1	Compartment 2
A	0P/0P	0P	0P	0 mg P/kg	0 mg P/kg
B	10P/50P	10P	50P	10 mg P/kg	50 mg P/kg
C	50P/200P	50P	200P	50 mg P/kg	200 mg P/kg

0P = no P added; 10P = 10 mg P/kg added; 50P = 50 mg P/kg added.

Fig. 1. Split-root experiments.

spectrophotometer at 535 nm (Petersen, 1996). The soil available K was extracted with 1N NH_4OAC and determined using an atomic absorption spectrometer (Biswas et al., 2012). The available P of the soil was determined using a spectrophotometer at a wavelength of 890 nm. The soil sample was extracted using the Olsen method with 0.5 M $NaHCO_3$ as outlined by Huq and Alam (2005). Zn in the soil sample was measured using an atomic absorption spectrophotometer after extracting with diethylenetriaminepentaacetic acid (Soltanpour and Workman, 1979).

Phosphorus determination in soil and plant tissue

The amounts of P in root, shoot, and soil were determined. After digestion in a mixture of concentrated nitric and perchloric acids (4:1), the concentrations of P in root and shoot materials were determined using the vanadomolybdate method (Michelsen, 1957) after digestion in a mixture of concentrated nitric and perchloric acids (4:1). A colorimetric method—the molydovanado-phosphate method (Association of Official Analytical Chemists, 1975)—for the determination of phosphorous concentrations in digest solutions was used. Briefly, phosphorous was assayed by adding 3 mL of digested solution, 2 mL of reagent and 5 mL of DI water. The absorbance reading was used at 470 nm (Iqbal, 2014a).

Statistical analysis

Shoot and root parameters were analyzed using two-way analysis of variance (Treatment × Variety), total P uptake and the distribution of P in different plant parts was analyzed using one-way analysis of variance with Genstat 11th edition for Windows (Lawes Agricultural Trust, Norwich, UK). Results were considered significant at the $p < 0.05$ level and highly significant at the $p < 0.001$ level.

Results

Growth response of wheat plant in the split-root system

Plants typically respond to P limitation by reducing their total plant biomass and diverting resources disproportionately towards root growth (Zhu and Lynch, 2004; Zhu et al., 2005). In many soil types, P is localized in the upper soil layers and immobilized with other molecules (Chu and Chang, 1966). Predictably, under limiting phosphorous conditions, plants that proliferate roots into these upper layers outperform varieties with deeper root systems (Zhu and Lynch, 2004; Zhu et al., 2005). The highly significant Treatment (T) interaction for plant growth in this study indicated that the plant growth responses of BARI-GOM 25 and BARI-GOM 26 seedlings were dependent on the level of added P (Table 3). In all treatments, there were no significant differences between BARI-GOM 25 and BARI-GOM 26 seedlings for any growth measurement.

Plant height is a genetic character of a variety but its potential can be achieved by adequate crop management. The data on the effect of different P levels on plant height is given in Fig. 2. The results showed for the variety BARI-GOM 25 that the maximum plant height (34.75 mm) was recorded in treatment C (50P/200 P mg/kg), while it was lowest (28.79 mm) in treatment A (control). Similarly, the results showed for the variety BARI-GOM 26 that the maximum plant height (35.89 mm) was recorded in

Table 3
Significance levels for the main and interactive effect of P and varieties on seedlings growth.

Source of variation	Plant height	Shoot dry weight	P concentration in shoot	Root dry weight	P uptake in root
Treatment (T)	***	***	***	***	***
Variety (V)	***	ns	***	*	*
Compartment (C)	–	–	–	***	***
T × V	**	ns	***	ns	ns
T × C	–	–	–	***	***
C × V	–	–	–	ns	ns
T × V × C	–	–	–	ns	ns

ns, ** and *** represent $p > 0.05$, $p < 0.01$ and $p < 0.001$, respectively. – = no data available.

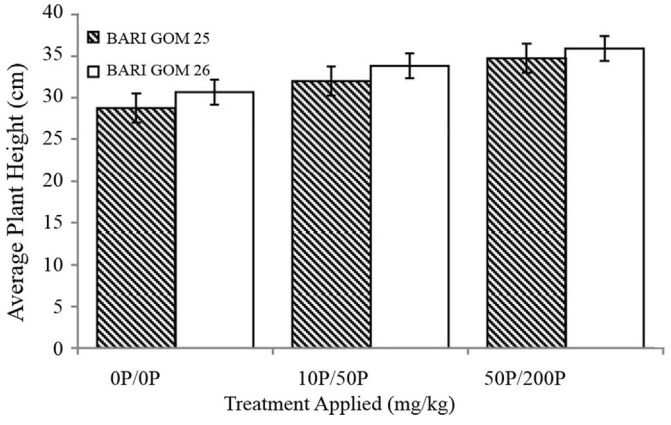

Fig. 2. Effect of P application on average plant height of the wheat seedlings grown under various level of P for 28 d.

Table 4
Total Plant biomass, total shoot and root biomass in different plant parts of the split-root system and distribution of biomass in shoot and two separate compartments.

Plant part /Variety	Total plant biomass (g/pot)		
	Treatment A	Treatment B	Treatment C
BARI-GOM 25	1.17	1.85	2.22
BARI-GOM 26	1.24	1.91	2.25
Total biomass (g/pot) in different plant parts of the split-root system			
BARI-GOM 25			
Shoot	0.55	0.80	0.90
Compartment-I	0.30	0.45	0.61
Compartment-II	0.32	0.60	0.70
BARI-GOM 26			
Shoot	0.57	0.83	0.91
Compartment-I	0.32	0.47	0.62
Compartment-II	0.35	0.61	0.71
Distribution of biomass (%) in shoot and roots grown in two separate soil compartments (I and II)			
BARI-GOM 25			
Shoot	47.0	43.2	40.8
Compartment-I	25.6	24.3	27.7
Compartment II	27.4	32.4	31.6
BARI-GOM 26			
Shoot	46.1	43.3	40.7
Compartment I	25.7	24.8	27.7
Compartment II	28.2	31.9	31.6

treatment C (50P/200 P mg/kg), while it was lowest (30.73 mm) in treatment A (control). Plant height was highly significantly affected among all the various P applications and varieties of wheat plants. It also increased with the increasing level of phosphorus application. Hence, among the low levels of various phosphorous application, phosphate had a gradually increasing effect on plant height with increasing P applications, while at high levels, P resulted in maximum plant height.

Like plant height, the plant biomass showed similar trends under different P applications. Total plant biomass in BARI-GOM 26 of treatment C increased 81% (2.25 g/pot) compared with the controlled treatment A (1.24 g/pot). Similarly, treatment B increased 54% (1.91 g/pot) compared with treatment A. Again, for BARI-GOM 25, the total plant biomass in treatment C increased 89% (2.22 g/pot) compared with the controlled treatment A (1.17 g/pot). Similarly, Treatment B increased 58% (1.85 g/pot) compared with treatment A. A similar trend was found in the shoot biomass and root biomass results of both wheat plant varieties in this study (Table 4), while the internal biomass distribution in the shoot and root showed a different trend among all treatments (Fig. 3). The shoot biomass was highest (47% of total plant biomass) in treatment A of BARI-GOM 25 and in treatment B and treatment C in decreasing order (43.2% and 40.8% of total plant biomass, respectively). For root biomass, the trend was in increasing order in both compartments among all treatments (Table 4). Similarly, in BARI-GOM 26, the highest percentage of shoot biomass was in treatment A (46.1% of total plant biomass) followed by treatment B and treatment C in decreasing order (43.3% and 40.7% of total plant biomass, respectively). For root biomass, the trend was an increasing order in both compartments among all treatments (Fig. 3). The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (*Triticum aestivum*) has been well documented (Ma and Rengel, 2008; Pedas et al., 2011; Iqbal, 2014b). In the current split-root study in acidic soil, the percentage distribution differences in the total root and shoot dry weights among the three P treatments were due to the elevated P supply which directly interfered with shoot root growth.

The root-shoot ratio is an important factor in understanding the growth responses of plants under elevated P applications in acidic soil. The root-shoot ratio of the wheat plants with and without treatments at the various level of P supply (Table 5) showed an increase with increasing P application in both varieties of wheat plant. This was supported by Shane et al. (2003) who reported that an increase in the phosphate supply in root halves influenced the root-shoot ratio of wheat; because root growth increased more than shoot growth. Similar results were observed in wheat plants by Bingham and Bengough (2003) and Qifu et al. (2011).

The relationship between shoot biomass and average plant height was analyzed to determine the effect of plant height on the production of biomass of the wheat plant. A significant correlation ($R^2 = 0.97$) between plant height and shoot biomass under elevated P supply indicated that plant development was enhanced with the application of P in soil (Fig. 4). Similarly, a significant correlation ($R^2 = 0.99$) between plant height and root biomass under elevated P application was observed. The increase in plant growth was largely due to the increased absorption of nutrients from the soil solution (Son and Smith, 1988). However, the elevated P played an important role in the growth of the wheat plants in the split-root system in acidic soil.

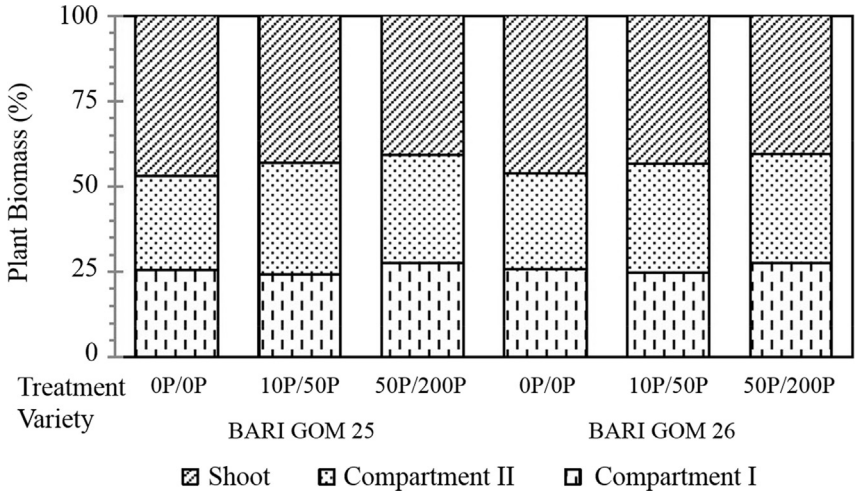

Fig. 3. Distribution of plant biomass in different plant parts of the split-root system.

Table 5
Root biomass, shoot biomass and root/shoot ratio of two wheat varieties across different P applications.

Variety	Treatment	P rate (mg/kg)	Biomass Production (mg/plant)		Root-shoot ratio
			Shoot	Root	
BARI-GOM 25	T1	0P/0P	0.55	0.62	1.13
	T2	10P/50P	0.80	1.05	1.31
	T3	50P/200P	0.90	1.31	1.45
BARI-GOM 26	T1	0P/0P	0.57	0.67	1.17
	T2	10P/50P	0.83	1.08	1.31
	T3	50P/200P	0.91	1.33	1.46

0P = no P added; 10P = 10 mg P/kg added; 50P = 50 mg P/kg added.

P distribution and the translocation in wheat plant within a split-root system

In general, plants grow better when partially soluble phosphate is applied in comparison with the soluble P source. Soil pH influences the charge of the P species in solution as well as the charge of the adsorbing particles in soils. The study was conducted in acidic soil with pH 5.2 and P doses were applied directly to the soil. The shoot and root P concentrations showed an increasing trend under different P applications to the wheat plants. Shoot and root P concentrations were highly significantly affected among all the various P applications to wheat plants. Again, similar trends in the total P uptake were found in both BARI-GOM 25 and BARI-GOM 26. Total plant P concentration in BARI-GOM 25 in treatment C increased more than eight times (8.63 g/kg) compared with the control treatment A (1.0 g/kg). Similarly, in treatment B, the total plant P concentration increased more than three times (3.61 g/kg) compared with treatment A. Again, for BARI-GOM 26, the total plant biomass in treatment C increased nine times (9.09 g/kg) compared with the control treatment A (1.06 g/kg). Similarly, in treatment B, the increase was more than three times (3.74 g/kg) compared with treatment A. A similar trend was recorded for the shoot biomass and root biomass of both wheat plant varieties (Table 6); while the internal P uptake by shoots and roots followed the same trend among all treatments (Fig. 5). The highest percentages of P uptake by shoots was in treatment C of BARI-GOM 25 (49% of total plant P uptake) followed by treatment B and treatment A in decreasing order (47.9% and 41.0% of total plant P uptake, respectively). Root P uptake increased with increasing P supply in both compartments (Table 6). Similarly, in BARI-GOM 26 the highest percentage of P uptake by shoots was in treatment C (48.3% of total plant P uptake) followed by treatment B and treatment A in decreasing order (47.3% and 40.6% of total plant P uptake, respectively). Again, root P uptake increased with increasing P supply in both compartments (Fig. 5). The percentage distribution differences in the total root and shoot P uptake

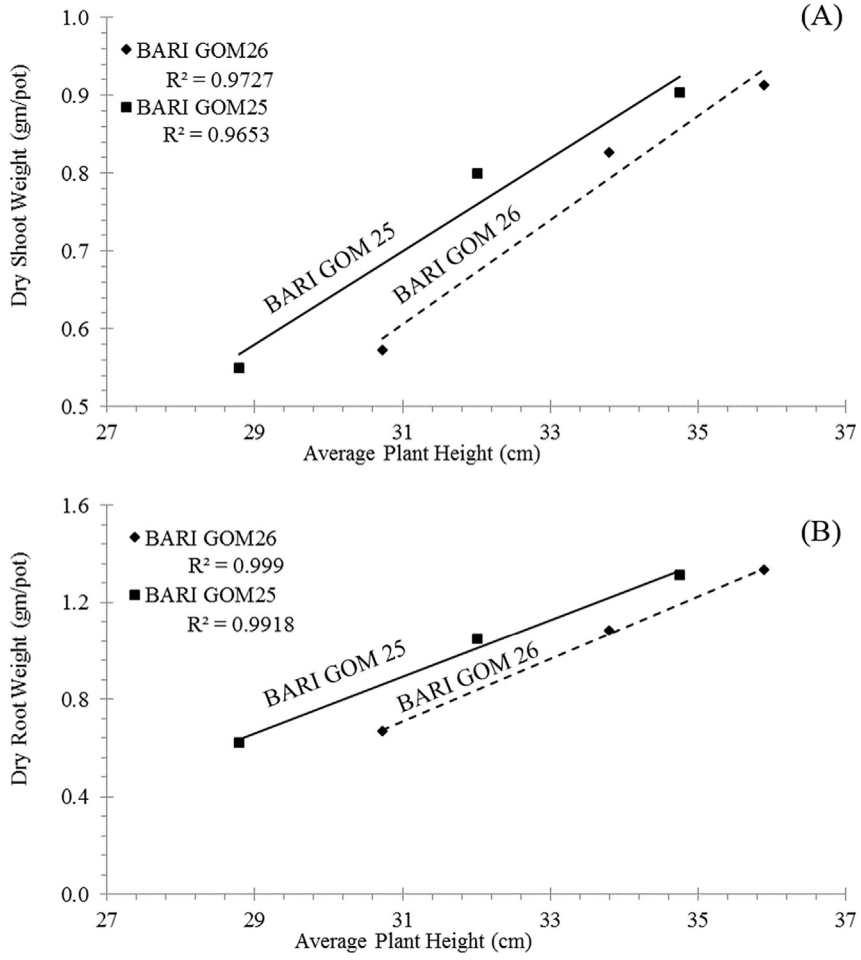

Fig. 4. Relationship in wheat plants between (A) shoot dry weight and average plant height; (B) root dry weight and average plant height.

Table 6
Total P uptake in different plant parts of the split-root system and distribution of P in shoot and root two separate compartments.

Plant part /Variety	Total P uptake (g/kg)		
	Treatment A	Treatment B	Treatment C
BARI-GOM 25	1	3.61	8.63
BARI-GOM 26	1.06	3.74	9.09
Total P uptake (g/kg) in different plant parts of the split-root system			
BARI-GOM 25			
Shoot	0.41	1.73	4.23
Compartment-I	0.29	0.49	1.43
Compartment-II	0.3	1.39	2.97
BARI-GOM 26			
Shoot	0.43	1.77	4.39
Compartment-I	0.31	0.53	1.58
Compartment-II	0.32	1.44	3.12
Distribution of P (%) in shoot and roots grown in two separate soil compartments (I and II)			
BARI-GOM 25			
Shoot	41.0	47.9	49.0
Compartment-I	29.0	13.6	16.6
Compartment-II	30.0	38.5	34.4
BARI-GOM 26			
Shoot	40.6	47.3	48.3
Compartment-I	29.2	14.2	17.4
Compartment-II	30.2	38.5	34.3

among the three P treatments were due to the elevated P supply which directly interfered with the shoot-root P status in this split-root system study in acidic soil.

Mimura et al. (1996) and Jeschke et al. (1997) described a picture of patterns of inorganic P movement in whole plants. In *P*-sufficient plants, most of the inorganic P absorbed by the roots is transported through the xylem to the younger leaves. Concentrations of inorganic P in the xylem range from 1 mm in inorganic *P*-starved plants to 7 mm in plants grown in solutions containing 125 μm inorganic P (Mimura et al., 1996). There is also the significant retrained location of inorganic P in the phloem from older leaves to the growing shoots and from the shoots to the roots. In inorganic *P*-deficient plants, the restricted supply of P to the shoots from the roots via the xylem is supplemented by increased mobilization of stored P in the older leaves and retranslocation to both the younger leaves and growing roots. This process involves both the depletion of inorganic P stores and the breakdown of organic P in the older leaves. A curious feature of *P*-starved plants is that approximately one-half of the inorganic P translocated from the shoots to the roots in the phloem is then transferred to the xylem and recycled back to the shoots (Jeschke et al., 1997).

Increasing the external P supply to the split roots from 0 mg P/kg to 200 mg P/kg significantly increased the P concentration in those roots and shoots, but had no significant effect on the P concentration of the controlled roots. This lack of response of controlled roots has been demonstrated in other split-root studies, including barley (Drew and Saker, 1984), subterranean clover (Scott and Robson, 1991), tomato (Burleigh and Harrison, 1999) and *Hakea prostrata* in the Proteaceae (Shane et al., 2003). In contrast with the results of split-root plants, the results in the current wheat plant split-root study and those of others using foliar spray (Marschner et al., 1987) demonstrate that P retranslocated in the phloem sap can result in increased root P concentrations. In the current study, a very high P supply (200 mg P/kg KH_2PO_4) to just one crown root of the wheat plant significantly increased the P concentration of compartment-I roots with regard to treatment B compartment II. It was expected that in treatment C, plants would be able to translocate P from the roots in compartment I to those in compartment II. Studies with barley (Greenway and Gunn, 1966; Clarkson and Scattergood, 1982) indicated that *P*-stressed leaves absorb P more rapidly than control leaves do, and they export much larger amounts to the roots. Higher P concentrations in the shoots of wheat plants in the acidic soil in the current study probably provided more P for shoot unloading of P and for P assimilation in the controlled roots, resulting in increased P concentrations in the roots of the wheat plants. In contrast, the split-root technique in acidic soil probably provides a more stable supply of P at a lower concentration.

Considering that P is an essential and often limiting nutrient for plant growth, it is surprising that many aspects of P uptake and transport in plants are not thoroughly understood. The current study investigated P uptake and P translocation in a split-root system of wheat plants in acidic soil and found that the added soluble P increases the absorption of nutrients from the soil solution. However, added P is efficient both for increasing shoot development and root growth. Moreover, no varietal difference was found in the various experiments. Again, elevated P concentrations in the shoots of the wheat plants probably provided more P for shoot unloading of P and for P assimilation in the controlled roots, resulting in increased P concentrations in the roots of the wheat plants in a split-root system in acidic soil.

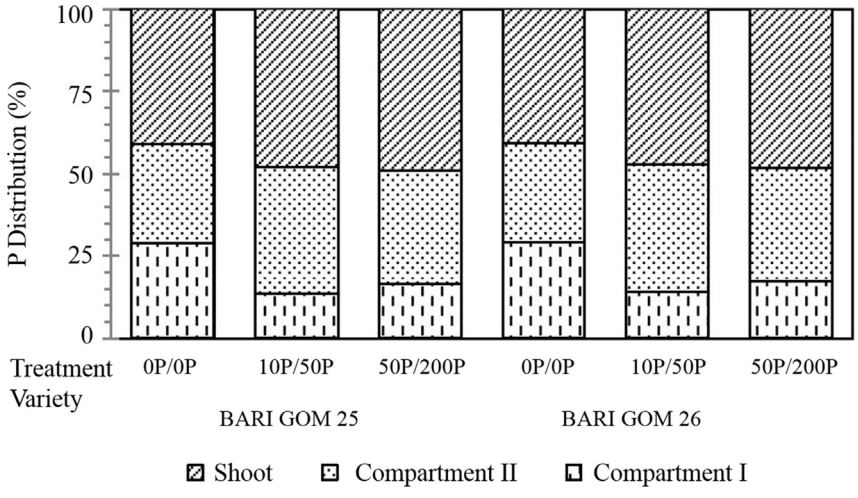

Fig. 5. P distribution in different plant parts of the split-root system.

Perhaps the next important leap in conceptual understanding in this area will come from the integration of these techniques to provide a comprehensive picture of the function of phosphate transporters and how they control of their spatial and temporal expression allows the plant to cope with changing environmental conditions.

Ethics statements

This study was approved by the academic committee of Institute of Biological Sciences, Rajshahi University, Bangladesh.

Conflict of interest

The authors declare that there are no conflicts of interest.

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