Understanding phosphorus status and P translocation within wheat plant in a split-root system

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Abstract

Plants are not uniform in their nutritional requirements, most of them survive under adverse conditions of humidity, temperature, and nutrients. Because they are genetically adapted to their habitats and even some varieties of the same species show differences in absorption, translocation, accumulation and nutrient use. This study is aimed at examining the phosphorus (P) status in the different parts of wheat (Triticum aestivum L.) plant and its influence on plant growth and P translocation in a split-root soil culture. KH₂PO₄ was used as the source of phosphorus for the different level of P application. Two recently BARI developed wheat varieties namely BARI GOM 25 and BARI GOM 26 were used as testing plants. Result showed the growth parameter increased with the increase of P application. Likewise, P uptake by wheat plant also increases with the elevated P application. However, no significant differences were observed between wheat varieties irrespective of growth and P uptake by wheat plant. Moreover, elevated P concentrations in the shoot of wheat plants probably provide more P for shoot unloading of P and for P assimilation in the controlled roots. This phenomenon results in increased P concentrations in the roots of wheat plants that mean translocation of P in the roots. These findings indicate that the added soluble P increases the absorption of nutrients from the soil solution. So, this study concluded that the application of elevated P in split-root system is efficient both for increasing shoot development and root growth and plays significant role in the P translocation within the wheat plants.

Keywords: Phosphorus uptake, P use efficiency, P translocation, xylem, phloem.

Introduction

Phosphorous (P) plays a key role in plant growth and is the major plant growth-limiting nutrient despite of its abundance in soils in both inorganic and organic forms (Gyaneshwar et al., 1999). It is absorbed by the plants, in the orthophosphate (H₂PO₄⁻ and HPO₄²⁻) forms (Hinsinger, 2001). The concentrations of inorganic P in soil solution are, however, typically very low, due to inorganic P's propensity 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.

The split-root system is the division of the plant root into two media. This system has been used by researchers (Shani et al., 1993; Zhu and Ito, 2000; Shen et al., 2005; Shu et al., 2005), but not for improving plant nutrition. Using localized fertilizer in row crops is a similar phenomenon as the split-root system, Tworkoski et al. (2003) report that the greatest number of roots grew at 43 to 46 cm from the root collar where localized, polypropylene, nonwoven fabric fertilizer was applied, resulting in rapid shoot growth as a result of increased nutrient supply.
response to daily fertilization. Ma and Rengel (2008) studied phosphorus distribution in split-root systems to examine the influence of plant phosphorus status and distribution in the root zone and phosphorus acquisition on the growth of root and shoot of wheat (*Triticum aestivum* L.). The results of their research suggest that root proliferation and greater phosphorus uptake in the phosphorus-enriched zone may meet the demand for phosphorus by phosphorus-deficient plants only for a limited period of time.

Our previous study showed that elevated P taken a significant part in the development of wheat plant in acidic soil (Shabnam and Iqbal, 2016a). Likewise, application of elevated P is efficient both for increasing shoot development and root proliferation and plays a significant role in the P dynamics within the wheat plant in split-root system in alkaline soil (Shabnam and Iqbal, 2016b). Further, a study found that translocated P does not alleviate aluminium toxicity within plant tissue (Iqbal, 2014). However, no study was undertaken acidic and alkaline soil combination within split-root system. Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms 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). Understanding the mechanisms controlling these traits is, therefore, of great importance in the pursuit of improved crop inorganic P uptake. Keeping in view of the above facts, the study aims at following objectives: to understand mechanisms involved in the utilization of inorganic phosphorus by wheat plant under various split-root systems to quantify how translocated phosphorus effects on wheat plant within split-root system under P efficient condition. It will be hypothesized that elevated phosphorus will be affected on root morphology and growth response to wheat plant.

**Material and Methods**

**Soil and Plant**

Two types of soils (acidic and alkaline) were used in this study. The soil type I (acidic) having pH 5.2 was collected from Thakurgaon district, acidic region of Bangladesh and the soil type II (alkaline) having pH 7.9 was collected from Ganges River Floodplain (Rajshahi District, Bangladesh) which soil types predominantly include calcareous grey floodplain soils. The basic properties of soil are out-lined in Table 1. From the soil texture analysis the acidic soil contains 59% clay content (sandy clay) where; the alkaline soil contains 72% sand (sandy loam). The sandy loam means alkaline soil (P: 14.3 mg kg⁻¹) has a higher P content in the soil than the clay loam means acidic soil (P: 10.4 mg kg⁻¹). BARI GOM 25 and BARI GOM 26 wheat (*Triticum aestivum* L.) varieties were used as testing plants.

| Properties          | Soil I, Acidic | Soil II, Alkaline |
|---------------------|----------------|-------------------|
| Soil pH             | 5.2            | 7.9               |
| Total N, %          | 0.05           | 0.03              |
| Available P, mg kg⁻¹| 10.2           | 14.3              |
| Exchangeable K, cmol kg⁻¹ | 0.2       | 0.21              |
| Available S, mg kg⁻¹| 19.5           | 5.6               |
| Available Zn, mg kg⁻¹| 0.59          | 11.55             |
| Organic matter, %   | 0.85           | 0.55              |
| Sand, %             | 55             | 92.2              |
| Silt, %             | 25.9           | 3.8               |
| Clay, %             | 19.1           | 4.0               |

**Experimental Design**

The split-root experiment was conducted with the treatments described in Table 2. The BARI GOM 25 and BARI GOM 26 were compared. The treatments were replicated three times. KH₂PO₄ chemical was used as P. To avoid the interactions between soil nutrients and added P, no basal nutrients were added. The plants were allowed to grow for 28 days and they had to depend on the reserved food of the seeds and the added P for their growth.

The soil was incubated at 30⁰C for 7 days and then KH₂PO₄ 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 and Agricultural Extension, Rajshahi University, Rajshahi.
Table 2. Split-root system with different treatments

| Treatment | Symbols | Compartment 1 | Compartment 2 | Compartment 1 | Compartment 2 |
|-----------|---------|----------------|----------------|----------------|----------------|
|           |         | Alkaline Soil  | Alkaline Soil  | Acidic Soil    | Acidic Soil    |
| A         | 0P/0P   | 0 mg P kg⁻¹   | 0 mg P kg⁻¹   | 0 mg P kg⁻¹   | 0 mg P kg⁻¹   |
| B         | 10P/50P | 10 mg P kg⁻¹  | 50 mg P kg⁻¹  | 50 mg P kg⁻¹  | 50 mg P kg⁻¹  |
| C         | 50P/200P| 50 mg P kg⁻¹  | 200 mg P kg⁻¹ | 200 mg P kg⁻¹ | 200 mg P kg⁻¹ |
| D         | 100P/400P| 100 mg P kg⁻¹ | 400 mg P kg⁻¹ | 400 mg P kg⁻¹ | 400 mg P kg⁻¹ |

Construction of 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. Each compartment was filled with 500g of experimental soil. The soil was compacted. The whole split root system with soil and plant continued for 28 days.

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 dark at 25°C for 70h. To produce young seedlings, the germinated seeds were allowed to grow for 5 days in those separate trays.

Cultivation of plant

To support the transplanted seedlings, five slots were made on each side of the partition-wall of the pot. Five days old healthy seedlings, were transplanted. Each seeding bearing four seminal roots, (6-7 cm long), after cutting one-uneven root was taken. A single-seeding 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. 20 ml water was added to each compartment every day and watering was stopped 3 days before harvesting.

Harvesting

Pots having two compartments or chambers with a fixed partition-wall at the middle of the pot were used for the treatment. Each compartment was filled with 500g of experimental soil. The soil was compacted. The whole split root system with soil and plant continued for 28 days. The experimental plants were harvested 27 days after transplanting. The shoots were cut 0.5cm above the base part of the stem uniformly. Then the roots were cut 0.5cm below the base part and separated carefully into two halves as previously marked. Soils from two root halves were removed carefully so that the roots could not be torned or left in the soil. Then the collected bulk soil was air dried and stored in a controlled room temperature (25°C) until analysis. Then the roots were washed with DI water to remove the adhered soil from roots. The washed roots were oven dried at 70°C for 3 days. Shoots were also oven dried at the same temp 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 by using an abbreviated version of the International Pipette method. Clay content was determined by a pipette method after pretreatment with H₂O₂ to remove organic matter (Gee and Bauder, 1986). The pH of the soil was determined before incubation in deionised water using a soil-to-solution ratio of 1:2.5. Organic carbon of the soil samples was determined by wet oxidation method (Walkley and Black, 1934). Soil organic matter content was determined by multiplying the percent value of organic carbon with 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 (Subhaiah and Asiya, 1956). The distillate was taken in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H₂SO₄) (Podder et al., 2012). Soil available S (ppm) was determined by calcium phosphate extraction method with a spectrophotometer at 535 nm (Petersen, 1996). The soil available K was extracted with 1N NH₄OAC and determined by an atomic absorption spectrometer (Biswa et al., 2012). The available P of the soil was determined by spectrophotometer at a wavelength of 890 nm. The soil sample was extracted by Olsen method with 0.5 M NaHCO₃ as outlined by Huq and Alam (2005). Zn in the soil sample was measured by an atomic absorption spectrophotometer (AAS) after extracting with DTPA (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; v/v), the concentration of P in root and shoot materials were determined using the vanadomolybdate method (Zheng et al., 2005). Colorimetric method for the determination of phosphorus concentrations in digest solutions was used. This method is called the molydovanado-phosphate method (AOAC, 1975). Briefly, phosphorous was assayed using the molydovanado-phosphate method adding 3-ml digested solution, 2-ml reagent and 5-ml distilled water. The absorbance reading was used at 470 nm (Iqbal et al., 2010).

Statistical analysis

Shoot and root parameters were analysed by three-way ANOVA (Treatment × Variety × Compartment), total P uptake as well as distribution of P in different plant parts were determined by one-way ANOVA using Genstat 11th edition for Windows (Lawses Agricultural Trust, UK).

Results

Effect on plant height

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 Figure 1. The results showed for the variety BARI GOM25 that the maximum plant height (30.83 mm) was recorded in treatment C (50P/200P mg kg⁻¹), while minimum (14.78 mm) was found in treatment D (100P/400P mg kg⁻¹). Again, the results showed for the variety Bari GOM26 that the maximum plant height (30.85 mm) was recorded in treatment C (50P/200P mg kg⁻¹), while minimum (16.77 mm) was found in treatment D (100P/400P mg kg⁻¹). Plant height was significantly (P≤0.001) affected among all the various P applications and variety of wheat plants. It also increased with the increasing level of P application, but at high level P resulted in minimum plant height. Hence, among low level of various P application, phosphate had the gradual increasing effect on plant height with increasing P applications, but at high level P resulted in minimum plant height.

Figure 1. Effect of P application on average plant height of the wheat seedlings grown in various level of P for 28 days.

All the plant growth and P-uptake parameters were highly significant (P ≤0.001) under P levels (Table 3). Similarly, significant differences among varieties were observed in relation with all the growth and P-uptake parameters.

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)       | ***          | ***              | ***                      | NS             | NS              |
| Variety (V)         | ***          | NS               | ***                      | NS             | NS              |
| Compartment (C)     | -            | -                | -                        | NS             | NS              |
| T×V                 | ***          | NS               | ***                      | NS             | NS              |
| T×C                 | -            | -                | -                        | NS             | NS              |
| C×V                 | -            | -                | -                        | NS             | NS              |
| T×V×C               | -            | -                | -                        | *              | NS              |

Where NS, ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively, '-' (dash) indicates no data available.
Shoot dry weight

Like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM25 that the maximum shoot biomass (0.85 g pot$^{-1}$) was recorded in treatment C (50P/200P mg kg$^{-1}$), while minimum (0.25 g pot$^{-1}$) was found in treatment D (100P/400P mg kg$^{-1}$). Again, the results showed for the variety BARI GOM26 that the maximum shoot biomass (0.87 g pot$^{-1}$) was recorded in treatment C (50P/200P mg kg$^{-1}$), while it was minimum (0.26 g pot$^{-1}$) in treatment D (100P/400P mg kg$^{-1}$). The shoot biomass was significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. The shoot biomass did not significantly ($P > 0.05$) differ between varieties of wheat plant. The shoot biomass also increased with the increased level of P application, but at high level P resulted in minimum shoot biomass (Figure 2).

![Figure 2](image2.png)

Figure 2. Effect of P application on dry shoot weight of the wheat seedlings grown in various level of P for 28 days.

Root dry weight

Total root biomass varied among the treatments. Total root biomass of BARI GOM26 in Treatment C on compartment II (200 mg kg$^{-1}$ P in acidic soil) was the highest (0.57 g pot$^{-1}$) and the lowest in Treatment D-II (400 mg kg$^{-1}$ P in acidic soil) (0.17 g pot$^{-1}$), followed by gradual increase in the Treatment A-I (0 mg kg$^{-1}$ P in alkaline soil) (0.26 g pot$^{-1}$), Treatment A-II (0 mg kg$^{-1}$ P in acidic soil) (0.29 g pot$^{-1}$), Treatment B-I (10 mg kg$^{-1}$ P in alkaline soil) (0.36 g pot$^{-1}$), Treatment C-I (50 mg kg$^{-1}$P in alkaline soil) (0.41 g pot$^{-1}$), Treatment B-II (50 mg kg$^{-1}$ P in acidic soil) (0.46 g pot$^{-1}$) and Treatment D-I (100 mg kg$^{-1}$ P in alkaline soil) (0.51 g pot$^{-1}$) (Figure 3). Again, for BARI GOM25 total root biomass was the highest in Treatment C on compartment II (200 mg kg$^{-1}$ P in acidic soil) (0.55 g pot$^{-1}$) and was the lowest in Treatment D-II (400 mg kg$^{-1}$ P in acidic soil) (0.15 g pot$^{-1}$), followed by gradual increase in the Treatment A-I (0 mg kg$^{-1}$ P in alkaline soil) (0.25 g pot$^{-1}$), Treatment A-II (0 mg kg$^{-1}$ P in acidic soil) (0.28 g pot$^{-1}$), Treatment B-I (10 mg kg$^{-1}$ P in alkaline soil) (0.35 g pot$^{-1}$), Treatment C-I (50 mg kg$^{-1}$ P in alkaline soil) (0.40 g pot$^{-1}$), Treatment B-II (50 mg kg$^{-1}$ P in acidic soil) (0.45 g pot$^{-1}$) and Treatment D-I (100 mg kg$^{-1}$ P in alkaline soil) (0.50 g pot$^{-1}$). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. But, the root biomass did not significantly ($P > 0.05$) differ between varieties of wheat plant.

![Figure 3](image3.png)

Figure 3. Effect of P application on dry root weight of the wheat seedlings was grown in various level of P for 28 days.
Shoot P concentration
In general, shoot P concentration was found in increasing trend under different P application on wheat plant. Shoot P concentration was significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. Total shoot P concentration in BARI GOM 26 was the highest and the lowest in Treatment C (50P/200P mg kg$^{-1}$) (2.74 g kg$^{-1}$) and Treatment A (0P/0P mg kg$^{-1}$) (0.41 g kg$^{-1}$) respectively, but intermediates in Treatments B (10P/50P mg kg$^{-1}$) (1.43 g kg$^{-1}$) and Treatment D (100P/400P mg kg$^{-1}$) (0.71 g kg$^{-1}$) (Figure 4). Again, for BARI GOM25 total shoot P concentration was the highest and the lowest in Treatment C (50P/200P mg kg$^{-1}$) (2.52 g kg$^{-1}$) and Treatment A (0P/0P mg kg$^{-1}$) (0.40 g kg$^{-1}$) respectively, but intermediates in Treatments B (10P/50P mg kg$^{-1}$) (1.30 g kg$^{-1}$) and Treatment D (100P/400P mg kg$^{-1}$) (0.63 g kg$^{-1}$). The shoot P concentration of BARI GOM25 and BARI GOM26 were dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments.

Figure 4. Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

Root P concentration
In general, root P concentration was found in increasing trend under different P application on wheat plant. Root P concentration was significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. Total root P concentration in BARI GOM 26 was the highest and the lowest in Treatment C in compartment II (200 mg kg$^{-1}$ P in acidic soil) (2.77 g kg$^{-1}$) and Treatment D-II (400 mg kg$^{-1}$ P in acidic soil) (0.19 g kg$^{-1}$) respectively, followed by gradual increase in Treatment A-I (0 mg kg$^{-1}$ P in alkaline soil) (0.29 g kg$^{-1}$), Treatment A-II (0 mg kg$^{-1}$ P in acidic soil) (0.31 g kg$^{-1}$), Treatment B-I (10 mg kg$^{-1}$ P in alkaline soil) (0.53 g kg$^{-1}$), Treatment B-II (50 mg kg$^{-1}$ P in acidic soil) (0.68 g kg$^{-1}$), Treatments C-I (50 mg kg$^{-1}$ P in alkaline soil) (1.63 g kg$^{-1}$) and Treatment D-I (100 mg kg$^{-1}$ P in alkaline soil) (2.22 g kg$^{-1}$) (Figure 5).

Figure 5. Effect of P application on P uptake of wheat root in various level of P for 28 days.
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Again, for BARI GOM 25 total root P concentration was the highest and the lowest in Treatment C in compartment II (200 mg kg\(^{-1}\) P in acidic soil) (2.61 g kg\(^{-1}\)) and Treatment D-II (0 mg kg\(^{-1}\) P in acidic soil) (0.27 g kg\(^{-1}\)) respectively, followed by gradual increase in Treatment A-I (0 mg kg\(^{-1}\) P in alkaline soil) (0.68 g kg\(^{-1}\)), Treatment A-II (0 mg kg\(^{-1}\) P in acidic soil) (0.28 g kg\(^{-1}\)), Treatment B-I (10 mg kg\(^{-1}\) P in alkaline soil) (0.49 g kg\(^{-1}\)), Treatment B-II (59 mg kg\(^{-1}\) P in acidic soil) (0.68 g kg\(^{-1}\)), Treatments C-I (50 mg kg\(^{-1}\) P in alkaline soil) (1.45 g kg\(^{-1}\)) and Treatment D-I (100 mg kg\(^{-1}\) P in alkaline soil) (2.07 g kg\(^{-1}\)). The root P concentration of BARI GOM25 and BARI GOM26 were dependent on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments.

**Total P uptake and P distribution**

In both varieties of BARI GOM25 and BARI GOM26 similar trend in total P uptake were found. The total P uptake by plant was significantly high in Treatment C (50P/200P mg kg\(^{-1}\)) from other treatments in both varieties. However, total P uptake was more than six times greater in Treatment C (50P/200P mg kg\(^{-1}\)) than control Treatment A (0P/0P mg kg\(^{-1}\)). The total P uptake was greater in BARI GOM26 than that of BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 of Treatment C(50P/200P mg kg\(^{-1}\)) and Treatment A (0P/0P mg kg\(^{-1}\)) were the highest (7.14 g kg\(^{-1}\)) and the lowest (1.01 g kg\(^{-1}\)) respectively, but intermediates in Treatment B (10P/50P mg kg\(^{-1}\)) (2.64 g kg\(^{-1}\)) and Treatment D (100P/400P mg kg\(^{-1}\)) (3.12 g kg\(^{-1}\)) (Figure 6). Again, for BARI GOM25 total P uptake in Treatment C (50P/200P mg kg\(^{-1}\)) and Treatment A (0P/0P mg kg\(^{-1}\)) were the highest (6.58 g kg\(^{-1}\)) and the lowest (0.95 g kg\(^{-1}\)) respectively, but intermediates in Treatments B (10P/50P mg kg\(^{-1}\)) (2.38 g kg\(^{-1}\)) and Treatment D (100P/400P mg kg\(^{-1}\)) (2.87 g kg\(^{-1}\)).

**Discussion**

**Growth response of wheat plant in split root system**

Plants typically respond to P limitation by reducing 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 to the upper soil layers and immobilized with other molecules (Chu et al., 1966). It is predicted that under limiting phosphorous condition, plants that proliferate roots into these upper layers outperform varieties with deeper root systems (Zhu and Lynch, 2004; Zhu et al., 2005). Root proliferation and greater P uptake per unit of root in the nutrient-rich zones are often considered to be compensatory responses. So, the study was conducted to examine the influence of plant phosphorus (P) status and P distribution in the root zone on root P acquisition and root and shoot growth of wheat (Triticum aestivum L.) in a split-root soil culture. To investigate growth response of recently BARI released wheat varieties under elevated P applied condition, all growth measurements, including root biomass, plant height and shoot biomass measures were taken. The highly significant Treatment (T) interaction for plant growth (P ≤ 0.001) in this study indicates that the plant growth responses of BARI GOM25 and BARI GOM26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM25 and BARI GOM26 seedlings for any growth measurement. Total plant biomass in BARI GOM 26 of Treatment C increased 74.5% (1.85 g pot\(^{-1}\)) in comparison with the controlled Treatment A (1.06 g pot\(^{-1}\)). Similarly in Treatment B increased 49.1% (1.55 g pot\(^{-1}\)) and in Treatment D decreased 11.3% (0.94 g pot\(^{-1}\)) in comparison with
Treatment A. Again, for BARI GOM 25 total plant biomass in Treatment C increased 74.7% (1.80 g pot⁻¹) in comparison with the controlled Treatment A (1.03 g pot⁻¹). Similarly in Treatment B increased 50.5% (1.55 g pot⁻¹) and in Treatment D decreased 12.6% (0.90 g pot⁻¹) in comparison with Treatment A. Similar trend was found in shoot biomass and root biomass of both wheat plant varieties in this study (Table 4). But internal biomass distribution in shoot and root was found no common trend among all treatments (Figure 7). The shoot biomass was found highest (48.5% of total plant biomass) in Treatment A of BARI GOM25 and in Treatment B, Treatment C and Treatment D were found in decreasing order 48.4, 47.2% and 27.8% respectively of total plant biomass. In this study of split root system both compartments were used different soil among all treatments. In compartment I of the split root system alkaline soil was used and in compartment II acidic soil was used. So, the trend in root biomass was found irregular order among all treatments (Figure 7).

![Figure 7. The distribution of plant biomass in different plant parts of the split-root system](image)

In alkaline soil in compartment I of the split root system, the highest percentage of root biomass was found in Treatment D 55.6% of total plant biomass and in Treatment A, Treatment B and Treatment C the percentages were found in decreasing order 24.3%, 22.6% and 22.2% respectively of total plant biomass. In acidic soil in compartment II of the split root system, the highest percentage of root biomass was found in Treatment C 30.6% of total plant biomass and in Treatment B, Treatment A and Treatment D the percentages were found in decreasing order 29.0%, 27.2% and 16.7% respectively of total plant biomass. Similarly, in BARI GOM26 the highest percentage of shoot biomass was found in Treatment A 48.3% of total plant biomass and in Treatment B, Treatment C and Treatment D the percentages were found in decreasing order 48.1%, 47.0% and 27.7% of total plant biomass respectively. In alkaline soil in compartment I of the split root system, the highest percentage of root biomass was found in Treatment D 54.3% of total plant biomass and in Treatment A, Treatment B and Treatment C the percentages were found in decreasing order 24.5%, 22.8% and 22.2% respectively of total plant biomass. In acidic soil in compartment II of the split root system, the highest percentage of root biomass was found in Treatment C 30.8% of total plant biomass and in Treatment B, Treatment A and Treatment D the percentages were found in decreasing order 29.1%, 27.3% and 18.1% respectively of total plant biomass (Table 4). The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (Triticum aestivum) is well documented (Ma et al., 2008; Pedas et al., 2011; Iqbal, 2014). In our split-root study, the percentage distribution differences in the total root and shoot dry weight among the three P treatments are due to elevated P supply which directly interferes with shoot root growth.

The root-shoot ratio is an important factor to understand growth responses of plants under elevated P applications. The root:shoot ratio of the wheat plant with and without treatments at the various level of P supply were analyzed (Table 5). Comparison of root: shoot ratio of different treatment showed an increase with increasing P application in both varieties of BARI released wheat plants. In the same line, Shane et al. (2003) reported that, the increase of 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 plant by Bingham et al. (2003) and Ma et al. (2011).
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 parts       | Variety       | Treatment A | Treatment B | Treatment C | Treatment D |
|-------------------|---------------|-------------|-------------|-------------|-------------|
| Total Plant Biomass (g pot⁻¹) | BARI GOM 25   | 1.03        | 1.55        | 1.80        | 0.90        |
|                   | BARI GOM 26   | 1.06        | 1.58        | 1.85        | 0.94        |
| Shoot             | BARI GOM 25   | 0.50        | 0.75        | 0.85        | 0.25        |
|                   | BARI GOM 26   | 0.51        | 0.76        | 0.87        | 0.26        |
| Compartment-I     | BARI GOM 25   | 0.25        | 0.35        | 0.40        | 0.50        |
|                   | BARI GOM 26   | 0.26        | 0.36        | 0.41        | 0.51        |
| Compartment-II    | BARI GOM 25   | 0.28        | 0.45        | 0.55        | 0.15        |
|                   | BARI GOM 26   | 0.29        | 0.46        | 0.57        | 0.17        |

Total Biomass (g pot⁻¹) in different plant parts of the split-root system

| Variety       | Treatment   | Shoot | Compartment-I | Compartment-II |
|---------------|-------------|-------|---------------|---------------|
| BARI GOM 25   | A           | 48.50 | 24.30         | 27.20         |
|               | B           | 48.40 | 22.60         | 29.00         |
|               | C           | 47.20 | 22.20         | 30.60         |
|               | D           | 27.80 | 55.60         | 16.70         |
| BARI GOM 26   | A           | 48.30 | 24.50         | 27.30         |
|               | B           | 48.10 | 22.80         | 29.10         |
|               | C           | 47.00 | 22.20         | 30.80         |
|               | D           | 27.70 | 54.30         | 18.10         |

The distribution of Biomass (%) in shoot and roots grown in two separate soil compartments (I and II)

| Variety       | Treatment | Shoot | Compartment-I | Compartment-II |
|---------------|-----------|-------|---------------|---------------|
| BARI GOM 25   | A         | 48.50 | 24.30         | 27.20         |
|               | B         | 48.40 | 22.60         | 29.00         |
|               | C         | 47.20 | 22.20         | 30.60         |
|               | D         | 27.80 | 55.60         | 16.70         |
| BARI GOM 26   | A         | 48.30 | 24.50         | 27.30         |
|               | B         | 48.10 | 22.80         | 29.10         |
|               | C         | 47.00 | 22.20         | 30.80         |
|               | D         | 27.70 | 54.30         | 18.10         |

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

| Variety       | Treatment  | Biomass Production (mg pot⁻¹) | Root-shoot ratio |
|---------------|------------|------------------------------|------------------|
|               |            | Shoot | Root                          |                  |
| BARI GOM 25   | 0P/0P      | A     | 0.50                          | 1.06             |
|               | 10P/50P    | B     | 0.75                          | 1.07             |
|               | 50P/200P   | C     | 0.85                          | 1.12             |
|               | 100P/400P  | D     | 0.25                          | 2.60             |
| BARI GOM 26   | 0P/0P      | A     | 0.51                          | 1.07             |
|               | 10P/50P    | B     | 0.76                          | 1.08             |
|               | 50P/200P   | C     | 0.87                          | 1.13             |
|               | 100P/400P  | D     | 0.26                          | 2.62             |

P distribution and translocation in wheat plant within 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 split-root system using both alkaline soil (compartment I) and acidic soil (compartment II) where P doses were applied directly to the soil. The shoot and root P fixation were found in increasing trend under different P application on wheat plant, except at highest level of P application in acidic soil. Shoot and root P fixation were significantly (P ≤0.001) affected among all the various P applications on wheat plant. Again, similar trend in total P uptake were found in both varieties of BARI GOM25 and BARI GOM26. Total plant P fixation in BARI GOM 25 of Treatment C increased about 7 times (6.58 g kg⁻¹) in comparison with the controlled Treatment A (0.95 g kg⁻¹). Similarly, in Treatment B and Treatment D total plant P fixation increased about 2.5 times (2.38 g kg⁻¹) and 3 times (2.87 g kg⁻¹) respectively in comparison with Treatment A. Again, for BARI GOM26, total plant biomass in Treatment C increased 7 times (7.14 g kg⁻¹) in comparison with the controlled Treatment A (1.01 g kg⁻¹). Similarly, in Treatment B and Treatment D the total plant P fixation increased about 2.5 times (2.64 g kg⁻¹) and 3 times (3.12 g kg⁻¹) respectively in comparison with Treatment A. Similar trend was found in shoot P fixation and root P fixation of both wheat plant varieties in this study (Table 6); while internal P uptake by shoot and root was found irregular pattern among all treatments (Figure 8). The highest percentages of P uptake by shoot was found in Treatment B of BARI GOM 25, 54.6% of total plant P uptake while in Treatment A, Treatment C and Treatment D was found in deceasing order 42.1%, 38.3% and 22.0% respectively of total plant P uptake. Root P uptake was found in different pattern between compartments with increasing P supply (Table
6). In alkaline soil in compartment I of the split root system, the highest percentage of root P fixation was found in Treatment D 72.1% of total plant P uptake and in Treatment A, Treatment C and Treatment B the percentages were found in decreasing order 28.4%, 22.0% and 20.6% respectively of total plant P uptake.

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 parts / Variety | Treatment A (g kg⁻¹) | Treatment B (g kg⁻¹) | Treatment C (g kg⁻¹) |
|-----------------------|-----------------------|-----------------------|-----------------------|
| BARI GOM 25           | 0.95                  | 2.38                  | 6.58                  | 2.87                  |
| Bari GOM 26           | 1.01                  | 2.64                  | 7.14                  | 3.12                  |

Total P uptake (g kg⁻¹) in different plant parts of the split-root system

| Variety          | Total P uptake (g kg⁻¹) |
|------------------|-------------------------|
| Shoot            | 0.40                    |
| Compartmen I     | 0.27                    |
| Compartmen II    | 0.28                    |
| BARI GOM 26      |                         |
| Shoot            | 0.41                    |
| Compartmen I     | 0.29                    |
| Compartmen II    | 0.31                    |

The distribution of P (%) in shoot and roots grown in two separate soil compartments (I and II)

| Variety          | Shoot | Compartmen I | Compartmen II |
|------------------|-------|--------------|---------------|
| BARI GOM 25      | 42.10 | 28.40        | 29.50         |
| Bari GOM 26      | 40.60 | 28.70        | 30.70         |

In acidic soil in compartment II of the split root system, the highest percentage of root P fixation was found in Treatment C 39.7% of total plant P uptake and in Treatment A, Treatment B and Treatment D the percentages were found in decreasing order 29.5%, 24.8% and 5.9% respectively of total plant P uptake. Similarly, in BARI GOM 26 the highest percentage of P uptake by shoot was found in Treatment B (54.2% of total plant P uptake), while in Treatment A, Treatment C and Treatment D were found in decreasing order (40.6%, 38.4% and 22.8% respectively of total plant P uptake). In alkaline soil in compartment I of the split root system, the highest percentage of root P fixation was found in Treatment D 71.2% of total plant P uptake and in Treatment A, Treatment C and Treatment B the percentages were found in decreasing order 28.7%, 22.8% and 20.1% respectively of total plant P uptake. In acidic soil in compartment II of the split root system, the highest percentage of root P fixation was found in Treatment C 38.8% of total plant P uptake and in Treatment A, Treatment B and Treatment D the percentages were found in decreasing order 30.7%, 25.8% and 6.1% respectively of total plant P uptake (Figure 8). This percentage distribution differences in the total root and shoot P uptake between the three P treatments are due to elevated P supply which directly interferes with shoot root P status.

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 125µm inorganic P (Mimura et al., 1996). There is also significant retained 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 and then transferred to the xylem and recycled back to the shoots (Jeschke et al., 1997).
Increase of the external P supply to split root from 0 mg P kg\(^{-1}\) to 400 mg P kg\(^{-1}\) 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 with, e.g. barley (Drew and Saker, 1984), subterranean clover (Scott and Robson, 1991), tomato (Burleigh and Harrison, 1999) and Hakea prostrata (Proteaceae) (Shane et al., 2003). In contrast with the results of split-root plants, the results of our wheat plant split-root study and those of others using foliar spray (e.g. Marschner et al., 1987) demonstrate that P retranslocated in the phloem sap can result in increased root P concentrations. In our study of split root system, alkaline soil (pH 7.9) was used in compartment I and acidic soil (pH 5.2) was used in compartment II. P uptake rates are highest between pH 5.0 and 6.0 (Ullrich-Eberius et al., 1984; Furihata et al., 1992), which suggests that P is taken up at higher rate in acidic soil. So, it was expected that P fixation in compartment II was higher than that of compartment I. But, the difference in percentage between the P fixations of compartment I roots and compartment II was much lower. It was due to plants that would be able to translocate P from the roots in compartment I to that 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 shoot of our 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 wheat plants. In contrast, the split-root technique probably provides a more stable supply of P at a lower concentration.

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